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BIOCOMPATIBILIDADE DE UMA RESINA ACRÍLICA E REEMBASADORES RÍGIDOS: ANÁLISE HISTOMORFOMÉTRICA EM RATOS

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Tese apresentada para obtenção do título de Doutor na Universidade Estadual de Ponta Grossa, no Curso de Doutorado em Odontologia Área de concentração em Clínica Integrada.

Orientadora: Prof[a] Dr[a]. Nara Hellen Campanha Bombarda
Co-orientador: Prof Dr. Eduardo Balm Campagnoli

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LISSANDRA MATOS BRÓL MEISTER

BIOCOMPATIBILIDADE DE UMA RESINA ACRÍLICA E REEMBASADORES RÍGIDOS: ANÁLISE HISTOMORFOMÉTRICA EM RATOS

Tese aprovada como requisito parcial para obtenção do título de Doutora em Odontologia, Área de concentração em Clínica Integrada na Universidade Estadual de Ponta Grossa.

Ponta Grossa, 13 de dezembro de 2012

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Dedico este trabalho,

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“Ser feliz é reconhecer que vale a pena viver
Apesar de todos os desafios,
Incompreensões e períodos de crise.
Ser feliz é deixar de ser vítima dos problemas
E tornar-se um autor da própria história.
É atravessar desertos fora de si,
Mas ser capaz de encontrar um oásis
No recôndito da sua alma.
É agradecer a Deus a cada manhã pelo milagre da vida.
Ser feliz é não ter medo dos próprios sentimentos.
É saber falar de si mesmo.
É ter coragem para ouvir um “não”.
É ter segurança para receber uma crítica,
Mesmo que injusta.
Pedras no caminho?
Guardo todas, um dia vou construir um castelo...”

Fernando Pessoa

RESUMO

MEISTER LMB. **Biocompatibilidade de uma resina acrílica e reembasadores rígidos: análise histomorfológica em ratos.** [Tese] Doutorado em Clínica Integrada. Ponta Grossa: Universidade Estadual de Ponta Grossa, 2012.

A ocorrência de reações adversas na mucosa provocadas pelo uso de próteses removíveis é comum. Esse fato tem despertado o interesse por este tópico e muitos estudos tem sido realizados para determinar o comportamento biológico desses materiais objetivando buscar materiais mais biocompatíveis com o meio bucal. Além disso, novas técnicas e tratamentos pós-polimerização de resinas de base e reembasadores são propostos para minimizar a quantidade de produtos liberados e os efeitos que provocam no epitélio bucal. Assim, a proposta do presente estudo foi avaliar a biocompatibilidade de uma resina acrílica e de reembasadores rígidos, em uma análise histomorfométrica em ratos. A hipótese de que um tratamento térmico pós polimerização poderia diminuir a citotoxicidade dessas resinas acrílicas foi avaliada. Para isso, foram utilizados 45 ratos fêmeas adultas (*Rattus Norvergicus albinus Wistar*), com 60 dias de idade, pesando de 150 g a 250 g, obtidos do Biotério Central da Universidade Estadual de Ponta Grossa. Este estudo foi aprovado e está de acordo com as recomendações do Comitê de Ética em Experimentação Animal (SCEEA-UEPG) e as normas de manipulação de animais do COBEA (Colégio Brasileiro de Experimentação Animal) foram seguidas durante todo o experimento. Placas palatais de resina acrílica termopolimerizável Lucitone 550 foram confeccionadas individualmente, e reembasadas com os reembasadores rígidos Tokuyama Rebase II, New Truliner e Kooliner. Para comparação dos dados, cinco animais que não receberam as placas foram mantidos sob as mesmas condições, durante o experimento (controle). As placas compreendiam a área do palato localizada na região entre os molares, cobrindo as faces oclusal e vestibular dos mesmos e estendendo-se anteriormente até a primeira prega palatina. Metade das placas com reembasadores recebeu tratamento térmico pós-polimerização de imersão em água a 55^{o}C por 10 min e metade das placas confeccionadas apenas com a resina de base receberam o mesmo tratamento térmico, porém pelo tempo de 60 min. As placas foram cimentadas aos molares, com resina fotopolimerizável, permanecendo por 14 dias. Os animais receberam dieta pastosa e água *ad libitum*. Foi realizada aferição do peso dos animais antes e após o período experimental, em balança de semi precisão. Os animais de todos os grupos foram sacrificados por eutanásia após 14 dias. O palato dos animais foi dissecado, fixado em formol tamponado a 10% durante 48 h e descalcificado em EDTA a 4,17% por aproximadamente quatro meses. As peças assim obtidas foram incluídas em parafina, de forma que a parte posterior do palato ficasse para baixo. As amostras foram cortadas em micrótomo em secções de 5 µm e coradas com hematoxilina e eosina (H&E). Foram realizadas cinco lâminas de cada animal, contendo cinco cortes histológicos. Foram feitas duas imagens, padronizadas da região central dos cortes, em aumento de 200x, para verificar as alterações histológicas. Foram calculadas espessuras de epitélio total, compartimento celular e camada de queratina. A análise estatística aplicada aos dados do peso (g) dos animais nos tempos 0 e 14 dias de utilização das placas foi ANOVA ($\alpha = 0,05$). Não houve diferença significativa na alteração do peso dos animais. Para comparação dos dados obtidos na análise histológica de todos os grupos analisados foi realizado teste ANOVA de um fator de variação (tipo de placa que

o animal recebeu). Em um dos estudos, o grupo sem placa (controle) foi comparado estatisticamente com palatos que receberam placas de resina termopolimerizável submetidas ou não a tratamento térmico. Em outro estudo, o grupo controle foi comparado estatisticamente com palatos que receberam placas reembasadas com os reembasadores submetidos ou não ao tratamento térmico. As médias dos grupos foram comparadas com Pós-teste de Tukey, com $\alpha = 0,05$. Os resultados da espessura de camada de queratina dos materiais New Truliner, Tokuyama Rebase II e Kooliner sem tratamento térmico adicional foi estatisticamente menor do que a do grupo controle ($p<0,0001$). Para esse mesmo parâmetro, o tratamento térmico alterou significativamente a média da resina de base Lucitone 550, reduzindo-a. As médias de espessura de tecido conjuntivo das resinas Lucitone 550 e Tokuyama Rebase II sem tratamento térmico adicional foram estatisticamente maiores do que o controle ($p<0,01$). As mesmas médias de Kooliner, sem tratamento térmico, foram menores do que o controle ($p<0,01$). O tratamento térmico foi capaz de alterar o comportamento das resinas Lucitone 550, Tokuyama Rebase II e New Truliner, reduzindo significativamente a espessura de tecido conjuntivo dos dois primeiros materiais e aumentando a do último material ($p<0,01$). As médias de espessura total de epitélio das resinas Kooliner e New Truliner sem tratamento térmico foram significativamente menores do que o controle ($p<0,001$ e $p<0,01$, respectivamente). Com relação ao tratamento térmico, esse foi responsável por alterar o comportamento dos materiais Lucitone 550 e Kooliner, aumentando, nos dois casos, a espessura desse tecido ($p<0,05$ e $p<0,001$, respectivamente). Com base nos resultados, pôde-se concluir que a utilização de impressões individuais em ratos Wistar, associadas a placas com cobertura oclusal sobre molares e o método de cimentação com resina composta sem o uso de sistema adesivo, proporcionaram boa estabilidade do dispositivo palatino desenvolvido neste estudo e a sua permanência na cavidade bucal dos animais não alterou os hábitos alimentares. Além disso, o tratamento térmico pós-polimerização proposto alterou a biocompatibilidade dos materiais avaliados, dentro das condições experimentais do presente estudo.

Palavras-chave: Ratos Wistar. Mucosa palatal. Citotoxicidade. Reembasadores. Tratamento térmico.

ABSTRACT

MEISTER LMB. Biocompatibility of an acrylic resin and a hard reliner: histomorphologic analysis in rats. [Tese] Doutorado em Clínica Integrada. Ponta Grossa: Universidade Estadual de Ponta Grossa, 2012.

The occurrence of adverse reactions in the mucosa caused by the use of removable dentures is very common. This fact raised an interest in this particular matter, and, because of that, a lot of studies have been done in order to determine the biological behavior of these materials, looking for materials that are biocompatible with the oral environment. Besides that, new techniques and treatments after-polymerization of denture base and reline resins are proposed in order to minimize the amount of released products and their effects in oral epithelium. Thus, the purpose of the current study was to test the biocompatibility of an acrylic base and hard chair-side reline resins, with an histopathological analysis in rats. The hypothesis that post-polymerization heat-treatments could reduce the cytotoxicity of these acrylic resins was evaluated. For this purpose, forty five adult female rats (*Rattus Norvergicus* Albinus Wistar), with 60 days old, weighing 150 g – 250 g, taken from the Biotério Central da Universidade Estadual de Ponta Grossa were used. This study received approval and is in accordance with the Comitê de Ética em Experimentação Animal (SCEEA-UEPG) recommendations, and, the animal manipulation rules of COBEA (Colégio Brasileiro de Experimentação Animal) were followed during the experiment. Palatal plates of heat-polymerized denture base acrylic resin Lucitone 550 (L) were custom-made and relined with Tokuyama Rebase II (TR), New Truliner (NT) or Kooliner (K) hard chair-side reline resins. For data comparison, five animals that did not receive the palates were kept under the same conditions during the experiment (control group). The plates embraced the palate area, in the region between the molars covering the occlusal and vestibular surfaces, being extended up to the first palatal fold. Half of the relined plates received a post-polymerization heat-treatment (immersion in water at 55°C for 10 min) and half of the acrylic resin denture bases received a similar post-polymerization heat-treatment, but for 60 min. The plates were cemented in the molar region with light-cured resin, thus being kept there for 14 days. The animals received a paste diet and water and libitum. Before and after the trial period, the rats were weighed individually on a precision scale. After 14 days, the animals of all groups were sacrificed by euthanasia. The palate of the animals were dissected, fixed in 10% buffered formalin for 48 h and decalcified in 4,17% EDTA for approximately four months. Then, the pieces were processed through a dehydration process to be embedded in paraffin, so that the back part of the palate lied down. The samples were cut with a microtome in sections of 5 µm and then stained with haematoxylin and eosin (H&E). Five histological cuts were done in each animal. Two standardized images from the central region of the cuts were made, referring to the rafe palatine, augmented 200x in order to verify histological changes. These include the thickness calculation of cellular compartment (TCC), total epithelial thickness (TET), and of keratin layer (TKC). Statistical analysis was performed using ANOVA ($\alpha = 0.05$) test for comparison of weigh (g) of the animals at times 0 and 14 days of device using. There was no significant difference in weight change observed in the animals. For histological data comparisons from all groups it was performed one-way ANOVA (for type of palatal plate used). In one of the studies, the control group (without plates) was statistically compared to acrylic resin denture base plates submitted or not to the post-polymerization heat-treatment. In another study, the control group was

statistically compared to palates fitted with relined plates submitted or not to the post-polymerization heat-treatment. Mean values from groups were compared with post-hoc Tukey with $\alpha = 0.05$. Results from Keratin layer thickness from materials New Truliner, Tokuyama Rebase II and Kooliner without post-polymerization heat-treatment were statistically lower than control group ($p<0.0001$). For the same parameter, the heat-treatment significantly changed the results from Lucitone 550, reducing it. Means of connective tissue from resins Lucitone 550 and Tokuyama Rebase II whithout treatment were statisticaally higher than the control ($p<0.01$). The same means from Kooliner, without heat-treatment, were lower than the control ($p<0.01$). The heat-treatment was able to change the behaviour of resins Lucitone 550, Tokuyama Rebase II and New Truliner, reducing significantly the connective tissue thickness from both the former and augmenting that from the last material ($p<0.01$). Means of total epithelial thickness from resins Kooliner and New Truliner without heat-treatment were significantly lowers than the control ($p<0.001$ and $p<0.01$, respectivelly). As regards to the heat-treatment, it was responsable for change the behavior from materiais Lucitone 550 and Kooliner, augmented, in both cases, the thickness of this tissue ($p<0.05$ e $p<0.001$, respectivelly). Based on the results, it was possible to conclude that the use of individual impressions in Wistar rats, associated to plates with occlusal coverage on molars and the cementation with resin method developed in this study did not change their eating habits. Besides that, the post-polymerization heat-treatment proposed changed the biocompatibility of the materials evaluated, under the experimental conditions of this study.

Keywords: Wistar rats. Palatal mucosa. Citotoxicity. Reliners. Heat-treatment.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

g	grama
mg	miligrama
ml	mililitro
cm	Centímetro
mm	milímetro
µm	micrometro
min	minuto
n^o	número
°C	temperatura Celsius
%	porcentagem
PEMA	polietil metacrilato
BMA	butil metacrilato
IBMA	isobutil metacrilato
HDMA	hexanediol dimetacrilato
PMMA	polimetil metacrilato
PB	peróxido de benzoíla
DPT	Dimetil p-toluidina
L	Lucitone 550
K	Kooliner
TR	Tokuyama Rebase II
NT	New Truliner

SUMÁRIO

1 INTRODUÇÃO

O material de escolha para reabilitação oral com prótese total ou prótese parcial removível em pacientes, desde a década de 30 até os dias atuais é a resina acrílica[1]. Utilizada para confecção de base de próteses e dentes artificiais, deveria possuir como propriedades essenciais para sua utilização na cavidade bucal algumas características importantes como cor e translucidez, ser quimicamente estável, ter suficiente dureza, ótima resiliência e resistência ao desgaste, ser impermeável, insolúvel aos fluidos bucais e biocompatível[2].

As resinas acrílicas são classificadas pela forma de ativação e processamento, sendo, quimicamente ativadas, termoativadas (convencionais ou polimerizadas por microondas) e fotoativadas[2]. São compostas por polimetil metacrilato, uma mistura de um polímero em pó contendo peróxido de benzoíla e um monômero líquido, geralmente o metacrilato de metila. A reação de polimerização do material ocorre com uma rápida decomposição do peróxido de benzoíla, após ter sido ativado, o que faz com que seja liberada uma quantidade grande de radicais livres, polimerizando o monômero. Mas existe a presença de uma quantidade de monômero que não é convertido em polímero, denominado monômero residual[3-5]. O monômero residual apresenta efeito negativo sobre as propriedades das resinas acrílicas[6-9] e pode ser liberado para o meio bucal, afetando a biocompatibilidade desses materiais. As reações mais frequentes encontradas na mucosa são vermelhidão, erosão na mucosa bucal, queimação na mucosa e na língua[10-12].

Há muitos trabalhos que relatam que o monômero residual liberado causa irritação e sensibilidade nos tecidos de suporte[1,2,5,11-15]. Fisher[11], em 1956, dividiu as reações às resinas acrílicas em dermatite alérgica de contato que atinge dentistas e técnicos de prótese e estomatite alérgica que afeta os pacientes usuários de prótese. Smith e Bains[16], em 1956, atribuíram ao metil metacrilato não convertido em polímero a irritação da mucosa bucal de pacientes portadores de próteses de resina acrílica. Kedjarune et al.[15], em 1999, verificaram que todas as resinas testadas apresentaram conteúdo de monômero residual e sua quantidade não depende somente do tipo de polimerização, mas também da proporção pó-líquido e do método de processamento. Quanto maior a quantidade de líquido adicionada na mistura maior será a quantidade de monômero residual. Além disso, quando foi aumentado o tempo de polimerização, a

quantidade de monômero reduziu e a citotoxicidade está relacionada com a concentração de metacrilato de metila da solução.

Além do monômero residual, também tem sido detectada a liberação de formaldeído, ácido metacrílico, e ácido benzóico desses materiais[16-19]. Ruyter[17], em 1980, realizou um estudo com objetivo de avaliar a liberação e a origem do formaldeído de resinas acrílicas para base de próteses, e verificou que a quantidade de formaldeído liberada pelas resinas termopolimerizáveis foi menor que nas resinas autopolimerizáveis. Em 1994, Tsuchiya et al[18] realizaram um estudo com os mesmos objetivos acima citados, porém foram testados discos de resina imersos em saliva artificial e em cavidade bucal de indivíduos. Observaram que houve liberação de formaldeído e metacrilato de metila de em todas as imersões, no entanto, a liberação in vitro foi maior que a in vivo, e que as resinas autopolimerizáveis liberaram mais produtos que as resinas polimerizadas convencionalmente em banho da água e por meio de microondas.

Com o passar tempo, as bases acrílicas das próteses podem ficar desadaptadas, perdendo retenção, estabilidade e suporte, como consequência da reabsorção do rebordo alveolar, que é um processo crônico e irreversível, provocando mobilidade e perda na efetividade mastigatória, gerando desconforto para o paciente. Além disso, essa prótese não intimamente ajustada pode intensificar a concentração de forças em regiões do rebordo, aumentando a reabsorção óssea. Portanto, há necessidade de retorno periódico do paciente ao consultório, para reavaliação das bases e, se necessário, reembasamento das próteses removíveis totais e parciais. Um método alternativo ao reembasamento do tipo mediato, em que há necessidade das fases de inclusão e prensagem laboratoriais, é a readaptação das bases que pode ser realizada diretamente na boca, no próprio consultório. Essa técnica, denominada reembasamento imediato ou direto, é realizada com materiais reembasadores resilientes ou rígidos, autopolimerizáveis, sendo considerada fácil, de rápida execução e baixo custo[20]. Segundo Christensen[21], em 1995, os materiais reembasadores devem apresentar baixa exotermia, manutenção de cor e sabor agradável. Entretanto, uma das mais importantes propriedades desses materiais é sua biocompatibilidade, que pode ser definida como a capacidade de um material artificial não causar reações nos tecidos ao seu redor e no organismo como um todo[22].

Os materiais reembasadores rígidos são fornecidos na forma de um pó geralmente composto de polietil metacrilato (PEMA) e de um líquido que pode ser à base de metacrilato butil (BMA), metacrilatoisobutil (IBMA) ou 1,6-hexanediol

dimetacrilato (1,6 - HDMA), apresentando, composição diferente das resinas acrílicas autopolimerizáveis convencionais. Arima et al.[23], em 1996, por consequência do grande crescimento de resinas autopolimerizáveis indicadas para reembasamento de próteses, analisaram algumas dessas resinas quanto à composição química, temperatura de transição vítrea, peso molecular e distribuição do tamanho das partículas do pó. Para a avaliação da composição do pó e do líquido, foi utilizado o método da espectrofotometria. Os autores observaram relação direta entre a composição química do material e suas propriedades. Essas diferenças na composição podem influenciar, significativamente, as propriedades físicas, mecânicas e biológicas dos materiais reembasadores[24-26]. Assim, estudos com a finalidade de testar novos materiais e técnicas para melhorar propriedades físicas, químicas e biológicas de resinas de base e reembasadores de prótese têm sido realizados[3,27-29].

As substâncias tóxicas e seus efeitos sobre os tecidos têm sido constatados por meio de estudos em animais, observações clínicas e cultura de células in vitro[30-31]. O teste de citotoxicidade, em que se utiliza o método de cultura de células, é considerado um teste preliminar para avaliar biocompatibilidade de um material, é simples, pode ser reproduzido, é efetivo e controlado[32,33]. Existem na literatura vários estudos com testes iniciais que determinam a citotoxicidade das resinas acrílicas[33-39]. Em 2001, Costa[40] relatou uma metodologia de avaliação de citotoxicidade de materiais odontológicos. O autor cita o teste MTT, que é baseado na contagem celular, no metabolismo celular, na verificação do halo de inibição e na morfologia celular por meio de utilização de microscopia eletrônica de varredura. Schuster et al.[35], em 1995, realizaram um estudo com o objetivo de analisar o efeito das substâncias liberadas pelas resinas para bases de próteses no metabolismo de lipídeos de células epiteliais orais e chegou-se a conclusão de que os componentes liberados pelas resinas para bases de próteses podem afetar o metabolismo de lipídeos e possibilitam alterações nas membranas celulares. Em outro estudo, realizado por Sheridan et al.[36] em 1997, foi verificado o efeito citotóxico de resinas acrílicas sobre fibroblastos gengivais. O estudo demonstrou que, dentre as resinas, a que apresentou maior efeito citotóxico foi a quimicamente ativada em relação a termoativada. O efeito citotóxico foi maior nas primeiras 24 horas e diminuiu ao longo do tempo para todas as resinas testadas. Com isso, concluiu-se que o armazenamento das próteses em água por longo período pode diminuir a citotoxicidade das substâncias liberadas e que as resinas acrílicas ativadas quimicamente são mais citotóxicas do que as termoativadas.

Poucas informações são encontradas relacionadas ao comportamento biológico dos materiais para base e reembasamento de próteses em testes secundários, isto é, realizados diretamente em animais[41-45]. As resinas acrílicas permanecem em contato direto com os tecidos da cavidade bucal, havendo necessidade de testes in vivo em animais para se observar as alterações histológicas que podem ocorrer devido à utilização prolongada desses materiais. Estudos in vivo, utilizando aparelhos adaptados no palato de animais experimentais, foram encontrados na literatura. Jorge et al.[46], em 2001, realizaram um estudo em ratos (*Rattus norvergicus*, Albinus, Wistar), para verificar os efeitos da xerostomia após sialoadenectomia, no desenvolvimento de candidose bucal, utilizando placas acrílicas para recobrimento do palato. Barclay et al.[42,43] em 1997, realizaram um estudo em ratos Wistar, com o objetivo de verificar os efeitos histológicos na mucosa do palato, tanto da utilização de placas palatinas (por 7 e 14 dias), quanto o tipo de dieta (três, ao todo). Observaram que é possível utilizar placas palatinas em ratos Wistar e que a dieta pastosa é uma alternativa para que haja uma redução no acúmulo de resíduos alimentares sob a placa. Nesse mesmo ano, em um segundo estudo realizado por Barclay et al.[43] foram avaliados os efeitos histológicos sobre a mucosa palatina de ratos Wistar por conseqüência do uso de um dispositivo palatino confeccionado de resina acrílica com a incorporação de dois materiais para reembasamento imediato de próteses removíveis, sendo um rígido, Kooliner, e outro resiliente, Coe-soft. No seu estudo, foi verificado que os materiais reembasadores não foram inertes sobre a mucosa e causaram um aumento na espessura da camada de queratina, porém não houve aumento do epitélio total. Maruo et al.[47], em 2003, em um estudo sobre diabetes mellitus, utilizou placas acrílicas cimentadas em ratos Wistar, para verificar reação tecidual. As placas ficaram cimentadas sem pressão, com pressão contínua e intermitente e verificaram que houve uma redução da espessura de epitélio total e os estímulos mecânicos diminuíram a susceptibilidade para alterações celulares. Tsuruoka et al.[45], em 2008, realizaram um estudo para determinar os efeitos da compressão mecânica de prótese mucossuportada no palato de ratos, em níveis fisiológico, histológico e molecular. Concluiu-se que o efeito da compressão mecânica foi maior nas células do periósteo e essas células sintetizam HSP70, que é responsável pela remodelação óssea e VEGF para manter homeostasia nessas condições. Devido às dificuldades inerentes a esse tipo de metodologia, tem sido buscados dispositivos palatais para serem utilizados em estudos em ratos. Lee et al.[48], em 2011, realizaram um estudo sobre *Candida* associada à estomatite devido ao uso de prótese total.

Desenvolveram um dispositivo de fácil confecção para pesquisas na cavidade bucal de animais, que consiste em uma placa acrílica estabilizada no palato de ratos. Johnson et al.[49] em 2012, utilizaram um sistema de dentadura em palato de ratos, com uma parte fixa e outra removível para realizar estudos longitudinais sobre estomatite protética relacionados com *Candida albicans*. Entretanto a perda de dispositivos palatais durante os experimentos, as dificuldades técnicas, o prejuízo nutricional provocado pelo uso das placas ainda tem sido relatados na literatura.

Têm sido investigadas formas de se melhorar a polimerização dos materiais para base e rembasamento de próteses e seu comportamento por meio de tratamentos para diminuir o efeito citotóxico de seus produtos e subprodutos oriundos da reação de polimerização[8,19,23,39,50-55]. Jorge et al.[38] em 2004, investigaram os efeitos de tratamentos térmicos na citotoxicidade de três resinas para bases de próteses, por meio de dos testes de incorporação de ^{3}H-timidina e MTT. Para a análise do efeito citotóxico das substâncias liberadas pelos corpos de prova, foram obtidos das amostras seus extratos das substâncias hidrossolúveis e verificaram que a citotoxicidade das resinas testadas não foi influenciada pelo tratamento térmico em microondas ou banho em água aquecida. A polimerização complementar em microondas ou em banho de água diminui a quantidade de monômero residual e, portanto, podendo diminuir a citotoxicidade das resinas acrílicas, sendo assim, Campanha et al.[39], em 2006, realizaram um estudo com teste de incorporação de ^{3}H-timidina e teste MTT, para avaliar o efeito da polimerização complementar em microondas ou em banho de água aquecida sobre a citotoxicidade de seis reembasadores diretos e verificaram que o tratamento em banho de água diminuiu a proliferação celular para os materiais Tokuso Rebase Fast, New Truliner e Light Liner. No tratamento em microondas, houve aumento na citotoxicidade dos materiais Kooliner, New Truliner e Light Liner. Jorge et al.[56] em 2009, em um estudo para avaliar o efeito do tratamento térmico em microondas e do tempo de armazenamento em água sobre a citotoxicidade de resinas acrílicas rígidas para base e reembasamento de próteses, testaram Kooliner, Tokuyama Rebase II, New Truliner, Acron MC, Lucitone 550 e QC 20 por meio de teste de incorporação de ^{3}H-timidina. Os resultados mostraram que o tratamento térmico em microondas e o armazenamento em água podem ser considerados uma alternativa para a redução da citotoxicidade da algumas resinas para base e reembasamento de prótese.

Enquanto poucas informações com estudos in vivo, relativas aos materiais reembasadores rígidos estão disponíveis, foi julgado oportuno testar o efeito do

tratamento térmico pós-polimerização dos dispositivos imersos em água aquecida sobre a citotoxicidade de resinas acrílicas para base e reembasamento imediato de próteses, sendo que para isso foi desenvolvido no presente estudo, um dispositivo de resina acrílica para fixação em palato de ratos que permite o reembasamento das resinas de base.

2 PROPOSIÇÃO

2.1 OBJETIVO GERAL

Avaliar a biocompatibilidade, por meio de estudo in vivo, de resinas acrílicas para base e reembasamento de próteses frente ou não a tratamento térmico pós-polimerização.

2.2 OBJETIVOS ESPECÍFICOS

a) Descrever um dispositivo palatal simples e funcional para ser utilizado em estudos de biocompatibilidade de materiais para base e reembasadores de prótese em ratos.

b) Avaliar a biocompatibilidade de uma resina acrílica para base e de materiais reembasadores rígidos para próteses, por meio de análise histopatológica em ratos Wistar.

c) Verificar o efeito de um tratamento térmico pós polimerização, que visa diminuir a quantidade de monômero residual presente na resina acrílica polimerizada, sobre a biocompatibilidade desses materiais.

3 MATERIAIS E MÉTODOS

3.1. SELEÇÃO DOS ANIMAIS

Para realização do estudo foram utilizados 45 ratos fêmeas adultas (*Rattus Norvergicus albinus, Wistar*), com 60 dias de idade, pesando de 150 g a 250 g, obtidas do Biotério Central da Universidade Estadual de Ponta Grossa. Os animais foram mantidos em gaiolas separadas em temperatura ambiente de 23°C com 56% de umidade relativa, alternando-se ciclos claro/escuro de 12 h. Eles foram alimentados com água *ad libitum* e com ração (Nuvilab CR-1, Nuvital, Brasil). Os animais foram submetidos a dieta pastosa uma semana antes ao início dos experimentos. Este estudo foi aprovado e está de acordo com as recomendações do Comitê de Ética em Experimentação Animal (SCEEA-UEPG) pelo parecer nº 11/2010 e Protocolo nº 12673/2010 e seguiu as normas de manipulação de animais do COBEA (Colégio Brasileiro de Experimentação Animal) durante todo o experimento.

3.2. PREPARAÇÃO DOS ANIMAIS

Os procedimentos para obtenção das moldagens para confecção das placas palatinas dos animais foi realizado sob anestesia, com administração de uma solução de anestésico quetamina (100 mg/mL) e relaxante muscular xilasina (100 mg/mL), diluindo-se 3,75 mL de solução de quetamina mais 0,5 mL de xilasina em 5,75 mL de água destilada. A administração de injeção intraperitonial foi na concentração de 0,2 mL/100 g para sedação dos animais[59]. O tempo de trabalho obtido com esta técnica foi de aproximadamente de 30 min. Os animais foram sedados em todos os procedimentos realizados.

3.3. MATERIAIS AVALIADOS

Para realização do experimento, todas as placas foram confeccionadas com a resina acrílica termopolimerizável para base de prótese Lucitone 550, as quais foram reembasadas ou não com uma das três resinas acrílicas rígidas autopolimerizáveis, indicadas para reembasamento imediato de próteses removíveis descritas nos Quadro 1.

Material	Sigla	Fabricante	Proporção pó/líquido (g/ml)	Condições de polimerização	Pó	Líquido
Lucitone 550	L	Dentsply Indústria e Comércio Ltda, Petrópolis, RJ, Brasil	0,42/0,2	90 min a 73°C e 30 min a100°C (ciclo curto)	Polimetil metacrilato – PMMA	Metil metacrilato etileno glicol dimetacrilato
Kooliner	K	GC América Inc, USA	1,4/1	10 min à temperatura ambiente	Polietil metacrilato – PEMA Peróxido de benzoíla – PB	Isobutil metacrilato
Tokuyama Rebase II	TR	Tokuyama Dental Corporation, Japão	2,05/1	5,5 min à temperatura ambiente	PEMA PB	1,6 Hexanediol dimetacrilato HDMA β-metacriloil oxietil propianato DPT-dimetil p-toluidina
New Truliner	NT	Bosworth Company, USA	1,34/1	10-15 min à temperatura ambiente	PEMA PB	Isobutil metacrilato Dibutil n-ftalato

Quadro 1- Materiais utilizados

Os animais foram divididos em nove grupos, com cinco animais cada, de acordo com o tipo de material a ser testado com a realização ou não de tratamento térmico, e um grupo sob as mesmas condições, porém, sem placa.

3.4. FABRICAÇÃO DAS PLACAS PALATINAS

Para obtenção da moldagem individual foram confeccionadas, previamente, 40 moldeiras individuais fabricadas com silicone de adição de consistência pesada (Futura AD, DFL Indústria e Comércio S.A., RJ, Brasil), que foi construído sobre um modelo de gesso obtido de uma moldagem preliminar em um palato de rato Wistar. Este animal apresentava idade e peso aproximados aos utilizados durante os experimentos. Assim, as moldeiras foram ajustadas e suas bordas testadas para cada rato a ser moldado de tal maneira que alcançassem o fundo de vestíbulo. Após a sedação dos animais, foi realizada a moldagem de trabalho com silicone de adição de consistência leve (Futura AD, DFL Indústria e Comércio S.A., RJ, Brasil) (Figura 1). Para isso, os animais foram colocados em uma placa estabilizadora, sendo a boca mantida aberta e presa pelos incisivos com auxílio de alças, facilitando a moldagem do palato (Figura 2).

Figura 1 - Molde

Figura 2 - Estabilização do animal

Os modelos de trabalho foram obtidos com gesso tipo IV (Herostone, Vigodent S/A Indústria e Comércio, RJ, Brasil) (Figura 3) e, sobre eles, foi realizado o enceramento da placa com uma lâmina de cera 7, sem qualquer alívio. O enceramento foi feito sobre a área chapeável que compreendia a região entre os molares, estendendo-se até a primeira prega palatina, cobrindo a face oclusal dos molares e passando para face vestibular dos mesmos (Figura 4).

Figura 3-Modelo de gesso

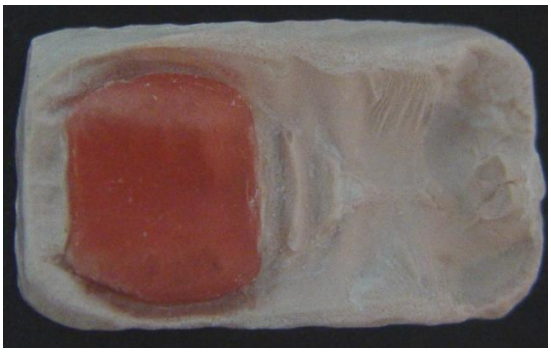

Figura 4-Modelo de gesso com enceramento com cera n° 7

Figura 5 - Placa palatina finalizada

Resina acrílica para base de prótese Lucitone 550 foi termopolimerizada convencionalmente em mufla de acordo com as recomendações do fabricante (Quadro 1). O pó foi pesado em balança de precisão e o monômero foi dispensado com auxílio de pipetas de vidro, individuais para cada material. As placas foram prensadas individualmente, e após o término da polimerização, a mufla foi resfriada por 30 min sobre a bancada e por 15 min em água corrente. Após a demuflagem, os dispositivos foram inspecionados visualmente, para verificação de presença de irregularidades, porosidades na superfície e em caso positivo eles seriam descartados. Foram removidos os excessos com fresa Maxicut e com um espessímetro foi verificada uniformidade na espessura da placa palatina, devendo possuir 1 mm de espessura em toda superfície. Em seguida, foi realizado o polimento somente na superfície externa da placa. Imediatamente após a fabricação das placas palatinas, as mesmas foram imersas em 50 mL de água destilada por 48 h a 37°C, anteriomente à instalação para eliminação de monômero residual[56], assim os dispositivos estavam concluídos para fixação (Figura 5).

3.5. FORMA DE FIXAÇÃO E REEMBASAMENTO DAS PLACAS PALATINAS

Uma semana após a obtenção das impressões, as placas palatinas em resina acrílica termopolimerizável foram fixadas no palato dos animais, todos os grupos da mesma maneira. Para promover uma retenção mecânica e estabilidade, foi realizado um desgaste na parte interna de todas as placas palatinas, na região dos dentes molares usando uma broca esférica nº6 em baixa rotação (Figura 6). Foi colocada uma pequena quantidade de resina composta (Opallis, FGM, Joinville, SC, Brasil), de modo a não haver excesso, na região dos molares para promover a fixação (Figura 7). A quantidade de resina composta utilizada foi mínima para não invadir a superfície palatina, não interferindo no contato da placa com o palato. Em seguida, a placa foi posicionada no palato do animal e a resina foi fotopolimerizada. As placas referentes aos grupos em que foram testados os materiais reembasadores K, TR e NT seguiram o mesmo procedimento, em seguida ao reembasamento dos dispositivos, que foi realizado de acordo com as instruções do fabricante (Quadro 1). Os excessos de material reembasador foram removidos e as placas foram colocadas em posição durante a polimerização (Figura 8), realizado com aparelho fotopolimerizador LED por 40 s (Gnatus, Ribeirão Preto, SP, Brasil). Após o término do procedimento, os ratos foram colocados em descanso para recuperação da anestesia. Foram mantidos em dieta pastosa para evitar muito acúmulo de resíduos de ração sob a placa palatina, com objetivo de não interferir na adequada avaliação histológica e também para facilitar a alimentação. Os animais permaneceram com as placas fixadas no palato durante 14 dias. Para comparação dos dados, cinco animais não receberam as placas e foram mantidos sob as mesmas condições, pelo mesmo período, denominados grupo controle.

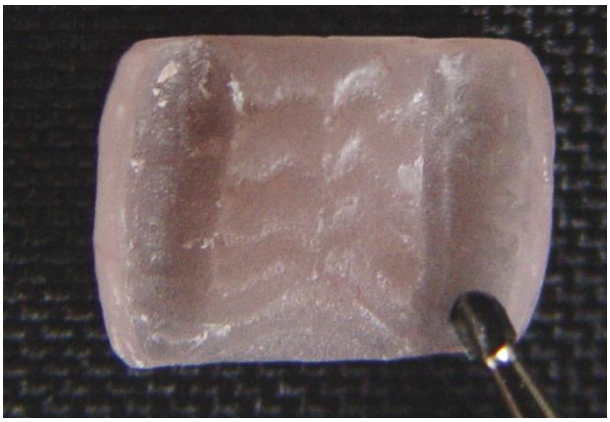

Figura 6-Retenção na região dos dentes molares

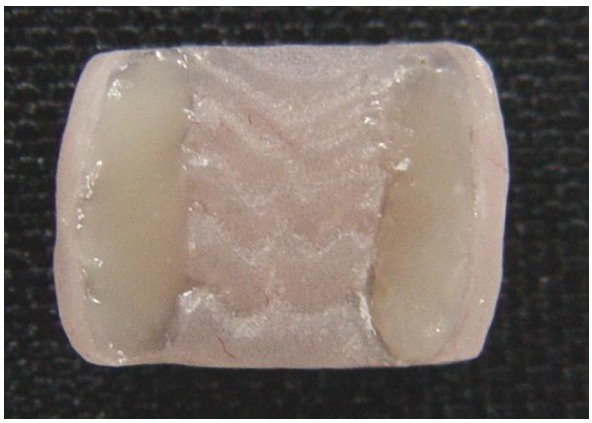

Figura 7-Resina composta para fixação

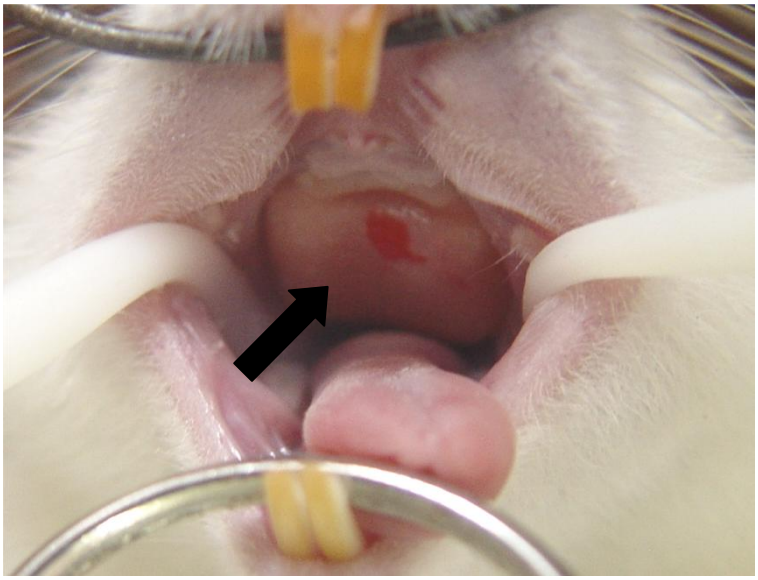

Figura 8 - Placa posicionada

3.6. TRATAMENTO TÉRMICO

Logo após a confecção e previamente à fixação, metade dos dispositivos com material reembasador, receberam tratamento térmico sendo imersos em água a 55^{o}C por 10 min em aparelho termopolimerizador[39]. Metade dos dispositivos confeccionados com Lucitone 550, receberam tratamento térmico imersos em água a 55^{o}C por 60 min em aparelho termopolimerizador[38].

3.7. PESO DOS ANIMAIS

Os ratos, dos diferentes grupos, foram pesados, individualmente, em balança de precisão (Gehaka, Ind. e Com. Eletro – Eletrônica Gehaka Ltda, São Paulo – Brasil), no momento em que foi realizada a moldagem para confecção dos dispositivos palatinos, e após quatorze 14 dias de utilização das placas.

3.8. ANÁLISE HISTOMORFOMÉTRICA

Os animais de todos os grupos foram sacrificados por eutanásia após 14 dias, de acordo com a determinação da resolução número 714 de 20 de Junho de 2002 do Conselho Federal de Medicina Veterinária (CFMV). Assim, cada animal foi anestesiado de acordo com a metodologia descrita anteriormente e em seguida foi aplicado cloridrato de potássio a 10% (1 mL/100 g), diretamente no coração, próximo ao ventrículo esquerdo. O palato dos animais foi dissecado, fixado em formol tamponado a 10% durante 48 h e descalcificado em EDTA a 4,17% por aproximadamente quatro meses. Desta forma, as peças foram processadas, passando por um processo de desidratação para serem incluídas em parafina, de forma que a face distal dos segundos ou terceiros molares ficasse para baixo. As amostras foram embebidas em parafina e cortadas em micrótomo (Leica, Berlin, Alemanha) em secções de 5 µm e coradas com hematoxilina e eosina (H&E). Foram realizadas cinco lâminas de cada animal, contendo cinco cortes histológicos. Lâminas com os cortes foram visualizados em microscópio de luz (Olympus Bx41^{TM}, DP-72, Japão). Foram feitas duas imagens, padronizadas da região central dos cortes, próxima ao plexo nasopalatino e na linha média da rafe palatina, em aumento de 200X, para verificar as alterações histológicas. Foram calculadas espessuras de epitélio total (TET), compartimento celular (TCC) e camada de queratina (TKC). Foram realizadas as mensurações do epitélio em programa denominado Image-Pro Plus (software versão 4.5.0.29-Media Cybernetics, Inc, Bethesda, EUA). Foi padronizada uma grade com dez linhas horizontais, em cada camada analisada, obtendo-se dez medidas (Figura 9) e, em seguida, passava-se as médias dessas medidas para programa Microsoft Excel obtendo, desta maneira, duas médias de cada animal para realização da análise estatística.

Os seguintes parâmetros histológicos foram analisados:

- Espessura da camada de queratina (hiperqueratose), TKC
- Espessura do compartimento celular (tecido conjuntivo), TCC
- Espessura total do tecido epitelial (hiperplasia epitelial), TET

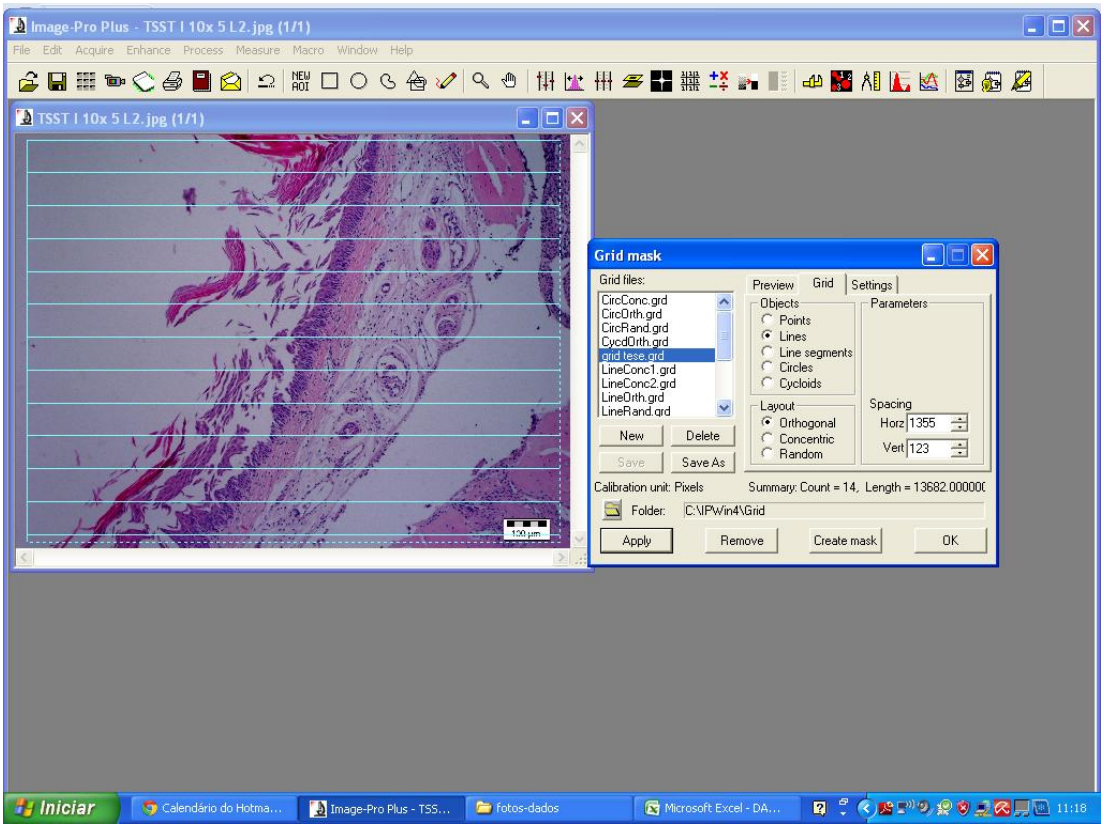

Figura 9 - Metodologia de quantificação em programa analisador de imagens Image Pro Plus

3.9. ANÁLISE ESTATÍSTICA

A análise estatística aplicada aos dados da massa corporal ou peso (g) dos animais no tempo 0 e 14 dias de utilização das placas foi ANOVA ($\alpha = 0,05$).

As médias das duas leituras (µm) de cada parâmetro (isto é, espessura de queratina, espessura de epitélio total e espessura de compartimento celular) para cada animal foram calculadas e comparadas com teste ANOVA de um fator de variação, que foi o tipo de placa que o animal recebeu.

Para análise dos dados obtidos com os grupos dos reembasadores comparados com grupo controle foi realizado teste ANOVA com um fator de variação e teste de comparação múltipla de Tukey. Significância estatística em nível de 5% ($p<0,05$).

4 CAPÍTULO

4.1 CAPÍTULO 1

Description of a rat palatal device for the study of denture base and reline resin biocompatibility.

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Abstract

The objective of this study was to describe a method to construct an intraoral acrylic device made of an acrylic plate which permits a reline material to be added to its inner surface. Fifteen sixty-day old adult female rats (*Rattus Norvegicus* Albinus Wistar), weighing 150 – 250 g were used for this study and allocated to three groups (n=5): G1, animals were sacrificed after 14 days of heat-polymerized, acrylic resin plate (Lucitone 550) use; G2, animals were sacrificed after 14 days of heat-polymerized, acrylic resin plate (Lucitone 550) use, relined with Tokuyama Rebase II; G3, animals were sacrificed after 14 days, without use of plates, being maintained under the same conditions as the experimental groups. The manipulation of the animals followed the guidelines of the Brazilian College of Animal Experimentation (COBEA), under the approval of the animal ethics committee of the State University of Ponta Grossa (UEPG). The plates covered all the palate and were fixed in the molar region with light-cured resin, thus being kept there for 14 days. The animals received a paste diet and water *ad libitum*. Before and after the trial period, the rats were weighed individually on a precision scale. Statistical analysis was performed using ANOVA ($\alpha = 0.05$) test for comparison of weight (g) of the animals at time 0 and 14 days of using the device. No statistical differences were observed regarding the weight of the animals among the experimental groups in the study. It was possible to observe changes in tissue morphology between animals that used the plate and animals that did not use this device. On this basis, we assume that the use of prostheses could bring about slight changes in the palatal mucosa of its users. We concluded that the individual master impressions in rats, associated with plates of molar teeth coverage and the method of cementation with composite without adhesive, provided good stability for the palatal appliance developed in this

study and its permanence in the oral cavity of the animals did not change the way in which the rats ate.

Keywords: hard reline, Wistar rat, palatal mucosa, acrylic resin

1) Introdution

The fit of dentures progressively declines as a result of time-dependent changes in the supporting tissues. Therefore, removable prostheses may require periodic relining to reestablish tissue support for the denture base. Some auto-polymerizing acrylic resins have been proposed for the temporary or permanent improvement of denture fit. These materials allow the clinician to reline a removable prosthesis directly in the mouth in intimate contact with a large area of the mucosa. Among other factors, the biocompatibility of auto-polymerizing reline and denture base acrylic resins is a major concern and should be considered when selecting these denture materials.[1]

Toxic substances and their effects on the tissues have been evaluated by *in vitro* cell cultures, animal studies and clinical observations. These several biocompatibility tests were divided into initial, secondary and application, the last one being described as a clinical pre-test.[2,3] Costa[4] stresses that the results of initial tests show limitations relating to their direct correlation with the clinical situation, but they are very important due to the fact that they provide fast and consistent results with respect to biological activity, enable the standardization of the methodology of the tests and also make them reproducible in different laboratories from different countries, allowing the comparison of the results obtained. With initial tests, one observes the proliferation or inhibition of cell growth due to the contact with cytotoxic substances.

Regarding the advantages of *in vitro* tests, several studies evaluating the biocompatibility of materials through the use of cell cultures were found in the literature.[5-8] Furthermore, *in vivo* studies using the method of subcutaneous implantation of materials in animals have also been described.[9-12] However, *in vitro* studies were not likely to reflect the true clinical situation. Pathological conditions of oral mucosa under denture bases, such as the deformation of mucosa, decubital ulcers or denture stomatitis,

are frequently found.[13,14] In addition, in clinical situations, biofilm formation on the surface of removable dentures plays a critical role. Therefore, biofilm should be considered in *in vitro* studies of biocompatibility due to the analyzed parameters, such as inflammatory response, infiltration of mast cells, fibroblast proliferation and vascular changes which may have been influenced by the existing biofilm.

In vivo studies of denture-associated biofilm showed the need for an intraoral apparatus in an animal model.[15] An animal model that uses an intraoral device is advantageous for the study of biofilm in denture stomatitis and for the study of biocompatibility of denture base and reline acrylic resins. However, it was not possible to develop an ideal model, because the previously described and used models still have limitations. Few *in vivo* studies, using apparatus adapted to the palate of experimental animals, were found in the literature.[15-19] This lack of information is largely a reflection of the methodological difficulties in working with animals. In addition, previously described intraoral devices were considered to be primitive and inadequate[15].

Among the studies of animal models using an intraoral device, the methodology used by Barclay et al.[17] should be emphasized. The latter investigated the effects of denture relining materials on oral mucosa *in vivo* in a rat model. For this, denture-like appliances covering the palate in Wistar rats were relined with either a resilient soft lining material or a hard chairside reline material. The devices, which were constructed using a band of 4 mm-wide stainless steel, were fixed in the rat's palate by a retentive element encompassing the upper incisor teeth. The wire loop acted as retention for the acrylic. However, with the use of the above methodology, there is the possibility of the plate breaking away from the palate, since it is retained by the clamp placed on the rat's incisors. Lee et al.[15] also described an inexpensive intraoral removable denture system for rodents that could be used in numerous oral health research applications, at the

forefront is biofilm research related to *Candida*-associated denture stomatitis. However, the apparatus developed does not allow relining, which prevents the study of rigid or resilient reline materials.

Aiming to improve the retention of the acrylic resin device on rat palates and allowing the relining of resins bases, a new animal acrylic appliance is described in this present research, regarding the study of *in vivo* biocompatibility. Using this methodology, the biological behavior of the denture base and reline acrylic resins and substances used for the treatment or prevention of denture stomatitis can be evaluated. The objective of this work was to describe the method of construction of the intraoral acrylic device, made by an acrylic plate, which permits a reline material to be added to its inner surface that can be used in experimental biological studies.

2) Materials and Methods

2.1) Animals used

Fifteen sixty-day old adult female rats (*Rattus Norvegicus* Albinus Wistar), weighing 150 – 250 g were used for this study, and allocated to three groups (n=5): G1, animals were sacrificed after 14 days of heat-polymerized, acrylic resin plate (Lucitone 550) use; G2, animals were sacrificed after 14 days of heat-polymerized, acrylic resin plate (Lucitone 550) use, relined with Tokuyama Rebase II; G3, animals were sacrificed after 14 days, without use of plates, being maintained under the same conditions as the experimental groups (control group). These experimental groups belong to histological studies of heat treatments thought to improve the biocompatibility of denture materials (unpublished data).

The animals in the different groups were maintained in separate cages at 23° C with 56% relative humidity, alternating light/dark cycles of 12 h. They were fed with a

nutritionally complete powdered diet (Nuvilab CR-1, Nuvital, Brazil) and were given water *ad libitum*. The animals were kept on a paste diet one week before the experiments and were allowed nothing orally except for free access to water 12 h before the procedure. This study was approved in accordance with the recommendations of the Ethics Committee on Animal Use (CEUA) of the State University of Ponta Grossa (File: 11/2010, Protocol: 12673/2010) and the guidelines on animal handling of the Brazilian College of Animal Experimentation (COBEA) were followed at all times.

2.2) Experimental setup of the animals

In order to take the master impressions and to fit the appliances, it was necessary to anaesthetize each animal group to be tested. They were anesthetized by the administration of a solution of ketamin (100 mg/ml) and xylasine (100 mg/ml) that was made by diluting 3.75 ml of ketamin plus 0.5 ml of xylasine in 5.75 ml of distilled water. An intraperitoneal injection of 0.2 ml/100 g of this solution[20] was used to sedate the rats. This technique enabled a working time of approximately 30 min.

2.3) Acrylic custom-made palatal device preparation

Initially, 15 trays were fabricated with putty consistency impression material, (polivinilsyloxane and Futura AD catalyzer, DFL Indústria e Comércio S/A, RJ, Brazil), and these were constructed on a master dental stone model (Herostone, Vigodent S/A Indústria e Comércio, RJ, Brazil) from a preliminary impression of a Wistar rat palate. This animal presented age and weight similar to those used during the experiments. Then, the trays were tested and their borders adjusted for each rat to be shaped in such a way that reached the bottom of the vestibule. After sedation, master impressions of the palates were taken with light-body impression addition silicone (Futura AD, DFL

Indústria e Comércio S/A, RJ, Brazil). For this purpose, animals were placed in a stabilizing plate, to permit their mouths to be opened, which allowed the impressions to be made. The working stone cast were obtained with type IV gypsum (Herostone, Vigodent S/A Indústria e Comércio, RJ, Brazil) (Fig. 1) and on them, the waxing of a plate was made with a layer of type 7 wax (Lysanda, Produtos Odontológicos Ltda, São Paulo, Brazil) without relief. The waxing was done on the bearing area comprising the region between the molars, extending to the first fold palate, covering the occlusal surface of molars and passing to the labial surface of the same (Fig. 2). Acrylic resin Lucitone 550 (Dentsply Indústria e Comercio Ltda, Petrópolis, RJ, Brazil) was heat-polymerized according to the manufacturer's protocol. The following polymerization cycle was employed: Lucitone 550 was processed for 90 min at 73° C and then in 100° C boiling water for 30 min (short cycle). After polymerization, the flasks were bench cooled at room temperature for 30 min, and 15 min under running water, before the specimens were removed from the flasks. The devices were visually inspected to verify that they presented a smooth surface and showed no voids or porosity; otherwise, they were discarded. The edges of the denture base were carefully finished and polished to remove irregularities (Fig. 3). Immediately after specimen fabrication, the acrylic resin devices were stored in distilled water for 48 h at 37° C prior to insertion, in order to eliminate the possible effects of any residual monomer.[21]

2.4) Acrylic custom-made palatal device direct reline and fixing

The acrylic resin palatal devices were relined with the hard chairside reline resin Tokuyama Rebase II (Tokuyama Dental Corporation, Tokyo, Japan) following the manufacturer's instructions and were held in position until their polymerization. The excesses of the reline material were removed. In order to promote a mechanical retention on the molars and to fix the palatal device, a groove in the molar coverage

region was made using a spherical bur # 6 (Fig. 4). Composite resin (Opallis, FGM, Brazil) was used to fill the molar region groove (Fig. 5) and the resin was photo-polymerized while the device was kept in position (Fig. 6). The amount of composite resin used was minimal, so as not to invade the palatal surface and affect the plate in contact with the palate. After the procedure, the rats were placed at rest to recover from the anesthesia. The rats were placed on a paste diet to avoid food debris gathering under the denture system.[15] The animals used the appliances for 14 days. For data comparison, 5 animals did not receive the plates and were kept under the same conditions for the same period. Before and after the trial period, the rats were weighed individually on a precision scale (Gehaka, Ind. e Com. Eletro – Eletrônica Gehaka Ltda, São Paulo – Brazil).

2.5) Weight of animals

The rats of different groups were weighed individually in a precision scale (Gehaka, Ind. e Com Electro - Electronic Gehaka Ltda, São Paulo - Brazil), at the time the master impressions were performed, and after fourteen days use of the plates.

2.6) Statistical analysis

Statistical analysis was performed using ANOVA ($\alpha = 0.05$) test for comparison of weight (g) of the animals at time 0 and 14 days of using the device (Table 1).

3) Results

The results in Table 1 show that all the rats maintained body weight as ANOVA showed no statistical differences between the weight of the animals in the experimental groups of the study.

4) Discussion

The rules concerning *in vivo* studies increasingly present difficulties for biocompatibility studies, especially using patients. For these reasons, the number of clinical studies is often restricted, potentially influencing the experimental outcomes, reproducibility and subsequent interpretation of data. The use of animals in *in vivo* research is essential for a better understanding of the mechanisms of disease and also to test new drugs before using them in human studies. A major advantage of animal research is related to ethical considerations that limit the realization of many studies in humans in situations that require more invasive procedures and also when there is no prior knowledge of the *in vivo* efficacy and toxicity of new drugs.[22] Numerous tests with cell cultures have been used to determine the cytotoxic effects of biomaterials because they are simple, effective and easily reproducible.[7,8,23] However, the diversity of experimental conditions in cell cultures makes it difficult to establish conclusive findings on the cellular activity in mucosal epithelium under denture bases.[14] Animal models are available to study *in vivo* disease processes and represent an important step to determine the biological behavior of biomaterials. In the present study, we suggest a new method of preparation of heat-polymerizable acrylic resin palatal plate, used in Wistar rats, which allows direct reline to be used in *in vivo* studies of biocompatibility of different materials and substances.

Some features are very important in order to define the mode of production of palatal devices for testing the biocompatibility of the biomaterials. Lee et al.[15] and Johnson et al.[24] reported the following specific requirements of the intraoral device for a study of *Candida* biofilm: to be retained in the oral cavity long enough for the biofilm to form and affect the oral mucosa; to be removable, so that longitudinal observations can be performed; to be biostable, so that it would not with interfere with the animal's

daily activities such as eating and drinking; to be easy to fabricate at a reasonable cost and be custom fitted to the palate to ensure adequate contact without altering the rat's dental architecture. The device presented here is consistent with the main characteristics mentioned above.

Few studies were found in the literature regarding the use of Wistar rats in biocompatibility and cytotoxicity tests.[18,25,26,27,28,19,15,12] One of the major problems of the use of palatal plates in rats is the difficulty in feeding the animals, which can result in weight loss and nutritional deficiencies that interfere in the results of studies related to biocompatibility and the virulence of microorganisms. To verify the effect of using the proposed device on animal nutrition, the weight of the rats at the time of installation was checked and compared with the weight after 14 days use of the plates. The permanency of the device in the oral cavity of the animals did not change their eating habits because there were no significant differences in weight. Thus, the architecture of the plate did not interfere with routine activities. This was possible due to the customized master impressions, promoting a better adaptation of the devices.

In this study, young animals were selected (aged 2 months, on average). The control group showed a mean variation of weight of 5.7 g. The group with plate and relined plate showed values of 14.6 g and 9.3 g, respectively. In summary, the fact that the animals demonstrated stability of weight indicates that the suggested device did not influence the nutrition of the animals. This may be accounted for by the fact that the plate covered only the molar region, therefore being smaller and more comfortable for the animal, which differs from the study by Barclay et al.[18], in which larger palatine plates were used (a stainless steel band on the upper incisors of the animal) which made the plate less stable.

In another study by Barclay et al.[17] the same model of palatal appliance was used, but in that study it was to test relining materials. The plate was made with a space in the intermolar region using a 1 mm thick spacer. This space was then filled with the soft lining material to be tested. The procedure described above differs from our study due to the method of manufacturing space to accommodate the soft lining, because we created a space in the same region, using a drill at low speed. This was carried out using a carbide bur in a standardized manner by the same operator. The methodology to prepare palatine plates described by Tsuruoka et al.[19] is very similar to our model, but with a different cementation technique, because these authors used acid conditioning prior to composite resin insertion and in the present study the devices were fixed with composite, in a non-adhesive cementation. During the completion of our pilot study, we tested different cements, and composite resin was the most effective in maintaining the retention of the device. Animals were tested with and without acid etching of the enamel of the molars, and this procedure was seen to be complicated and unnecessary to promote satisfactory retention of the device. Jorge et al.[16] used acrylic resin for retaining the plate: however, the heat generated by exothermic reaction and possible residual monomer of the material could lead to histological alterations, thus interfering with the results. In the study by Barclay et al.[17], the authors used resin to cement the device, but they encountered deficient retention, losing a significant number of devices. Consequently, the authors replaced the acrylic cement with zinc oxide and eugenol cement. Both an acrylic resin, such as zinc oxide, and eugenol cement could cause histological changes, affecting the results. Jorge et al.[16] conducted a study using palatal appliances, but with a significant difference. The device was made of acrylic resin on a decalcified palate and fixed in formalin, and then the plates were adapted, relined and cemented with the same acrylic resin. The incisors were included in the cement. This

preparation procedure differs from our study. Because we performed impressions of each individual animal, it promoted a better fit, making it easier to feed and did not affect the occlusion. Jorge et al.[16] reported weight loss and significant systemic involvement of animals, possibly because of a change in the rats' diet, even though the animals were provided with food.

5) Conclusion

The use of individual impressions in Wistar rats, associated with the occlusal cover plates on molars and non-adhesive cementation method, provided good stability of the palatal device developed in this study. There was no significant difference in weight change observed in the animals. The *in vivo* model described for testing the biocompatibility of acrylic resins and of substances used for the treatment or prevention of denture stomatitis seems to be reproducible and offers an adequate histopathological evaluation of this issue. This opens up opportunities for using this approach to study the *in vivo* biocompatibility of resins and liners and the effectiveness of new drugs prescribed for the treatment of infections such as denture stomatitis. It was possible to observe changes in tissue morphology between the animals that used the plate and the animals that did not use this device. On this basis, we assume that the use of prostheses could bring slight changes in the palatal mucosa of its users.

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Table 1: Results of 2-way ANOVA for weight of animals (g)

Source	*df*	SS	MS	F	*p* value
Time (A)	1	580.83	580.83	2.77	0.132
Denture base material (B)	2	400.90	200.45	0.90	0.429
A x B	2	194.97	97.48	0.44	0.341
Error	16	3573.47	223.34		
Total	29	6425.50			

df, degrees of freedom; SS, sum of squares; MS, mean square.

Figures

Fig. 1. Stone cast

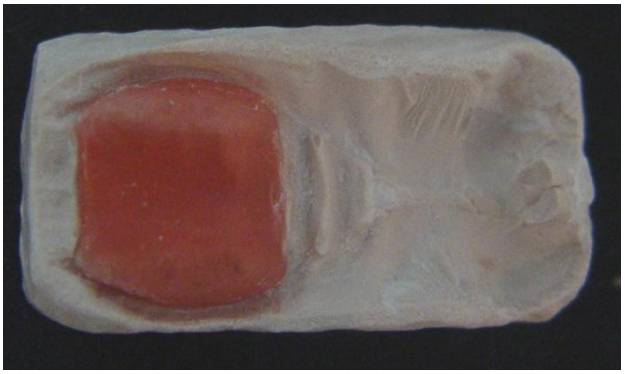

Fig. 2. The waxing was done on the bearing area comprising the region between the molars, extending to the first fold palate, covering the occlusal surface of molars and passing to the labial surface of the same

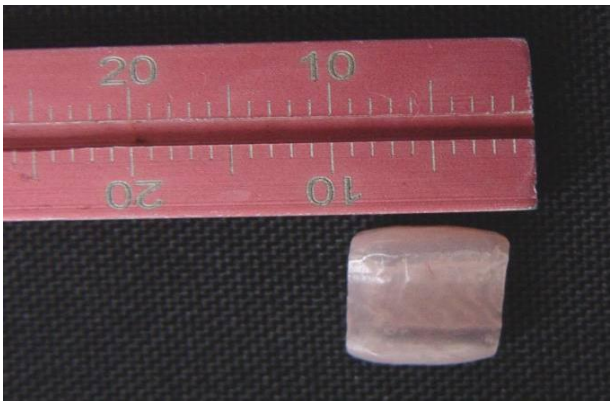

Fig. 3. The edges of the denture base were carefully finished and polished to remove irregularities

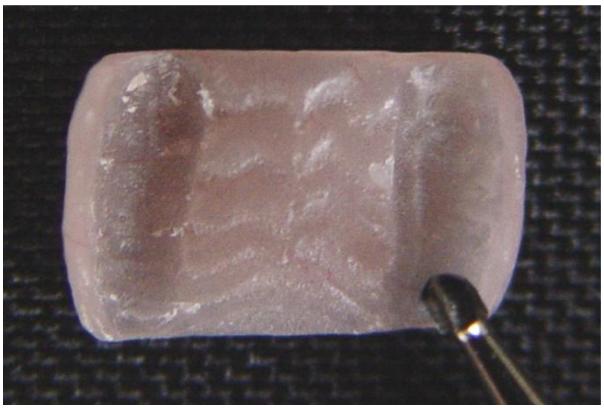

Fig. 4. Mechanical retention on the molars and to fix the palatal device, a groove in the molar coverage region was made using a spherical bur # 6

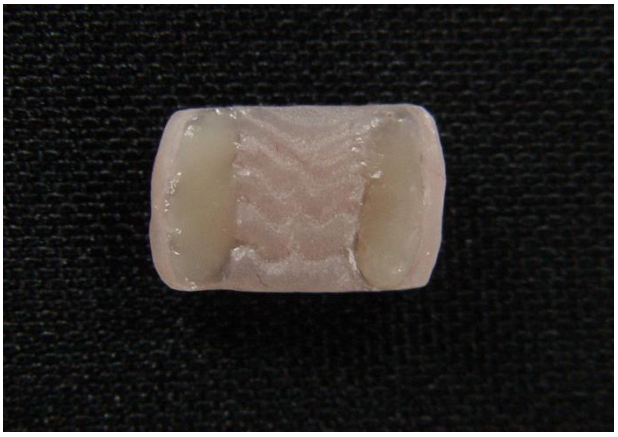

Fig. 5.Composite resin was used to fill the molar region groove

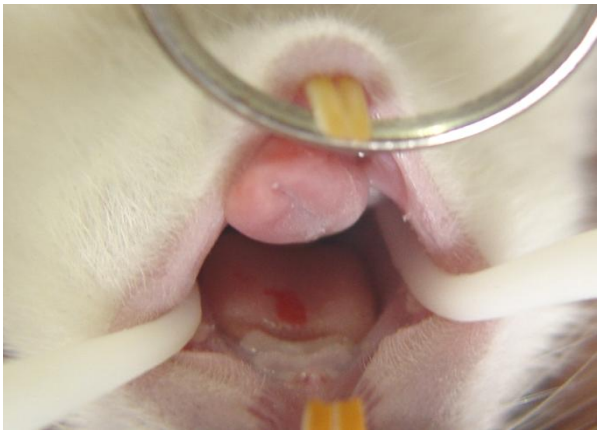

Fig. 6. The device was kept in position

4.2 CAPÍTULO 2

Effect of post-polymerization heat treatment on a denture base acrylic resin in rat palatal mucosa

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Abstract

Aims: This work examined the histological effects, on the rat palatal mucosa, of a denture base acrylic resin, submitted or not to a post-polymerization heat-treatment. Methods: Fifteen adult female Wistar rats, with sixty days old, weighting 150 g – 250 g were divided in G1: animals being maintained under the same conditions as the experimental groups following described, but without the use acrylic palatal plates (control group); G2: use of heat-polymerized acrylic resin palatal plates made of Lucitone 550; G3: use of palatal plates identical to G2, but subjected to a post-polymerization treatment in a water bath at 55° C for 60 min. The plates covered all the palate and were fixed in the molar region with light-cured resin, thus being kept there for 14 days. After the sacrifice, the palate was removed, fixed in formaldehyde 10% and decalcified with EDTA. Sections were stained using haematoxylin and eosin. Images in duplicate were made from the central region of the cuts, to measure the thickness (μm) of the keratin layers (TKC), epithelium total (TET) and connective tissue (TCC). Statistical analyses were carried out by one-way ANOVA and Tukey post-tests (α=0.05). Results: According to the results there was significant difference in the thickness of keratin between G2 and G3, with G1 having the intermediate value and similar to the other groups. There was a significant difference in the connective tissue with G3 <G1 <G2 (p <0.0001). Conclusion: Regarding the total epithelium, group G3 presented a statistically significant difference with both G1 and with G2 (p <0.0001), which were similar to each other. When using the proposed heat treatment it was found to be effective, from the viewpoint of biocompatibility, for the acrylic resin denture base investigated.

Keywords: biocompatibility, acrylic resin, Wistar rat, palatal mucosa, palatal appliances

Introduction

The material of choice for oral rehabilitation of partially or totally edentulous patients from the 1930s to the present day has been acrylic resin[1]. Used as the basis for full and partially removable dentures, and artificial teeth, when used in the oral cavity important features need to be taken into consideration such as: color and translucency, chemical stability, sufficient hardness, good resistance and resilience to wear, it should be insoluble and impervious to oral fluids, and should be biocompatible. However, one of the major factors limiting the use of this material is their biocompatibility. Biocompatibility can be defined as the acceptance (or rejection) of artificial materials to perform with an appropriate host response when applied as intended. Thus, studies in order to test new materials and techniques to improve physical, chemical and biological properties have been performed[2-5].

Denture resins may be classified by polymerization mode and include those that are heat-polymerized (by microwave or conventionally), auto-polymerized, and visible light-polymerized[6]. Acrylic resins consist of polymethylmethacrylate, a mixture of a polymer powder containing benzoyl peroxide and a liquid monomer, typically methyl methacrylate. The polymerization of the material occurs with a rapid decomposition of benzoyl peroxide, after being activated, which releases a large amount of free radicals, polymerizing the monomer. The process of formation of polymers and the growth of chains continues at a considerable speed. Theoretically, these reactions should proceed with increased heat until all the monomer had been converted to polymer. However, with a decrease in the polymerization temperature, polymer formation also decreases and, consequently, an amount of residual monomer remains in the polymerized resin[6]. There are many reports suggesting that residual monomer in the denture base is related to mucosal irritation and sensitization of tissues[1,6-12]. The leaching of formaldehyde,

methacrylic acid, and benzoic acid from acrylic resin dental materials has also been detected[13-16]. There have been several investigations into ways of improving the polymerization of these materials and their behavior in relation to treatments to decrease the cytotoxic effect of their products and by-products from the polymerization reaction[5,16,24-35].

Toxic substances and their effects on tissues have been observed through animal studies, clinical observations and *in vitro* cell cultures[17,18]. A cytotoxicity assay which uses the cell culture method is considered to be a preliminary test for assessing the biocompatibility of a material; it is simple, reproducible, effective and controlled[19,20]. There are several studies in the literature with initial tests which determine the cytotoxicity of acrylic resins[21-24]. Acrylic resins remain in direct contact with the tissues of the oral cavity, requiring *in vivo* tests in animals to observe the histological changes that may occur due to prolonged use of these materials. The reactions most frequently found in the mucosa are redness, erosion in the oral mucosa, and burning in the mucosa and tongue [7,8,36] . However, little information can be found related to the biological behavior of these materials in secondary tests, i.e. made directly in animals[37-39]. Few *in vivo* studies using experimental apparatus adapted for the palate of animals were found in the literature[37,41,42]. Therefore, the purpose of the study was to examine the histological effects of post-polymerization treatment on an acrylic resin denture base, on palatal mucosa in a Wistar rat model. The null hypotheses investigated in the present study were that: 1. palatal plates of heat-polymerized acrylic resin would not result in histological changes in the palatine mucosa of rats; 2. post-polymerization heat treatment of palatal plates would not diminish histological changes in the palatine mucosa of rats, relative to the untreated group.

Materials and Methods

Fifteen sixty-day old adult female rats (*Rattus Norvegicus* Albinus Wistar), weighing 150 – 250 g were used for this study. The animals were maintained in separate cages at 23° C with 56% relative humidity, alternating light/dark cycles of 12 h. They were fed with water *ad libitum* and a nutritionally complete powdered diet (Nuvilab CR-1, Nuvital, PR, Curitiba, Brazil). The animals were kept on a paste diet one week before the experiments and were allowed nothing orally except for free access to water for 12 h before the procedures. This study was approved in accordance with the recommendations of the Ethics Committee on Animal Use (CEUA) of the State University of Ponta Grossa (File: 11/2010, Protocol: 12673/2010) and the guidelines on animal handling of the COBEA (Brazilian College of Animal Experimentation) were followed.

A preliminary impression of a Wistar rat palate of an animal that had the approximate age and weight to those used in the experiments was taken. Ten trays were then constructed with putty silicone on a master dental stone cast from this preliminary impression. To take the master impressions and to fit the appliances, it was necessary to anaesthetize all the animals. This was accomplished by the administration of a solution of ketamin (100 mg/mL) and xylasine (100 mg/mL) that was made by diluting 3.75 mL of ketamin plus 0.5 mL of xylasine in 5.75 mL of distilled water. Intraperitonial injection of 0.2 mL/100 g of this solution[43] was used to sedate the rats. The time of work obtained by this technique was approximately 30 min.

After the sedation of the animals, molding, using an addition silicone material of light consistency, was performed (Futura AD, DFL Indústria e Comercio S.A., RJ, Rio de Janeiro, Brazil). For this, the animals were placed in a stabilizing plate, with the

mouth kept open and secured by incisors with the aid of handles, to facilitate the molding of the palate.

Palatal appliances, customized for each animal, were made using Lucitone 550 (Dentsply Indústria e Comercio Ltda, Petrópolis, Brazil) heat polymerized acrylic resin. The appliances were constructed over the plate area, comprising the region between the molars, extending to the first palatal fold, covering the occlusal surface of the molars and extending to the buccal surface of the molars. The acrylic resin denture material was polymerized according to the manufacturer's specifications (90 min at 73° C and 30 min at 100° C - short cycle) and the quantity used was 0.42 g of powder and 0.2 mL of liquid. Immediately after specimen fabrication, the acrylic resin devices were stored in distilled water for 48 h at 37° C prior to insertion, in order to eliminate the residual monomer[49].

At this moment, the animals were randomly allocated into three test groups: G1: without the use of plates, being maintained under the same conditions as the experimental groups (control group); G2 using Lucitone 550 heat polymerized acrylic resin palatal plates; G3: using plates identical to G2, but subjected to a post-polymerization treatment in a water bath at 55° C for 60 min[34].

In order to promote a mechanical retention of molars and fixing of the palatal device, a groove in the molar coverage region was made using a spherical bur #6. Composite resin (Opallis, FGM, SC, Joinville, Brazil) was used to fill the molar region groove and the resin was photo-polymerized while the device was set in position. Upon completion of the procedure, the rats were placed at rest to recover from anesthesia. From this moment, the rats were placed on a paste diet to avoid food debris gathering under the denture system[45]. The animals, and both groups, remained with the plates in the palate for 14 days.

After 14 days, the animals of all the groups were killed by euthanasia, according to resolution number 714 of 20/6/02 of the Federal Council of Veterinary Medicine (FCVM). The animals were anesthetized in accordance with the methodology, and under deep anesthesia, intracardiac perfusion with potassium chloride 10% (1 ml/100 g) was applied through the left ventricle. The palates of the animals were dissected, fixed in 10% buffered formalin for 48 h and decalcified in 4.17% EDTA for approximately four months. The pieces went through a dehydration process, embedded in paraffin, so that the back part of the palate stayed down. The samples embedded in paraffin were cut with a microtome (Leica, Berlin, Germany) in sections of 5 µm and then stained with haematoxylin and eosin (H&E). Slides were mounted and observed by using light microscopy (Nikon, model YS 100, Tokyo, Japan). Five ribbons were made with five sections of each animal, then, for analysis, two images, standardized in the central region of the cuts, near the nasopalatine plexus and the midline of the palatine raphe, at 200x magnification to check for changes histological were made. Overall thickness of the epithelium (TET), cellular compartment (TCC) and keratin layer (TKC) were calculated. For the measurement, the Image-Pro Plus (software version 4.5.0.29-Media Cybernetics, Inc, Bethesda, USA) program was used in groups G1 (control group), G2 (Lucitone without heat treatment) and G3 (Lucitone with heat treatment) with a grid with ten horizontal lines for each analyzed layer, obtaining ten measurements, and then the averages of the measurements were analyzed using Microsoft Excel. This resulted in two averages for each animal, to perform statistical analysis.

The averages of the two readings (µm) of each parameter (i.e. keratin thickness, epithelium thickness, and connective tissue thickness) were calculated and compared for each animal by ANOVA of one variation factor, which was the type of plate that the animal used (no plate, control - G1; palatal plate with no heat treatment - G2; palatal

plate with heat treatment - G3). The averages of groups G1, G2 and G3 were compared by Tukey's post-test, with $\alpha = 0.05$.

Results

According to the results, there was significant difference in the thickness of keratin between G2 and G3, with G1 being the intermediate value and similar to the other groups (Fig 1). There was a significant difference in the connective tissue with G3 <G1 <G2 (p <0.0001) (Fig 2). Regarding the total epithelium, group G3 presented a statistically significant difference with both G1 and with G2 (p<0,0001), which were similar to each other (Fig 3).

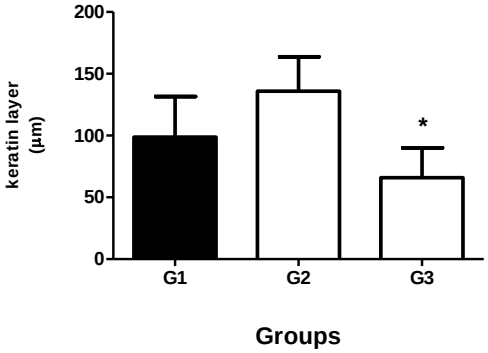

Fig. 1. Mean and ±SD of keratin layer thickness (µm). G1- Control; G2- Lucitone 550 without heat treatment; G3- Lucitone with heat treatment.
* Significant difference (P<0.0001) with G2. ANOVA and Tukey post hoc test.

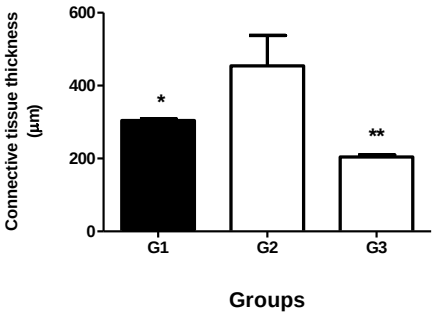

Fig 2. Mean and ±SD of connective tissue thickness (µm). G1- Control; G2- Lucitone 550 without heat treatment; G3- Lucitone with heat treatment.
* Significant difference (p<0,0001) with G2 e G3 and ** with G2. ANOVA and Tukey post hoc test Tukey.

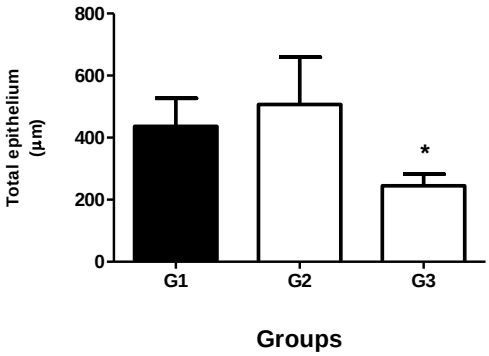

Fig. 3. Mean and ±SD of total epithelium thickness (µm).
G1- Control; G2- Lucitone 550 without heat treatment;
G3- Lucitone with heat treatment.
* Significant difference (p<0,0001) with G1 e G2.
ANOVA and Tukey post hoc test Tukey.

Discussion

A number of studies have carried out *in vitro* tests to evaluate the biocompatibility of acrylic resins through the use of cell cultures, in which it was possible to observe the proliferation or inhibition of cell growth resulting from contact with cytotoxic substances [23,27,34,46]. Whilst acknowledging the value of such studies, they are recommended mainly for the initial selection of materials and techniques and their results should not be directly extrapolated clinically [20-24,47,48]. Animal model studies have been used to clarify the pathogenesis of human diseases, as well as evaluating new therapeutic targets, and they represent an efficient and accessible way to study disease processes *in vivo*, since ethical issues preclude the use of some invasive procedures in humans[49]. Among these, *in vivo* studies using the method of the subcutaneous implantation of materials in animals have been described [38,39,50,51]. However, these studies were not likely to reflect the true clinical situation. Pathological conditions of oral mucosa under denture bases, such as the deformation of mucosa, decubital ulcers or denture stomatitis, are frequently found[52]. Little information is available regarding the biological behavior of materials in secondary tests, performed

directly on animals with the use of palatal devices[37,40-42,45,53]. Mucous membrane irritation tests by means of removable partial palatal dentures or fixed-bridge type appliances in small animals were considered to be extremely difficult, expensive, and time consuming[54] mainly due to the fact that previously described intraoral devices were considered primitive and inadequate[42].

Due to the difficulties associated with palatal devices described in the literature, and in view of the importance of performing *in vivo* studies, in the present study with regard to the preliminary tests prior to the clinical tests, palatine plates, fixed in Wistar rats, made with Lucitone 550 acrylic resin according to the methodology described by Meister et al., 2012 (article submitted) were used. The material selected for use in the present study was a heat-polymerized acrylic resin consisting of polymethyl methacrylate (PMMA) and ethylene glycol dimethacrylate, and it presents two possible polymerization cycles. One is called 'short cycle', where the dental flask is immersed in water at 73° C for 90 min and then the temperature is raised to 100° C and the flask is maintained for an additional 30 min period at this temperature. The other possible polymerization technique is called 'long cycle' and the flask is kept for 9 hours at 71° C, not submitting to a final polymerization in boiling cycle. It has been found that the 'short cycle' promoted a lower amount of residual monomer (0.08%) when using Lucitone 550 compared with the 'long cycle' (0.24%)[4] and also a lower level of toxicity *in vitro*[34]. Consequently, the 'short cycle' was selected for the present study. However, under the conditions of this study, using the same 'short cycle' described above, the use of palatal plates needed changes to some of the assessed parameters, e.g. group G2 showed higher levels of keratin thickness and connective tissue compared to G1, i.e. the control group, consisting of animals without plates. This increase in tissue thickness can be explained by the cytotoxic effect of the denture base resin used, already widely

studied by *in vitro* tests, which has been proven to release residual monomers and other substances [1,6-12,24,34], as confirmed in the present study. Our results are in agreement with Axelsson and Nyquist[25], who evaluated the levels and the effects of residual monomer on the mucosa due to the fact that they had observed a hyperkeratosis in various patients over a period of years. In 1963, Kapur and Shklar[55] also observed keratinization and a slight increase in connective tissue, suggesting that the tissue response might be a consequence of a chemical irritation.

However, the results for the groups G2 and G3 might also be a response to the continuous pressure exerted by the prosthesis on the oral mucosa due to occlusal forces exerted during mastication. More specific and more recent tests, such as the study of diabetes mellitus by Maruo *et al*.[53], used acrylic plates cemented in Wistar rats, to verify tissue reaction. The plates were cemented without pressure, with continuous and intermittent pressure, and it was found that there was a reduction in the total thickness of the epithelium and also that mechanical stimuli decreased susceptibility to cellular changes. Tsuruoka *et al*.[41] conducted a study on the physiological, histological and molecular levels to determine the effects of mechanical compression on prosthesis mucus supported on the palate of rats, They concluded that the effect of mechanical compression was greatest in cells of the periosteum and that these cells synthesize HSP70, which is responsible for bone remodelling and vascular endothelial growth factor (VEGF). VEGF is a protein that is produced by cells which stimulate angiogenesis and vasculogenesis. It is an integral part of the system that provides oxygen to tissues when blood circulation is insufficient and also if it is abundant it may contribute to diseases.

Analyzing data from the G3 group, values very close to normal (G1) were observed, meaning that the post-polymerization heat treatment that was performed to decrease the release of toxic compounds on the mucosa was probably efficient.

In the present study, a post-polymerization treatment in a water bath at 55° C for 60 min was performed, in accordance with Jorge *et al.*[34], a study which evaluated the cytotoxicity of several hard chairside reline resins assessed *in vitro* by MTT and by ^{3}H-thymidine. The null hypothesis formulated for this present study was not accepted and differences were observed between groups G2 and G3 when comparing thickness of connective tissue, keratin and epithelium. In all cases, the measurements of Group G3 always showed smaller thicknesses compared with G1 and G2. This was probably because there was a reduction in the irritation resulting from the use of plates compared to G2 due to the heat treatment that was tested. Regarding group G1, one can say that the pressure exerted on the overall thickness of epithelium was in agreement with Maruo *et al.*[53].

In carrying out post- polymerization treatments, heating the samples provides an increase in the mobility of monomer molecules, which are at rest in the polymeric mass, increasing the degree of conversion[56]. Vallittu *et al.*[28] demonstrated that the release of residual compounds is a process which depends on temperature; thus, if temperature is increased, greater diffusion occurs. It has been shown that the release mechanisms of monomers and other substances can be induced by treatments performed after polymerization, such as immersion in hot water or irradiation with microwave energy, thus significantly reducing the amount of monomer present in heat-polymerized acrylic resins and conventional auto-polymerized acrylic resins[2,15,57,58]. Methods for reducing the monomer contents of polymerized acrylic resins have been described in the

literature, to minimize the risk of adverse reactions for those who wear acrylic resin dentures.

Analyzing the image of the G1 group (Fig.4), the histological section displays orthokeratinized stratified squamous epithelium. The granular layer is very clear and the epithelial ridges are discrete. The lamina propria is with normal characteristics and there is the presence of vital bone tissue (HE 200X) There was a significant difference in the thickness of the epithelium compared with G2. Analyzing the image obtained from G2 (Fig. 5), the histological section displays hyper- orthokeratinized stratified squamous epithelium and there was an increase in the thickness of the cell compartment and also the keratin layer in the epithelial tissue, showing that there was a difference when compared with the other groups. Note also the absence of inflammatory infiltrate in the connective tissue. The lamina propria is normal and without the presence of significant inflammatory infiltrate. Bone tissue is vital and with normal characteristics. (HE, 200X magnification). In the image from G3 (Fig. 6), there is a smaller increase in the thickness of the cell compartment and also in the keratin layer in the epithelial tissue. In this group, there was little change in tissue morphology. There was also the absence of inflammatory infiltrate in the connective tissue (HE, 200X magnification), confirming the hypothesis that the heat treatment decreases cytotoxicity.

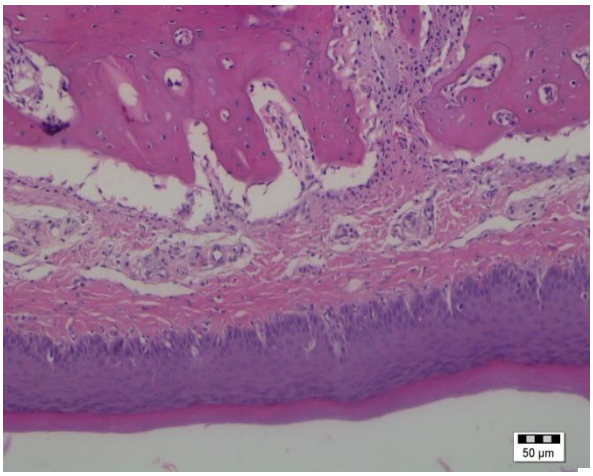

Fig. 4. The histological section displays orthokeratinized stratified squamous epithelium. The granular layer is very clear and the epithelial ridges are discrete. The lamina propria is with normal characteristics and there is the presence of vital bone tissue (Control HE 200X)

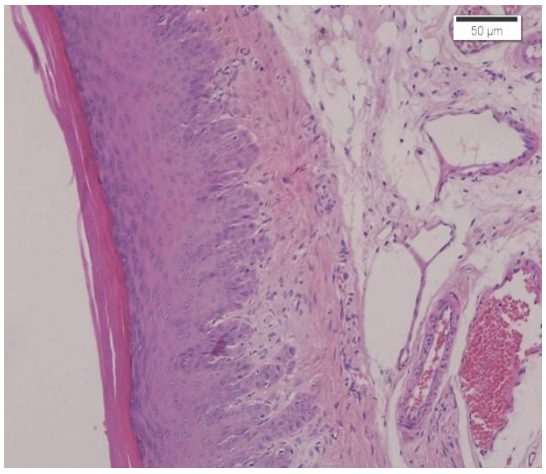

Fig. 5. Hyper-orthokeratinized stratified squamous epithelium, increase in the thickness of the cell compartment and also the keratin layer in the epithelial tissue. Absence of inflammatory infiltrate in the connective tissue. The lamina propria is normal, without the presence of significant inflammatory infiltrate. Bone tissue is vital and with normal characteristics (Lucitone 550 without heat treat HE, 200X)

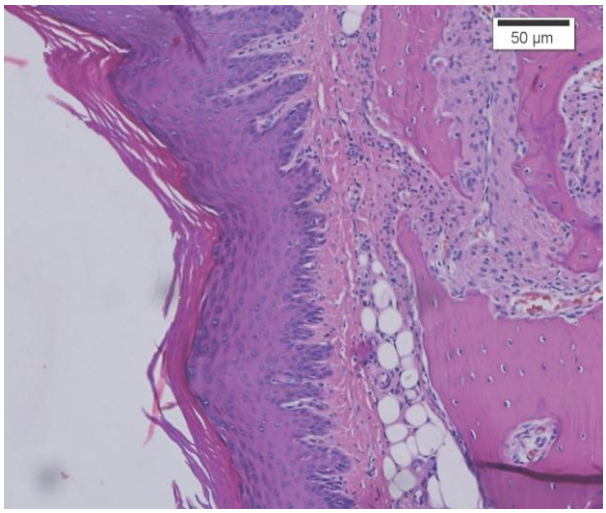

Fig. 6. There is a smaller increase in the thickness of the cell compartment and also in the keratin layer in the epithelial tissue. There was little change in tissue morphology. There was also the absence of inflammatory infiltrate in the connective tissue (Lucitone 550 with heat treat. HE, 200X)

Conclusion

Thus, in accordance with the present results, the importance of adequate processing of material and histological analysis for comparison among treatments is evident. When using the proposed heat treatment it was found to be effective, from the viewpoint of biocompatibility, for the acrylic resin denture base investigated. Future studies are needed to detect, quantify and qualify the effects in the oral mucosa of substances released by acrylic resins from denture bases as well as to determine the cytotoxic effect of these substances.

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4.3 CAPÍTULO 3

Biocompatibility of hard chairside reline resins: histomorphologic analysis in rats

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Abstract:

This study examined the histological effects, on the palatal mucosa of rats, of an acrylic resin denture base, submitted (or not) to post-polymerization heat-treatment. Thirty five adult female Wistar rats, sixty days old, weighing 150 g - 250 g were used in this study. Palatal appliances, customized for each animal, were made using Lucitone 550 heat-polymerized acrylic resin. The animals were divided into seven groups of five animals each, according to the type of material to be tested, with or without heat treatment. The appliances were relined (or not) with one of the following three hard chairside denture reline acrylic resins, Tokuyama Rebase II, New Truliner and Kooliner. Five animals were maintained under the same conditions as the experimental groups, but without the use of acrylic palatal plates (control group). Half the plates that were relined were subjected to a post-polymerization treatment in a water bath at 55°C for 10 min. The plates covered all the palate and were fixed in the molar region with light-cured resin. They were kept in place for 14 days. After the sacrifice of the animals, the palate was removed, fixed in formaldehyde 10%, and decalcified with EDTA. Sections were stained using haematoxylin and eosin. Images in duplicate were made of the central region of the cuts to measure the thickness (μm) of the keratin layers (TKC), total epithelium (TET) and connective tissue (TCC). Statistical analyses were carried out by one-way ANOVA and Tukey post-tests (α=0.05). The keratin thickness layer of the materials New Truliner ($p < 0.001$), Tokuyama Rebase II ($p < 0.001$) and Kooliner ($p < 0.05$), without additional heat treatment, was statistically lower than the control group. The average tissue thickness of the resin Tokuyama Rebase II, without further heat treatment, was significantly higher than control ($p < 0.01$). The resin Kooliner, also without heat treatment, was lower than control ($p < 0.01$). Heat treatment altered the behavior of the resins, Tokuyama Rebase II and New

Truliner, significantly reducing the tissue thickness of the first material and increasing that of the latter material ($p < 0.01$). The results demonstrate that the average total thickness of epithelium for the resins Kooliner and New Truliner, without heat treatment, was significantly lower than control ($p < 0.001$ and $p < 0.01$, respectively). With regard to heat treatment, this was responsible for changing the behavior of Kooliner, increasing, the thickness of the tissue ($p < 0.001$). According to the results, using the proposed heat treatment was found to be effective from the viewpoint of biocompatibility for the acrylic resin denture base investigated.

Keywords: hard reline, cytoxicity, palatal appliances

1) Introduction

The use of both partial and total prostheses can result in the loss of retention, stability and support, as a consequence of the resorption of the alveolar ridge, which is a chronic and irreversible process, causing mobility and the loss of masticatory effectivity and also generating discomfort for the patient[1]. Furthermore, a prosthesis that is not properly adjusted can intensify the concentration of forces on the rim regions, increasing osseous resorption. Consequently, the patient will need to periodically return to the dental surgery, for re-evaluation and, if neccessary, relining of the removable prosthesis. An alternative method to the indirect type of reline, which requires inclusion phases and laboratory-processing, is the re-adaptation of bases, which can be done directly, orally and in the surgery. This technique, called 'immediate' or 'direct' relining, is carried out with autopolymerized, resilient or hard reline materials and is considered to be easy, quick and low-cost[2]. According to Christensen[3], reline materials should have low exothermic properties, keep their color and have a pleasant taste. However, one of the most important properties of these materials is their biocompatibility.

Acrylic resins remain in direct contact with the tissues of the oral cavity, making it necessary to carry out *in vivo* tests in animals to observe the histological changes that can happen due to the prolonged use of these materials. Methods of optimizing the materials used for the polymerization of base and relining of prostheses and also methods of decreasing the cytotoxic effects of products and sub-products that arise from the polymerization reaction have been investigated[4-12]. The results of these studies show that post-polymerization heat treatment with microwaves and water bath can be an alternative for the reduction of cytotoxicity of some resins used for base and prosthesis relining. However, there is little information related to the biological behavior of

materials used for prosthesis relining in secondary tests, i.e. tests carried out directly with animals[13-17]. Since there is very little data related to hard reline materials available[15,18,19] it was considered appropriate to test the effect of post-polymerization heat treatment and the cytotoxicity of the acrylic resins for base and prosthesis relining of devices that were immersed in water. Consequently, the current study developed an acrylic resin device for palatal fixation of rats, which allowed the relining of base resins.

2) Materials and Methods

2.1) Animals used

Thirty five adult female rats (*Rattus Norvergicus* Albinus Wistar) sixty days old, weighing 150-250 g were used for this study. The animals were maintained in separate cages at 23°C with 56% relative humidity, alternating light/dark cycles of 12 h. They were fed with water *ad libitum* and a nutritionally complete powdered diet (Nuvilab CR-1, Nuvital, Brazil). One week before the experiments, the animals were kept on a paste diet and were allowed nothing orally, except for free access to water for 12 h before the procedure. This study was approved in accordance with recommendations of the Ethics Committee on Animal Use (CEUA) of the State University of Ponta Grossa (File: 11/2010, Protocol: 12673/2010) and the guidelines on animal handling of the COBEA (Brazilian College of Animal Experimentation) were followed.

2.2) Experimental setup of the animals

To take the master impressions and to fit the appliances, it was necessary to anaesthetize each animal group that were tested. This was achieved by the administration of a solution of ketamin (100 mg/mL) and xylasine (100 mg/mL) that was made by diluting 3.75 mL of ketamin plus 0.5 ml of xylasine in 5.75 mL of distilled water. Intraperitonial injection of 0.2 mL/100 g of this solution[20] was used to sedate the rats. The working time obtained by this technique was nearly 30 min. Custom-made

palatal appliances were fabricated using the heat-polymerized acrylic resin Lucitone 550 (Dentsply Indústria e Comercio Ltda, Petrópolis, Brazil), as described in Table 1. The appliances were relined (or not) with one of three hard chairside denture reline acrylic resins.

Table 1- Materials evaluated in this study

Brand name	Code	Manufacture	Powder (P)/ Liquid (L) ratio (g/mL)	Polimerization condition	Composition	
					Powder	Liquid
Lucitone 550	L	Dentsply Indústria e Comércio Ltda, Petrópolis, RJ, Brasil	0.42/0.2	90 min at 73°C and 30 min at 100°C (short cycle)	PMMA	MMA and EGDMA
Tokuyama Rebase II	TR	Tokuyama Dental Corporation, Japan	2.05/1	5.5 min at room temperature	PEMA PB	1.6 HDMA MAOP DPT
New Truliner	NT	Bosworth Company, USA	1.34/1	10-15 min at room temperature	PEMA PB	IBMA N-dibutyl phthalate
Kooliner	K	GC America Inc, USA	1.4/1	10 min at room temperature	PEMA PB	IBMA

PEMA, poly (ethyl methacrylate); IBMA, isobutyl methacrylate; MAOP, methacryloyl oxyethyl propionate; 1.6-HDMA, 1.6-hexanediol dimethacrylate; PMMA, poly (methyl methacrylate); MMA, methyl methacrylate; EGDMA, ethylene glycol dimethacrylate.

The animals were divided into seven groups of five animals each, according to the type of material to be tested, with or without heat treatment (Table 2).

Table 2- Experimental groups, material/hard chairside denture reline resin

Groups (n = 5)	Material
Group 1	Control group: without appliance
Group 2	L + TR without heat treatment
Group 3	L + TR with heat treatment
Group 4	L + NT without heat treatment
Group 5	L + NT with heat treatment
Group 6	L + K without heat treatment
Group 7	L + K with heat treatment

2.3) Acrylic custom-made palatal device preparation

Initially, thirty trays were fabricated with putty consistency impression material, (polivinilsyloxane and Futura AD catalyzer, DFL Indústria e Comércio S/A, RJ, Brazil), and these were constructed on a master dental stone model (Herostone, Vigodent S/A Indústria e Comércio, RJ, Brazil) from a preliminary impression of a Wistar rat palate. These animals presented age and weight similar to those used during the experiments. The trays were then tested and their borders adjusted for each rat to be shaped in such a way that reached the bottom of the vestibule. After sedation, master impressions of the palates were taken with light-body impression addition silicone (Futura AD, DFL Indústria e Comércio S/A, RJ, Brazil). For this purpose, the animals were placed in a stabilizing plate, to avoid their mouths from opening, which allowed the impressions to be made. The working models were obtained with type IV gypsum (Herostone, Vigodent S/A Indústria e Comércio, RJ, Brazil) and on them, the waxing of a plate was made with a layer of type 7 wax (Lysanda, Produtos Odontológicos Ltda, São Paulo, Brazil) without relief. The waxing was done on the bearing area, comprising the region between the molars, extending to the first fold palate, covering the occlusal surface of the molars and passing to the labial surface of the same. Acrylic resin Lucitone 550 (Dentsply Indústria e Comercio Ltda, Petrópolis, RJ, Brazil) was heat-polymerized according to the manufacturer's protocol (Table 1). The following polymerization cycle was employed: Lucitone 550 was processed for 90 min at 73°C and then in 100°C boiling water for 30 min (short cycle). After polymerization, the flasks were bench cooled at room temperature for 30 min, and 15 min under running water, before the specimens were removed from the flasks. The devices were visually inspected to verify that they presented a smooth surface and showed no voids or porosity; otherwise, they were discarded. The edges of the denture base were carefully finished and polished to

remove irregularities. Immediately after specimen fabrication, the acrylic resin devices were stored in distilled water for 48 h at 37°C prior to insertion, in order to eliminate the possible effects of any residual monomer[21]. Shortly after preparation, and prior to the attachment of the devices, groups 3, 5 and 7 received heat treatment, being immersed in water at 55°C for 10 min[22].

2.4) Acrylic custom-made palatal device direct reline and fixing

A week after obtaining the prints, the palatine plates in heat-polymerized acrylic resin were fixed on the palate of the animals and all of the groups were treated in the same way. In order to promote mechanical retention and stability of the molars and fixing of the palatal device, a groove in the molar coverage region was made using a spherical bur #6. Composite resin (Opallis, FGM, SC, Joinville, Brazil) was used to fill the molar region groove and the resin was photo-polymerized while the device was set in position. The amount of composite resin used was minimal, so as not to invade the palatal surface and affect the plate in contact with the palate. The acrylic resin palatal devices were relined with the hard chairside reline resin Kooliner, Tokuyama Rebase II e New Truliner, following the manufacturers' instructions and were held in position until their polymerization (Table 1). The excesses of the reline material were removed and the resin was photo-polymerized while the device was kept in position, performed with LED curing for 40 s (Gnatus, Ribeirão Preto, Brazil). Upon completion of the procedure, the rats were placed at rest to recover from anesthesia. From this moment, the rats were placed on a paste diet to avoid food debris gathering under the denture system. The animals, and both groups, remained with the plates in the palate for 14 days. Comparisons were made with five animals without the use of plates, being maintained under the same conditions as the experimental groups (control group).

2.8) Histomorphometric analysis

After 14 days, the animals of all groups were sacrificed by euthanasia, in accordance with resolution 714 of 20th June 2002 of the Federal Council of Veterinary Medicine (FCVM). After deep anesthesia, the mice were killed by intracardiac perfusion with potassium chloride 10% (1 ml/100 g) through the left ventricle. The palates of the animals were dissected, fixed in 10% buffered formalin for 48 h, and decalcified in 4.17% EDTA for approximately four months. The pieces went through a dehydration process, embedded in paraffin, so that the back part of the palate stayed down. The samples embedded in paraffin were cut with a microtome (Leica, Berlin, Germany) in sections of 5 μm and then stained with haematoxylin and eosin (H&E). Slides were mounted and observed by using light microscopy (Nikon, model YS 100, Tokyo, Japan). Two images, standardized in the central region of the cuts, near the nasopalatine plexus and the midline of the palatine raphe, at 200x magnification to check for changes histological, were made. Overall thickness of the epithelium (TET), cellular compartment (TCC) and keratin layer (TKC) were calculated.

Image-Pro Plus software (version 4.5.0.29-Media Cybernetics, Inc, Bethesda, USA) was used for measurement. For each experimental group, (NT, K, TR, with and without heat treatment, and control) a grid was used with ten horizontal lines crossing the layers to be analyzed. For each analyzed layer, ten measurements were obtained and then the averages of the measurements were calculated.

Comparisons were made of the various measured epithelial parameters. Thickness of keratin, connective tissue and total epithelial, individually and in combination, were also calculated. Statistical analysis was carried out by a one-way analysis of variance ANOVA and post hoc Tukey test (α=0.05) using the SPSS 18.0 program.

3) Results

Figures 1-6 show the results obtained by histomorphometric analysis of the tissue layers.

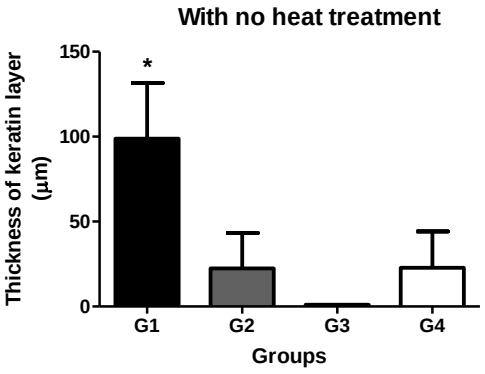

Fig. 1. Mean values and standard deviation of thickness of keratin layer (µm) with no heat treatment. G1- Control; G2- Tokuyama; G3- Truliner; G4- Kooliner. Significant difference (*) with all groups, p<0.0001. One-way ANOVA and Tukey's Post Hoc.

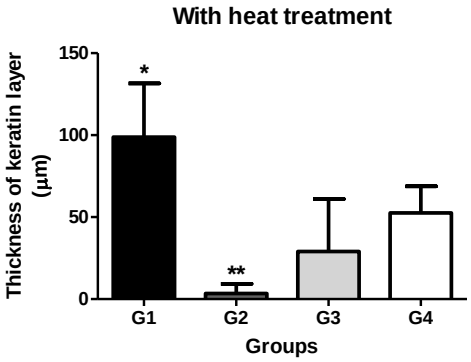

Fig. 2. Mean values and standard deviation of thickness of keratin layer (µm) with heat treatment. G1- Control; G2- Tokuyama; G3- Truliner; G4- Kooliner. Significant difference (*) with all groups; (**) with G4, p=0.0001. One-way ANOVA and Tukey's Post Hoc.

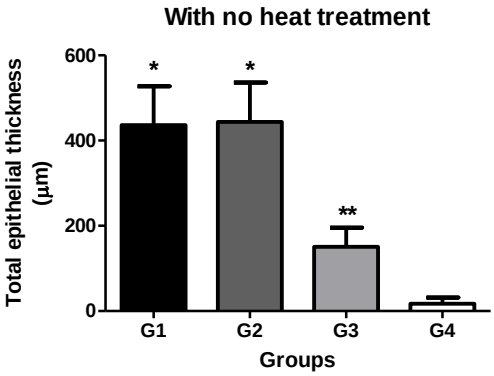

Fig. 3. Mean and deviation of total epithelium thickness (µm) dos reembasadores sem tratamento térmico. G1- Controle; G2- Tokuyama; G3- Truliner; G4- Kooliner. Significant difference (*) with G3 and G4; (**) with G4, p<0.0001. One-way ANOVA and Tukey's Post Hoc.

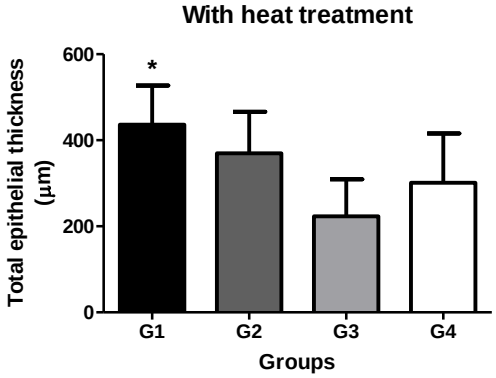

Fig. 4. Mean and deviation of total epithelium thickness (µm) dos reembasadores com tratamento térmico. G1- Controle; G2- Tokuyama; G3- Truliner; G4- Kooliner. Significant difference (*) with G3, p=0.0193. One-way ANOVA and Tukey's Post Hoc.

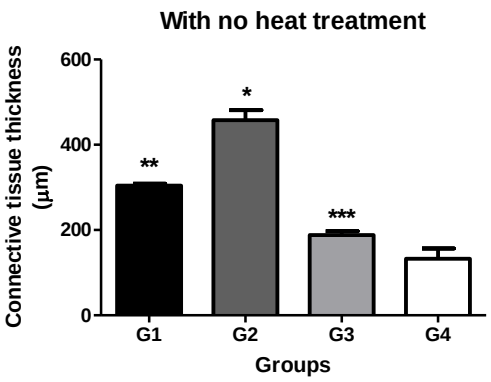

Fig. 5. Mean values and standard deviation of connective tissue thickness (µm) with no heat treatment. G1- Control; G2- Tokuyama; G3- Truliner; G4- Kooliner. (*) Significant difference between all groups; (**) G3 and G4; (***) G4. p<0.0001. One-way ANOVA with Tukey's post hoc test.

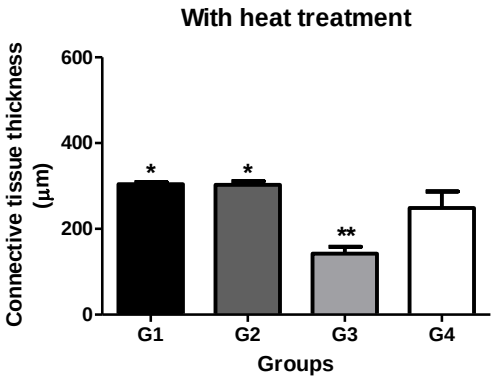

Fig. 6. Mean values and standard deviation of connective tissue thickness (µm) with heat treatment. G1- Control; G2- Tokuyama; G3- Truliner; G4- Kooliner. Significant difference (*) with G3 and G4; (**) with G4, p<0.0001. One-way ANOVA with Tukey's post hoc test.

The results of the Tukey post hoc test are show in Figures 1-6. As it can be seen in Figure 1, the thickness of the keratin layer for Tokuyama Rebase II, New Truliner and Kooliner, without additional heat treatment, was statistically lower than the control group (p<0.0001). Quite similar behavior can be seen in figure 2, but additionally the thickness of this layer in the Tokuyama Rebase II group was statistically different from Kooliner group (p<0.05).

In Figure 3 one can see that the average tissue thickness for Tokuyama Rebase II and control group, without further heat treatment, were significantly higher than Truliner and Kooliner (p<0.0001). The results for Truliner were lower than Kooliner (p<0.05). Heat treatment altered the behavior of reline materials, significantly increasing the tissue thickness of the Tokuyama Rebase II and Kooliner Tokuyama Rebase II and New Truliner, becoming them similar to the control group. Truliner carried on different from control (p<0.05, Figure 4).

The results shown in Figure 5 demonstrate that the average total thickness of epithelium for Kooliner and New Truliner, without heat treatment, was significantly lower than control (p<0.0001) and for Tokuyama was significant higher than control

(p<0.0001). With respect to heat treatment, this was responsible for changing the behavior of Tokuyama which became similar to the control group.

4) Discussion

Taking into consideration the release of residual monomer and other components by acrylic resins and also the fact that there have been reports of allergic reactions in patients that use both partial and total removable prostheses[23], various studies that quantify and identify such components[19,25] and that determine the cytotoxicity of acrylic resins[11,22,26] became necessary. This current *in vivo* study investigated the effect of a heat treatment on the behavior of hard chairside reline acrylic resins on the palate of rats. One of the main advantages of research using animals is related to ethical considerations; there are limits on studies involving humans in situations that require more invasive procedures and when there is no previous knowledge of the efficiency and toxicity of new drugs[27]. Animal models are available to study the process of diseases and they represent an important step in determining the biological behavior of biomaterials. Studies to verify irritation in the oral mucosa, using removable palatine devices in small animals, are considered extremely difficult and time consuming[28], mainly due to the fact that the devices that have been described in the literature are considered primitive and inadequate[29]. Considering the difficulties associated with palatal devices described in the literature, and bearing in mind, not only the importance of conducting *in vivo* studies as preliminary tests, this present study used an alternative palatine plate of heat-polymerizable acrylic resin in Wistar rats, which allowed direct reline for the study of the biocompatibility of different kinds of materials and substances. In this way, it was possible to evaluate the biocompatibility of acrylic resins for base and prosthesis relining, with and without post- polymerization heat treatment.

Using histomorphometric analysis, comparisons were made of the measured tissue parameters, i.e. thickness of the keratinized layer, connective tissue and total epithelium after fitting palatal appliances made from heat-polymerized acrylic resin, relined with one of three hard chairside reline resins (Tokuyama Rebase Fast, New Truliner and Kooliner) polymerized according to the manufacturer´ instructions and submitted or not to a post-polymerization heat treatment in water bath.

The normal human palatal epithelium is an orthokeratinizing tissue with a well-developed stratum corneum[29]. Clinically healthy denture-bearing mucosa is seldom histologically normal, usually showing disturbances of keratinization[30]. The results of hyperkeratosis have been found for some time[4] in assessment of levels the effects of the residual monomer on the oral mucosa in patients that have had a total prosthesis. Kapur and Shklar[31] observed keratinization, although other authors have stated that either complete or partial removable dentures reduce the quantity and/or quality of the keratin layer of the denture-bearing mucosa[29,32]. In a human histochemical and histopathologic study of alveolar mucosa under complete dentures, Razek and Shaaban[33] verified an increase in keratinization during the first three years of prosthesis use, and in the three years that followed it was verified that the subjects' epithelia gradually became thicker, the horny layer was nearly absent, and parakeratosis was spread all over the surface of the mucosa. Differences between results in the literature are due to differences in experimental conditions, such as the type of examination, the region of the palate where the sample was collected, the type of research (human or animal), among others.

Two reline materials (Truliner and Kooliner) promoted decreasing in the thickness of keratin layer as the reline materials presented lower keratin thickness than when compared to control. Comparing the reliners, New Truliner promoted the lowest keratin thickness. Of all the studied reliners, New Truliner showed the lowest powder-

liquid ratio, which might be partially responsible for the obtained results. In an *in vitro* study[24], it was verified that the lower the proportion of powder/liquid the greater the amount of residual monomer in the released material. However, one may take into consideration that the influence of the denture base on the keratine layer has been associated with mechanical factors[17,34], microbiological factors[28,35-37] and superficial factors[14,15] of the resin base. The present study did not aim to verify the influence of mechanical and biological factors on tissues. Nevertheless, once the palatal devices, according to the methodology of construction in the present study, were cemented on the molars, it was hoped that the devices would promote compression forces that would be distributed on the palate and on the teeth. This might partially explain the reduction of keratin layer in all of the studied groups due to the lack of mechanical stimuli as compared to the control group. Another aspect to be taken into consideration is that although all the studied materials might be rigid after polymerization, the superficial hardness and modulus of elasticity[2,24] of the reline materials are heterogeneous, leading to the conclusion that the distribution of tension compression on the mucosa might not be similar in all of the experimental groups. In fact, the devices had their internal portion relined. For this relining, standardized relief of the internal surface of the devices was carried out to generate enough space for the reline material. The achievement of relief, the difference in rigidity of all the materials studied, as well the distribution of the occlusal forces within the rats' molar teeth, might explain the results obtained in the present study in relation to the control group and also the differences of the materials between each other. A decrease of the keratin thickness after heat treatment, in the material Tokuyama Rebase II as compared to the other reliner materials was observed, confirming the *in vitro* studies that found that post-

polimerization in a water bath modified the biological properties of acrylic resins[11,12,21,22].

In the evaluation of total epithelium a different behavior was verified before and after heat treatment for material Kooliner as only before heat treatment the thickness of this layer was thinner than the control. In the evaluation of the connective tissue the heat treatment changed the behavior of Tokuyama and Kooliner, bringing them more similar to the control, or closer to normal.

Barclay *et al.*[14] carried out a study using Wistar rats with the objective of verifying the histological effects on the palatal mucosa, both using palatine plates (for 7 and 14 days), and the type of diet (three in total). It was observed that a paste diet was an alternative that might result in a reduction in the accumulation of food waste under the plate. In this same year, a second study by Barclay *et al.*[15] evaluated the histological effects on the palatine mucosa of Wistar rats of the use of a palatine device made with acrylic resin and the incorporation of two reline materials one hard, Kooliner, and the other resilient, Coe-Soft, both of which are materials that are used for the immediate reline of removable prosthesis. In that particular study it was verified that the reline materials were not inert in the mucosa and caused an increase in the keratin thickness layer, however, without resulting in an increase in the total epithelium. Another hypothesis might be the influence of the composition of the resultant liquid; this was observed in a study[38] where the materials Kooliner and New Truliner liberated high amounts of IBMA, in comparison with the 1.6 HDMA liberated by the resin Tokuso Rebase. The IBMA present in the liquid of Kooliner and New Truliner is a monofunctional monomer, while the 1.6 HDMA contained in the liquid resins and Tokuso Rebase Fast are dimethacrylate. The presence of bifunctional monomers can improve the polymerization process, compared to the systems based on monofunctional

monomers, because they provide a large number of reactive groups in the polymerization reaction, so there might not be the necessity of using heat treatment in some reline materials.

When analyzing Figures 7-13, no inflammatory cells such as lymphocytes, plasmocytes, leukocytes, and inflammatory infiltrate were found in the studied groups. The liberation of toxic substances, such as residual monomers, formaldehyde, and others from acrylic resins is already well established. However, it has been verified that this *in vivo* liberation is much inferior to that which occurs *in vitro*[48]. This may explain the lack of inflammatory responses in the present study.

5) Conclusion

In accordance with the results of this study, the importance of adequate processing of material and the use of histological analysis for comparison among treatments is evident. The proposed heat treatment was found to be effective, from the viewpoint of biocompatibility, for the acrylic resin hard reline Tokuyama Rebase II that were investigated. Prostheses appear to mostly modify the keratin layer. Future studies are needed to detect, quantify and qualify the effects in the oral mucosa of substances released by acrylic resins from denture base as well as to determine the cytotoxic effect of these substances.

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Figures

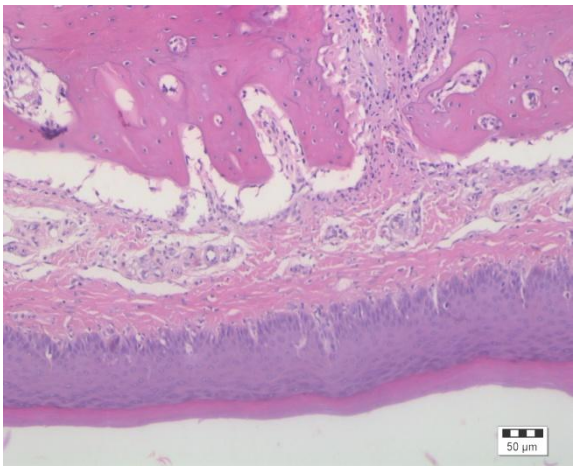

Fig. 7. The histological section displays orthokeratinized stratified squamous epithelium, and the granular layer is very evident and discrete epithelial ridges. The lamina propria is normal and there characteristics presence of vital bone tissue (Control, HE 200X).

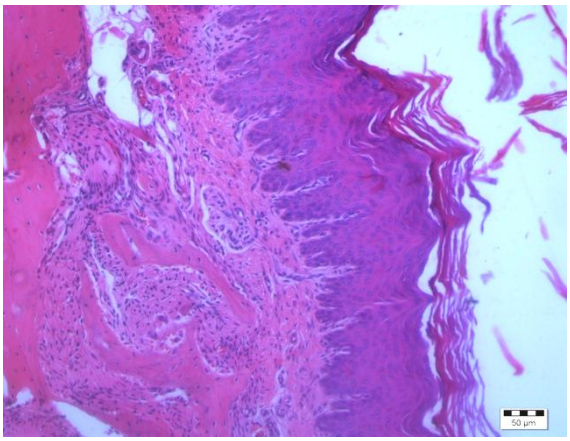

Fig.8. Hiperothoqueratinized stratified squamous epithelium, the presence of large amount of flaking layers of keratin. Epithelial tissue also presents hiperplásicio, and the epithelial ridges are evident. The lamina propria is with normal characteristics, without significant inflammatory infiltration. (Tokuyama Rebase II, HE 200X)

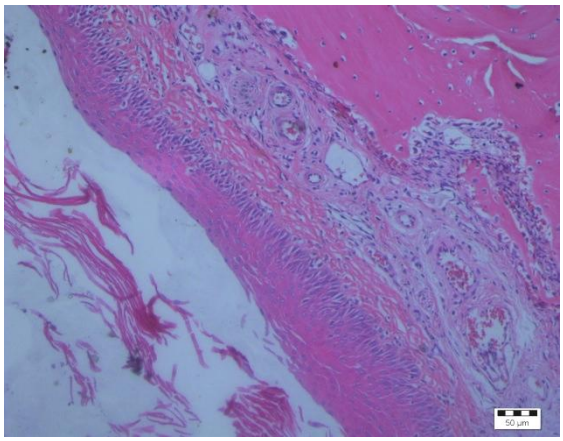

Fig.9. Atrophic stratified squamous epithelium keratinized (absence of epithelial crests and decreased thickness of the epithelial tissue). Presence of blades of keratin desgarrradas epithelial tissue. The lamina propria colagenizada well and without significant inflammatory infiltration. The tissue deeper presents collagenized and rather less vascularized. (Tukuyama Rebase II heat treatment, HE 200X).

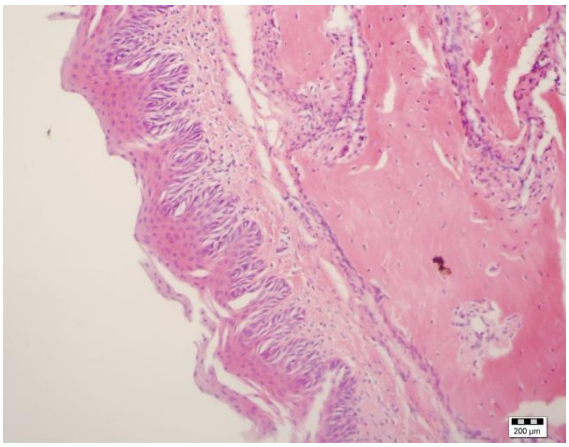

Fig 10. Stratified squamous epithelium without keratinization. Epithelium seems to be falling off. The cells of the basal layer of the epithelium are changed. No note is significant inflammatory infiltrate in the lamina propria and bone tissue is vital and with normal characteristics. (New Truliner HE 200X)

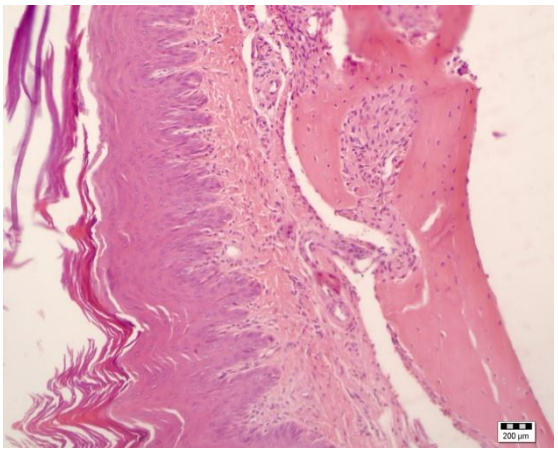

Fig.11. Hiperorhtoqueratinized stratified squamous epithelium, lamina of keratin in loosening. The epithelial ridges are more evident as compared to the control group. The lamina propria colagenizada well and without significant inflammatory infiltration. Bone tissue is vital and with normal characteristics (New Truliner heat treatment, HE 200X ?).

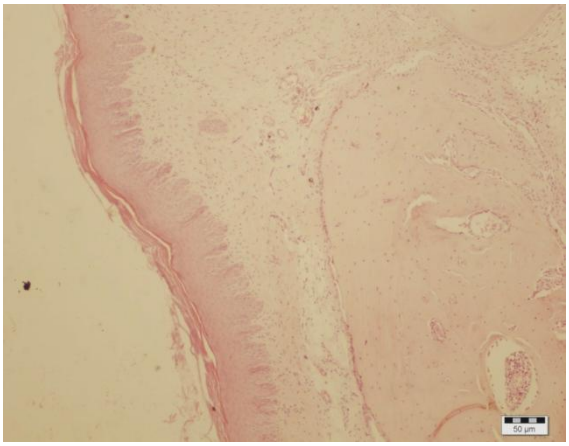

Fig 12. Decrease of the keratin layer and scaling, as well colagenizada The lamina propria and no significant inflammatory infiltration. Kooliner HE 200X

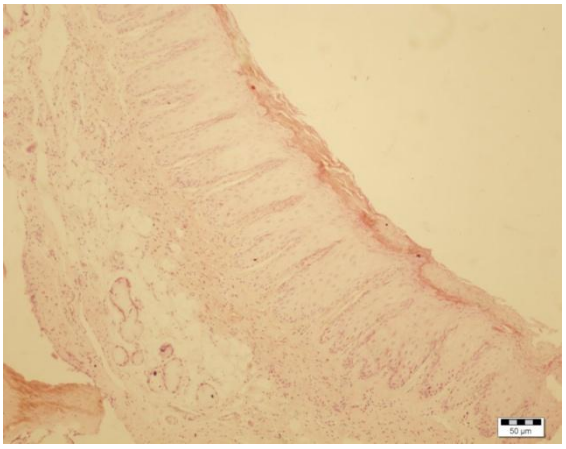

Fig 13. Epithelial ridges more evident epithelial tissue also presents hiperplásicio, and the epithelial ridges are evident. Kooliner heat treatment HE 200X

5 DISCUSSÃO

Em consequência da liberação do monômero residual e de outros componentes pelas resinas acrílicas e havendo relatos de reações alérgicas em pacientes que fazem uso de próteses removíveis totais ou parciais[1,2,5,11-15], vários estudos que quantificam e identificam tais componentes[57,60] e que determinam sua citotoxicidade tornaram-se necessários[38,39,54]. O presente estudo in vivo identificou o efeito de resinas acrílicas para base de prótese e reembasamento sobre as alterações histopatológicas no palato de ratos. Uma das principais vantagens da pesquisa em animais está relacionada às considerações éticas, que limitam a realização de muitos estudos em humanos, em situações que necessitam de procedimentos mais invasivos e quando não existe conhecimento prévio da eficácia e toxicidade de novos fármacos[61]. Modelos animais estão disponíveis para estudar processos de doenças e representam uma etapa importante para determinar o comportamento biológico de biomateriais. Estudos para verificar irritações na mucosa bucal, utilizando dispositivos palatinos removíveis em animais de pequeno porte, são considerados extremamente difíceis e consomem muito tempo para execução[62]. Ademais, os dispositivos descritos na literatura foram considerados primitivos e inadequados[48]. Devido a essas dificuldades associadas aos dispositivos palatais pré-existentes e tendo em vista a importância de se realizar estudos in vivo, como testes preliminares aos clínicos, foi descrita e utilizada no presente estudo uma alternativa de confecção de placa palatina em resina acrílica termopolimerizável, utilizadas em ratos Wistar, a qual permitiu o reembasamento direto para estudo da biocompatibilidade de diferentes tipos de materiais. Assim, foi possível avaliar a biocompatibilidade de resinas acrílicas para base e reembasamento de próteses frente ou não a tratamento térmico pós-polimerização. O baixo índice de perda das placas durante o experimento e a facilidade de execução permitiu a obtenção de resultados com baixo custo utilizando-se de materiais acessíveis, como a resina composta, utilizada na fixação das placas aos molares dos animais.

Algumas características são relevantes para definição do modo de confecção de dispositivos palatinos para testes de biocompatibilidade de biomateriais. Lee et al.[48], em 2011, descreveram algumas características que os dispositivos intraorais devem possuir: ter retenção, ser removível, não interferir na atividade normal de alimentação dos animais, ser bioestável, ser de fácil fabricação e possuir perfeito contato com a mucosa sem alterar a arquitetura dental. O dispositivo aqui apresentado está de acordo com as

principais características citadas acima, pois sua retenção na cavidade dos animais, no período proposto, foi possível utilizando resina composta fotopolimerizável, promovendo assim, fixação por meio de retenção mecânica envolvendo os dentes molares do palato dos ratos.

O material selecionado para utilização no presente estudo trata-se de uma resina acrílica termopolimerizável, constituída por polimetil metacrilato – PMMA e metil metacrilato e etileno glicol dimetacrilato, e apresenta dois possíveis ciclos de polimerização. Um deles é denominado ciclo curto, onde a mufla é imersa em água à temperatura de 73^{o}C, permanecendo por 90 min e, em seguida, a temperatura é elevada até a ebulição e a mufla, mantida por mais 30 min, em aparelho termopolimerizador. A outra técnica de polimerização possível é denominada ciclo longo e a mufla é mantida 9 h a 71ºC, não contendo uma polimerização final com água em ebulição. Em nosso estudo, utilizamos o ciclo curto, que inclui uma temperatura final em ebulição, o que pode ter sido suficiente para converter todo o monômero. Esta hipótese é apoiada nos resultados obtidos por Urban et al.[28] em 2007, que observaram que o ciclo curto promoveu menor quantidade de monômero residual livre (0,08%) para Lucitone 550 comparado com o ciclo longo (0,24%) e está também de acordo com estudos realizados por Jorge et al., em 2006, que verificaram diferenças no ciclo de polimerização, quando avaliaram o número de células viáveis, e concluíram que ciclo longo aumentou a citotoxicidade de Lucitone 550.

Um dos principais problemas da utilização de placas palatinas em ratos, como descrito na literatura, é a dificuldade de alimentação dos animais, podendo acarretar em perda de peso e deficiências nutricionais, interferindo nos resultados de estudos de biocompatibilidade e virulência de microrganismos. Para a verificação do efeito da utilização do dispositivo proposto sobre o comportamento na alimentação dos animais, verificamos o peso dos ratos Wistar no momento da instalação e o comparamos com o peso após 14 dias de utilização das placas. No presente estudo, foram selecionados animais com peso e idade iniciais de 200 g e três meses, em média, respectivamente. Os animais do grupo controle apresentaram variação média de peso de 5,7 g. Os do grupo com placa de Lucitone 550 e com placa reembasada apresentaram os valores 14,6 g e 9,3 g, respectivamente. Em resumo, os resultados mostraram que os animais apresentaram estabilidade de peso, o que indicou que o dispositivo sugerido não influenciou na nutrição dos animais. A permanência do dispositivo na cavidade bucal dos animais não alterou seus hábitos alimentares, pois não houve diferença significante

no peso dos ratos, não interferindo nas atividades rotineiras. Isso foi possível devido à realização de moldagens individuais, bem como a fixação com resina composta, promovendo uma melhor adaptação dos dispositivos.

Para a obtenção do dispositivo aqui descrito, utilizamos uma placa cobrindo somente a região dos molares, portanto menor e mais confortável para o animal, o que difere do estudo de Barclay et al.[42], em 1997, no qual os autores utilizaram placas palatinas maiores e uma banda de aço inoxidável nos incisivos superiores dos animais. No entanto, este dispositivo mais extenso poderia se soltar, além dos animais terem que superar o efeito da placa sobre oclusão e contínua erupção dos incisivos. Nesse primeiro estudo, os autores verificaram a influência de três diferentes dietas, examinando seu efeito histológico em palatos de ratos Wistar. Concluíram que a dieta pastosa reduz o acúmulo de resíduos sob a placa, diminuindo também seus efeitos sobre a mucosa. Com base nesse estudo, optou-se, no presente estudo, por dieta pastosa, por meio da mistura do pó da ração com água, facilitando a alimentação, quando comparada com a ração sólida.

Num segundo estudo realizado por Barclay et al.[43], em 1997, foi utilizado o mesmo modelo de dispositivo palatino, mas agora para testar materiais reembasadores, portanto o mesmo objetivo do presente estudo. Porém, foi obtido na placa, no momento da prensagem, um espaço na região intermolares, utilizando um espaçador de 1 mm de espessura. Em seguida, esse espaço foi preenchido com o material reembasador a ser testado. O procedimento descrito acima difere do do presente estudo pelo modo de confecção do espaço para acomodação do material que seria testado, pois neste, foi criado um espaço na mesma região, desgastando com uma broca esférica em baixa rotação nº 6, sendo introduzida meia ponta ativa. Assim, foi obtido um desgaste com espessura padronizada, sempre realizado pelo mesmo operador. Jorge et al.[46], em 2001 realizaram um estudo utilizando placas palatinas, mas com uma diferença significativa. O dispositivo foi confeccionado em resina acrílica sobre um palato descalcificado e fixado em formol. Em seguida, as placas eram adaptadas, reembasadas e cimentadas com a mesma resina acrílica. Os incisivos foram incluídos na cimentação. Esse procedimento de confecção difere do do presente estudo, primeiramente porque neste foram realizadas moldagens individuais de cada animal, visando melhor adaptação, facilitando a alimentação e não interferindo na oclusão. Os autores relataram que houve perda de peso e comprometimento sistêmico significativos dos animais, possivelmente porque houve alteração no hábito alimentar, mesmo sendo administrada ração moída e

molhada. Olsen e Bondevik[63] em 1978 descreveram um modelo similar ao descrito acima e também relatam perda de peso dos animais.

A metodologia para confecção de placas palatinas descrita por Tsuruoka et al.[45], em 2008, assemelha-se mais ao modelo do presente estudo, tendo como diferença a cimentação adesiva utilizada pelos autores. Além disso, foi colocada cera sobre os dentes molares para estimulação mecânica da mucosa e não houve desgaste interno para fixação. Lee et al.[48], em 2011, desenvolveram um dispositivo de fácil confecção para pesquisas na cavidade bucal de animais, que consiste em uma placa acrílica estabilizada no palato de ratos, para estudos sobre *Candida* associada à estomatite devido ao uso de prótese total. Johnson et al.[49], em 2012, utilizaram um sistema de base acrílica em palato de ratos, com uma parte fixa e outra removível para realizar estudos longitudinais sobre estomatite protética relacionados com *Candida albicans*. Entretanto a perda de dispositivos palatais durante os experimentos, as dificuldades técnicas, o prejuízo nutricional provocado pelo uso das placas ainda têm sido relatados na literatura.

Inicialmente, para o presente estudo, foram testados diferentes agentes cimentantes, tendo sido a resina composta mais efetiva na manutenção da retenção do dispositivo. Foram testados animais com e sem condicionamento ácido do esmalte dos molares e percebeu-se dificuldade nessa etapa, além de ter-se mostrado desnecessária para promover retenção satisfatória do dispositivo. Barclay et al.*[42]*, em 1997, e Jorge et al.*[46]*, em 2001, utilizaram resina acrílica autopolimerizável para retenção da placa. No entanto, o calor gerado pela reação exotérmica do material poderia gerar alterações histológicas, interferindo assim no resultado. Em outro estudo realizado por Barclay et al.[43] em 1997, os autores escolheram uma resina acrílica para cimentação dos dispositivos, mas se depararam com deficiência de retenção, perdendo um número significativo de dispositivos, sendo necessário um reforço com cimento de óxido de zinco e eugenol (IRM). Tanto a resina acrílica como o cimento de óxido de zinco e eugenol poderiam causar alterações histológicas, influenciando nos resultados. Porém, não foram encontrados na literatura dispositivos com a possibilidade de reembasamento e fixação efetivos como o que foi descrito, portanto foi possível avaliar, por meio de análise histomorfométrica espessuras da camada de queratina, tecido conjuntivo e epitélio total, da resina acrílica para base de prótese Lucitone 550 e reembasadores rígidos Tokuyama Rebase II, New Truliner e Kooliner antes a após tratamento térmico pós-polimerização.

O epitélio normal da região do palato é um tecido ortoqueratinizado com uma camada de estrato córneo bem desenvolvida[64]. A mucosa sob próteses removíveis raramente é normal, apresentando distúrbios de queratinização[65]. Resultados de hiperqueratose vêm sendo encontrados desde muitos anos[50] em pacientes portadores de próteses totais, em avaliações dos níveis, bem como os efeitos do monômero residual sobre a mucosa. Kapur e Shklar[66], em 1963, também observaram queratinização, embora outros autores afirmem que o uso de próteses reduz e/ou altera a camada de queratina[64,67]. Em um estudo sobre histoquímica e histologia em humanos, analisando mucosa alveolar sob próteses totais, Razek e Shaaban[68] em 1978, verificaram um aumento da queratinização nos primeiros três anos de uso das próteses, e nos três anos seguintes foi verificado que o epitélio tornou-se mais espesso, a camada córnea estava quase ausente e havia paraqueratose sobre toda superfície da mucosa. Diferenças entre os resultados da literatura devem-se às diferenças de condições experimentais, tais como tipo de exame, região do palato de onde foi coletada a amostra, sujeitos da pesquisa (humanos ou animais), entre outras.

A influência das bases de próteses sobre a camada de queratina tem sido mais associada a fatores mecânicos[45,47], microbiológicos[46,48,49,63] e superficiais[42,43] da resina de base do que propriamente sua toxicidade. Não foi objetivo do presente estudo verificar a influência desses fatores sobre os tecidos. Apesar disso, uma vez que os aparelhos palatais, de acordo com a metodologia de construção descrita no presente estudo, foram cimentados sobre os molares, espera-se que os aparelhos tenham promovido forças de compressão que fossem distribuídas sobre o palato e sobre os dentes, ou seja, os aparelhos eram dentomucossuportados. Isso pode explicar a ausência de hiperqueratinização em todos os grupos estudados, devido à falta de estímulo mecânico, quando comparada sua espessura com o grupo controle. Outro aspecto a ser levado em consideração é que embora todos os materiais estudados, seja a resina de base ou os reembasadores, sejam rígidos após polimerização, a dureza superficial e o módulo de elasticidade[20] dos materiais reembasadores são menores, esperando-se que a distribuição de tensões de compressão sobre a mucosa não seja similar em todos os grupos experimentais. Assim, foi verificado um maior grau de queratinização sob as bases de Lucitone 550 quando comparado com as placas reembasadas com quaisquer dos reembasadores estudados. Também pôde-se observar diminuição na espessura de queratina após o tratamento térmico, nos materiais Lucitone 550, Tokuyama Rebase II,

sendo confirmada os estudos in vitro de que banhos térmicos modificam as propriedades biológicas das resinas acrílicas[39,54-56].

Deve-se levar em consideração que, ao contrário dos aparelhos compostos apenas pela resina acrílica termopolimerizável, os outros tiveram sua porção interna reembasada. Para esse reembasamento foi realizado alívio padronizado da superfície interna dos aparelhos para prover espaço suficiente para o material reembasador. A realização do alívio, a diferença de rigidez de todos os materiais estudados, bem como a distribuição das forças oclusais com os dentes molares dos ratos, pode explicar os resultados obtidos no presente estudo em relação ao controle e as diferenças dos materiais entre si. Apesar de que em estudos prévios[42,43] os materiais reembasadores tenham promovido aumento da espessura de queratina, os materiais reembasadores apresentaram espessuras menores de queratina quando comparados ao controle e à resina de base. Entre os reembasadores, New Truliner foi que o apresentou menor espessura de queratina. Esse material foi o que apresentou a menor proporção pó-líquido, o que pode ter sido responsável pelos resultados obtidos. Em um estudo in vitro sobre citotoxicidade[60], foi verificado que quanto menor a proporção de pó/líquido maior seria a quantidade de monômero residual liberado deste material.

Na avaliação do tecido conjuntivo e epitélio total, observou-se aumento da espessura em quase todos os materiais previamente ao tratamento térmico em comparação ao grupo controle, e após tratamento térmico verificou-se uma redução significativa, ficando mais próximo da normalidade. No entanto, os materiais reembasadores Kooliner e New Truliner apresentaram um comportamento diferente, pois, após terem sido tratados termicamente, mostraram aumento da espessura tecido conjuntivo e epitélio total. Barclay et al.[43] em 1997, realizaram um estudo em ratos Wistar, com o objetivo de verificar os efeitos histológicos na mucosa do palato, por consequência do uso de um dispositivo palatino confeccionado de resina acrílica com a incorporação de dois materiais reembasadores, sendo um rígido, Kooliner, e outro resiliente, Coe-soft, que são materiais utilizados para reembasamento imediato de próteses removíveis. No seu estudo foi verificado que os materiais reembasadores não foram inertes sobre a mucosa e causaram um aumento na espessura da camada de queratina, sem, no entanto haver aumento do epitélio total. Uma hipótese para explicar esse comportamento, seria a influência da composição do líquido, uma vez que foi observado em um estudo[28] que os materiais Kooliner e New Truliner liberam quantidades altas de IBMA em comparação com o 1,6 HDMA liberado a partir da

resina Tokuso Rebase . O IBMA presente no líquido dos materiais Kooliner e New Truliner é um monômero monofuncional, enquanto o 1,6 HDMA contido no líquido das resinas e Tokuso Rebase Fast é um dimetacrilato. A presença de monômeros bifuncionais pode melhorar o processo de polimerização comparado com os sistemas baseados em monômeros monofuncionais, pois proporcionam um número maior de grupos reativos na reação de polimerização, assim talvez não haja necessidade de tratamentos térmicos em alguns materiais reembasadores.

Tem sido verificado que o "ciclo curto" (0,08%) promoveu uma menor liberação de monômero residual para Lucitone 550 comparado com "ciclo longo" (0,24%)[70] e menor nível de toxicidade *in vitro*[54], por isso a seleção desse ciclo para o presente estudo. Entretanto, dentro das condições do presente estudo, mesmo utilizando o ciclo curto descrito acima, a utilização das placas palatinas promoveu alterações para alguns dos parâmetros avaliados, isto é, o grupo com a resina de base Lucitone 500 apresentou maiores espessuras de tecido conjuntivo quando comparado com o grupo controle. Este aumento tecidual pode ser explicado devido ao estímulo sofrido pela mucosa por ação da resina de base utilizada, já amplamente estudada em testes in vitro, pela liberação de monômeros residuais e outras substâncias[1,2,5,11-15,39,54]. Segundo Carter, Kerr and Shepherd[69], 1986, a simples presença de uma prótese mucossuportada promove alterações no epitélio, e conforme descrição de Kapur e Shklar[66], em 1963, ocorre infiltrado inflamatório, na área em contato com a prótese.

Não foram encontradas células inflamatórias como linfócitos, plasmócitos e leucócitos, infiltrado inflamatório, em nenhum dos grupos estudados. A liberação de substâncias tóxicas, tais como monômeros residuais, formaldeído, e outras, a partir das resinas acrílicas já está bem estabelecida, porém tem sido verificado que essa liberação in vivo tem sido muito inferior à que ocorre in vitro, o que pode explicar a ausência dessas alterações celulares observadas[18].

6 CONCLUSÃO

A utilização de impressões individuais em ratos Wistar, associada a placas com cobertura oclusal sobre molares e o método de cimentação utilizado proporcionaram boa estabilidade ao dispositivo palatino desenvolvido neste estudo, sem interferência no peso nos animais, podendo ser uma alternativa para o estudo in vivo de resinas de base e reembasadores de próteses.

A presença de próteses removíveis pode alterar histologicamente as camadas epiteliais da mucosa palatina.

Foi encontrado benefício do ponto de vista de biocompatibilidade para resina acrílica de base de prótese Lucitone 550 e reembasador Tokuyama Rebase II quando se utilizou o tratamento térmico proposto.

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ANEXO 1 Comitê de Ética em Experimentação Animal (SCEEA-UEPG)

UEPG
UNIVERSIDADE ESTADUAL DE PONTA GROSSA

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMISSÃO DE ÉTICA EM PESQUISA ANIMAL

PARECER CONSUBSTANCIADO

PROCESSO Nº 12673 | Data de Apresentação : 02/09/2010

O parecer consubstanciado do relator será utilizado como subsídio para a COMISSÃO elaborar seu parecer final

1 – Identificação do Projeto de Pesquisa/

Título:
Biocompatibilidade de reembasadores para base de próteses: análise histopatológica

Coordenador do Projeto:

Prof Dra Janaina Habib Jorge

Local onde será realizado:
Biotério, Laboratório de Histologia da UEPG

2 – Sumário do Projeto de Pesquisa

2.1 – Objetivos:

Avaliar a biocompatibilidade de resinas acrílicas para base de próteses e de materiais reembasadores por meio de análise histopatológica em ratos. Além disso, este estudo também tem por objetivo verificar o efeito de tratamentos térmicos, que visam diminuir a quantidade de monômero residual, sobre a biocompatibilidade desses materiais.

2.2 – Revisão Bibliográfica:

Pertinente e atual

2.3- Descrição e caracterização da amostra:

Neste estudo serão utilizados 75 ratos (*Rattus Norvergicus albinus Wistar*) com 60 dias de idade, pesando em média 270-300 gramas, obtidos do Biotério Central do Departamento de Odontologia UEPG. Esses animais serão mantidos em gaiolas, a 23ºC com 56% de umidade relativa do ar. Além disso, os animais serão alimentados com água e ração (PURINA) *ad libitum*

2.4- Avaliação coerentemente estatística do número (N) de animais utilizados:

Os animais serão divididos em 15 grupos, de cinco animais cada, de acordo com o tipo de material a ser testado com e sem tratamento térmico.

2.5 – Adequação metodológica (Verificar a exequibilidade da proposta, isto é, se existe clareza do objetivo, compatibilidade entre os objetivos, a fundamentação teórica e a metodologia ou plano de ação, evidenciando consistência entre objetivos, procedimentos, ações de execução da pesquisa e capacidade do proponente, demonstrada por outros trabalhos similares.)

A metodologia está clara e compatível com os objetivos

2.5- Adequação das condições para a realização da pesquisa:

3- Parecer conclusivo, recomendações e/ou sugestões:

Considerando os protocolos apresentados sou de parecer favorável à execução da pesquisa

4- Parecer Consubstanciado

Aprovado X ☐ Aprovado com adequação do N. experimental ☐ Pendências ☐ Não aprovado ☐

5- Dados Pessoais do Relator

Nome Completo: MARIA MARTA LODDI		
Telefone(s): 3222-3062	Departamento: ZOOTECNIA	
Local: PONTA GROSSA	Data: 09/12/2010	Assinatura:
Assinatura do Coordenador do CEP:	Data da reunião: / /	

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