Pontifícia Universidade Católica do Rio Grande do Sul
Faculdade de Odontologia
Programa de Pós-Graduação em Odontologia
Mestrado em Odontologia
Área de Concentração em Estomatologia Clínica

ESTUDO EXPERIMENTAL EM RATOS SUBMETIDOS À INJEÇÃO INTRAVASCULAR DE POLIMETILMETACRILATO: AVALIAÇÃO CLÍNICA E DA TOXICIDADE SISTÊMICA

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FACULDADE DE ODONTOLOGIA

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PORTO ALEGRE

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Estudo Experimental em Ratos Submetidos à Injeção Intravascular de Polimetilmetacrilato: Avaliação Clínica e da Toxicidade Sistêmica

Linha de Pesquisa: Enfermidades da Região Bucomaxilofacial – Estudos Clínicos, Imunológicos e Anátomo-Patológicos

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Dedico este trabalho os meus queridos pais, Zilma e Laerte, por me amarem e apoiarem incondicionalmente.

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"*Nem tudo que se enfrenta pode ser modificado, mas nada pode ser modificado até que seja enfrentado*."

Albert Einstein

RESUMO

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Os materiais de preenchimento dermatológicos tem sido amplamente utilizados na região da face, com finalidade de reparo estético ou funcional. O uso inadvertido desses produtos pode promover efeitos adversos variados envolvendo, muitas vezes, a área anatômica de atuação do cirurgião-dentista. Ocasionalmente as complicações presentes sugerem um diagnóstico clínico incorreto, uma vez que podem simular outras patologias. Estes produtos são distintos e apresentam-se de formas variadas, podendo ter na sua composição, por exemplo, fragmentos biológicos, suspensões de partículas ou microesferas. A técnica de implantação tecidual e os resultados possíveis são específicos para cada tipo de material. Quando inseridos nos planos tissulares ou de forma acidental, no interior dos vasos sanguíneos, podem favorecer respectivamente a migração local e/ou sistêmica da substância. O polimetilmetacrilato (PMMA) é um dos materiais de preenchimento estético de maior emprego no Brasil, sendo francamente utilizado na região facial e perioral. O seu baixo custo, duração permanente nos tecidos, além da fácil técnica de aplicação, favorecem esta escolha. O objetivo desta pesquisa foi verificar em modelo murino a presença de reação local e toxicidade sistêmica, causadas pela injeção intravascular de PMMA em duas concentrações extremas regularmente utilizadas nos pacientes (2 e 30%). Os animais foram divididos randomicamente em 3 grupos contendo 10 animais cada (PMMA 2%, PMMA 30% e controle) e 2 tempos de observação (7 e 90 dias). O material (0,05mL) foi aplicado na veia ranina do lado direito, localizada no ventre lingual. Durante a avaliação clínica realizada nos respectivos tempos do estudo, não foram observadas lesões na área da língua onde o produto foi aplicado. Na análise microscópica dos órgãos distantes (fígado, pulmão e rim direito) não foi constatada a presença de microesferas do produto ou inflamação. Os resultados da análise sérica dos níveis de aspartato aminotransferase (AST) e creatinina entre os grupos não

demonstraram diferença estatisticamente significativa nos tempos do estudo. Aos 90 dias do experimento, o grupo 2 (PMMA 30%) apresentou níveis mais elevados de alanina aminotransferase (ALT) quando comparado aos demais grupos. Podemos constatar que na sua maior concentração, o PMMA induziu alteração estatisticamente significativa (P = 0.047) nos níveis de ALT quando comparado com o grupo controle ao longo do tempo. A análise da variação do peso dos órgãos não demonstrou que as mudanças verificadas estavam relacionadas a sinais de toxicidade oriundos do PMMA. Frente aos inúmeros relatos de efeitos adversos decorrentes de injeções de PMMA no terço inferior da face, principalmente em região nasolabial, é de suma importância que os cirurgiões-dentistas estejam aptos a identificar e manejar suas possíveis complicações. A deficiência de informações científicas sobre eventuais manifestações locais e sistêmicas relacionadas a utilização de produtos de preenchimento estético facial contendo PMMA motivou a realização desta pesquisa e deverá estimular outros estudos favorecendo uma melhor compreensão sobre o tema. Os resultados obtidos neste estudo não confirmam que os preenchedores faciais contendo PMMA possuam potencial efeito tóxico, nas concentrações utilizadas.

Palavras-chave: estomatologia; polimetilmetacrilato; efeitos adversos; toxicidade; ratos.

ABSTRACT

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Dermal fillers are being vastly applied at the face region in an attempt to achieve aesthetic or functional repair. When used inadvertently those products may promote a variety of adverse effects, sometimes involving the dentists' anatomical area of expertise. Occasionally these complications suggest an incorrect clinical diagnosis since it can simulate other types of pathologies. These materials are distinct and may contain in its composition biological fragments, suspensions of particles or microspheres, for example. The technique of implantation and the possible outcomes are specific of the type of substance. Once these products are inserted into the tissues or accidentally inside of a blood vessel, local and/or systemic migration of the particles may occur. The polymethylmethacrylate (PMMA) is one of aesthetic filling materials of greater employment in Brazil, being broadly used in tissues of the facial and perioral region. The choice to use these products is encouraged by its low financial cost, long time of duration and simple technique of implantation. This research aimed to evaluate, in a murine model, the local reaction and possible systemic toxicity caused by intravascular injection of PMMA in two extreme concentrations (2 and 30%) that are often applied. The animals were randomly divided into 3 groups (2%PMMA, 30%PMMA and control) and 2 observation times (7 and 90 days). The material (0.05 mL) was applied into the ranine vein located at the ventral surface of the tongue on the right side. Lesions were not observed during clinical evaluation of the tongues at any time of the study. There was no presence of microspheres or inflammation in distant organs (liver, right lung and kidney) during microscopic evaluation. The levels of aspartate aminotransferase (AST) and creatinine were not significantly different between groups at any time of the study. Group 2 (30% PMMA) showed higher levels of alanine aminotransferase (ALT) at day 90 when compared to the other groups. It could be assumed that PMMA's higher concentration induced statistically

significant change in ALT levels compared with the control group over time (P = 0.047). The variation in the weight of the organs has not demonstrated that the changes were related to signs of toxicity to PMMA. Faced with several reports of adverse effects from injections of PMMA in the lower face, especially nasolabial region it is of great importance that dentists are able to identify and manage possible complications. The lack of scientific data regarding local and systemic adverse effects related to cosmetic fillers containing PMMA, motivated this study and should be a stimulus for this issue to be addressed in further research in order to achieve a better comprehension about this subject. The data obtained in this study do not confirm that PMMA fillers have a potential toxic effect, after an intravascular injection, at the concentrations studied.

Keywords: oral medicine, polymethylmethacrylate, adverse effects, toxicity; rats.

SUMÁRIO

INTRODUÇÃO

A humanidade busca constantemente atingir padrões de beleza idealizados. A manutenção da juventude é um objetivo almejado de forma incessante por muitos indivíduos. Apesar de ser uma tendência mundial, o culto à beleza torna-se ainda mais evidente no Brasil, onde a cada dia cresce o interesse da população em manter uma aparência mais jovem.

O envelhecimento é um processo biológico que ocorre inexoravelmente com o passar do tempo, combinando fatores intrínsecos ou geneticamente determinados e extrínsecos, tais como exposição solar, tabagismo, dieta e estilo de vida. As características desse fenômeno estão bem descritas na literatura e incluem perda da elasticidade da pele, despigmentação cutânea, presença de linhas de expressão e rugas. Existem inúmeras opções corretivas disponíveis no mercado, que vão desde os tratamentos cirúrgicos até a aplicação de materiais de preenchimento facial, os quais prometem amenizar ou até mesmo reverter algumas destas mudanças (DE MAIO, 2004; DE MAIO, RZANY, 2007; DASTOOR, 2007; COX, 2009). Muitos dos defeitos dérmicos associados à pele envelhecida aparentemente são causados pela perda de colágeno e por sua menor produção (BAUMANN *et al*., 2006; BRANDT, CAZZANIGA, 2007; ATIYEH, DIBO, 2009). Esta será gradativamente reduzida tanto *in vitro* quanto *in vivo*, em virtude do envelhecimento dos fibroblastos (BAUMANN *et al*., 2006; ATIYEH, DIBO, 2009). Ocorre cerca de 20% de diminuição da espessura dérmica sendo a mesma também observada extrinsecamente, revelando quadro de atrofia epidérmica com feixes de colágeno mais espessos, fragmentados e irregulares (BAUMANN *et al*., 2006; BRANDT, CAZZANIGA, 2007; ATIYEH, DIBO, 2009).

As técnicas cirúrgicas invasivas foram, por muito tempo, as únicas opções disponíveis para minimizar as rugas características do envelhecimento facial, bem como, para realizar correções de defeitos estéticos (DE MAIO, 2004; ATIYEH, DIBO, 2009; DE LORENZI *et al.*,

2009). Desde a década de 50, tenta-se buscar uma alternativa aos procedimentos cirúrgicos invasivos. Estes necessitam ser realizados sob anestesia geral, além de possuírem um custo elevado e lenta recuperação pós-operatória. No mesmo período, o silicone líquido foi introduzido por dermatologistas visando o aumento de volume em tecidos moles e o preenchimento de defeitos cutâneos. Entretanto, surgiram inúmeros problemas relacionados à sua permanência nos tecidos, tais como a formação de granulomas e migração do material.

Nos anos 80, foram criadas técnicas operatórias apropriadas para utilizar materiais contendo colágeno bovino como por exemplo, o Zyderm Collagen™ que era o material de preenchimento cutâneo aprovado pelo *Food and Drug Administration* (FDA) preferido nos Estados Unidos da América (EUA) (COLEMAN *et al.*, 2000; BOWLER, 2009). Por apresentar uma taxa de reação alérgica de 3%, seu uso foi sendo substituído com o passar do tempo pelo colágeno humano injetável, o qual não possui potencial alergênico (COLEMAN *et al.*, 2000; DE MAIO, 2004; BOWLER, 2009).

A procura por métodos de fácil execução e indolores, buscando uma aparência jovial e saudável, sem as intercorrências inerentes a uma cirurgia cosmética invasiva, continua sendo um estímulo para o desenvolvimento de novas técnicas e substâncias de preenchimento facial (DE MAIO, 2004; ATIYEH , DIBO, 2009). A bioplastia é descrita como um método não-cirúrgico, que não exige o afastamento dos pacientes de suas atividades habituais e acaba sendo o tratamento escolhido por muitos (DE MAIO, 2004).

As substâncias de preenchimento facial possuem técnicas de aplicação, tempos de permanência nos tecidos e efeitos adversos inerentes à sua composição (DASTOOR, 2007; DE MAIO, RZANY, 2007; COX, 2009). Tais materiais podem ser fluidos ou compostos por fragmentos biológicos, suspensões de partículas ou microesferas (LEMPERLE *et al.*, 2004; ROSA; DE MACEDO; CHRISTENSEN *et al.*, 2005). A técnica de implantação e os resultados

obtidos são específicos para cada tipo de substância, podendo ocorrer migração sistêmica de partículas ou microesferas quando estes produtos são inseridos dentro de vasos sanguíneos (LEMPERLE *et al.,* 2003; LEMPERLE *et al.,* 2004).

Os materiais para preenchimento dermatológico podem ser classificados de acordo com o seu tempo de permanência nos tecidos como biodegradáveis, não-biodegradáveis ou permanentes e ainda como uma associação entre ambos (LEMPERLE *et al.,* 2003; WIEST *et al.,* 2009). Entre os produtos biodegradáveis descritos estão o colágeno de origem bovina, o colágeno de origem humana, o ácido hialurônico, o ácido polilático, a hidroxiapatita de cálcio e o álcool polivinílico (DE MAIO; RZANY, 2007). O silicone, o qual teve seu uso proibido pela FDA em 1991, faz parte do grupo de materiais de preenchimento permanentes, em conjunto com a poliacrilamida e polialquilamida (ERSEK, 1997). As associações dos 2 tipos de substâncias disponíveis para comercialização são o hidroximetilmetacrilato com ácido hialurônico, o polimetilmetacrilato (PMMA) em gel de colágeno bovino (Artefill®), o PMMA em gel de carboximetilcelulose (Metacrill®; Biossimetric®) e o PMMA em gel de hidroxietilcelulose (Newplastic®).

Dentre os materiais em uso, o PMMA é um produto amplamente utilizado em virtude do seu baixo custo financeiro, fácil acesso e técnica de aplicação simples (MIGUEL *et al.,* 2009; VARGAS *et al.,* 2011). Foi sintetizado inicialmente pelo químico alemão O. Rohm e foi patenteado sob o nome Plexiglas em 1928. Atualmente é empregado nos mais diversos dispositivos, tais como próteses dentárias, cimentos ósseos e lentes intraoculares (CALDELLAS *et al*., 2010). Em 2006, o FDA aprovou nos EUA um único produto utilizado em técnicas de preenchimento facial permanente contendo microesferas de PMMA suspensas em um gel de colágeno bovino (LEMPERLE *et al.,* 2003). O material denominado Artefill® foi liberado exclusivamente para correção de rugas na região nasolabial. As micropartículas permanecem no tecido após o gel carreador ser reabsorvido, induzindo uma reação de corpo estranho intencional,

resultando em fibrose dos tecidos (WIEST *et al.,* 2009). A substância deve ser injetada no terço inferior da derme, com uma agulha de 26 a 27 G, através da técnica de tunelização. Uma massagem cuidadosa deverá ser realizada na área imediatamente após a aplicação, para que haja uma melhor distribuição do material. Em alguns casos, indica-se uma segunda aplicação após o período de 3 meses (LEMPERLE *et al.*, 2003; DADZIE *et al.,* 2008).

A taxa de sucesso do preenchimento facial com materiais que contenham PMMA depende de uma minuciosa técnica de aplicação. Portanto falhas podem ocorrer, especialmente no início da curva de aprendizado do profissional (LEMPERLE *et al.,* 2003; LEMPERLE *et al.,* 2010). Em 2003, Lemperle *et al.* avaliaram a biocompatibilidade e o transporte de 5 tipos de microesferas não reabsorvíveis de PMMA de vários tamanhos, suspensas em diferentes veículos, injetados em ratos. Os locais de implantação foram bochecha, axila, virilha, uretra e músculo quadríceps da coxa. Essas estruturas foram extraídas juntamente com os linfonodos locais, pulmões, fígado e baço aos 1º, 3º, 6º e 9º mês após a injeção. No estudo foram observados especificamente 3 aspectos: resposta do sítio de implantação, fagocitose e migração. Os órgãos que não receberam implante (linfonodos locais, pulmões, baço e fígado) foram avaliados para presença de sinais de migração das microesferas. Partículas de PMMA com 4.3 µm e 20 µm de diâmetro foram detectadas no pulmão de 2 ratos após 6 meses da implantação, enquanto que as de tamanho igual ou maior que 40 µm não estavam presentem em nenhum dos órgão avaliados. Um aglomerado de centenas de esferas foi encontrado em uma artéria pulmonar maior de outro animal. Devido à ausência de partículas nos linfonodos e a presença no pulmão, é provável que a mesma tenha sido transportada hematologicamente e depois fagocitada. Segundo alguns autores, houve uma redução do número de microesferas de PMMA com tamanho menor que 20 µm para menos de 1% do total de partículas do produto e por tal motivo acredita-se que não tenham sido observadas novas complicações (DADZIE *et al.,* 2008). Após um período de 20 anos, a maioria dos efeitos

colaterais do Artefill® foram eliminados, buscando atingir os mesmos padrões do ácido hialurônico (AH). Este é um polissacarídeo presente na matriz extracelular, de ocorrência natural, altamente hidrofílico. Os produtos disponíveis para serem empregados nos procedimentos de aumento tecidual, contêm AH de origem aviária ou bacteriana (LEMPERLE *et al.,* 2003; BRANDT *et al.,* 2007; LEMPERLE *et al.,* 2010, BORGHETTI *et al.,* 2011).

Para a maioria dos autores (LEMPERLE *et al.,* 2003; CHRISTENSEN *et al.,* 2005; ZIELKE *et al.,* 2008; BACHMANN *et al.,* 2009; PARK *et al*., 2012) as substâncias de preenchimento facial são consideradas seguras para o uso em humanos. Entretanto, os mesmos admitem que significativas reações adversas podem ocorrer. Nas primeiras 72 horas após a injeção, eritema, edema, equimose, endurecimento e prurido são achados comuns. Reações tardias podem ocorrer, tais como eritema persistente, edema, prurido, descoloração, endurecimento, formação de nódulos, reação granulomatosa de corpo estranho persistente, ulceração e risco de complicação vascular com necrose associada à injeção. Algumas complicações frequentemente associadas ao uso do PMMA são a fagocitose das microesferas e as reações adversas locais com formação de granulomas (ERSEK, 1997; LEMPERLE *et al.,* 2003; CHRISTENSEN *et al.,* 2005; ANWAR, SHARPE, 2007 DE CASTRO *et al*., 2007; DADZIE *et al.,* 2008; ZIELKE *et al.,* 2008; BACHMANN *et al.,* 2009; WIEST *et al.,* 2009; LEMPERLE *et al.,* 2010). Os produtos permanentes devem ser cuidadosamente monitorados, em virtude da dificuldade e/ou impossibilidade de tratar seus possíveis efeitos adversos (ZIELKE *et al.,* 2008; LEMPERLE *et al.,* 2010).

No estudo realizado por Zielke *et al*. (2008), 6 pacientes receberam injeções de PMMA em 21 locais, com maior prevalência no vermelhão do lábio. O efeito adverso mais frequente foi o aparecimento de nódulos na área de aplicação do material (66.7%), eritema (33.3%) e edema

(19.9%). O maior intervalo observado entre a injeção da substância e o aparecimento de reação foi de 6.5 anos.

Foram relatados na literatura casos de cegueira após a infiltração de PMMA na região glabelar, suspeitando-se que as microesferas tenham sido injetadas muito próximas a ramos da artéria oftálmica (SILVA & CURI, 2004; BACHMANN *et al.*, 2009). Outro caso envolvendo complicação vascular após a implantação do material resultou na embolia de um vaso sanguíneo e consequente necrose na hemiface da paciente, após 24 horas da aplicação. Acredita-se que a injeção tenha sido realizada no sulco nasogeniano ou diretamente na artéria facial ou ainda em região muito próxima a ela (DE CASTRO *et al*., 2007).

No estudo realizado por Bachmann *et al.* (2009), foram registrados 139 pacientes com algum tipo de reação adversa causada por substâncias de preenchimento facial. Quarenta e oito mulheres receberam aplicações destes produtos na região glabelar sendo que 40 delas reportaram reações adversas após o preenchimento. Foram documentados inúmeros sintomas nestas pacientes, sendo mais comum o endurecimento ou nódulos teciduais, eritema e edema. Até o momento, as causas de necrose na região glabelar não são totalmente compreendidas. Consideram-se hipoteticamente, injeções intra arteriais e/ou compressão de diversos pequenos vasos sanguíneos. As complicações vasculares são raras, porém importantes, uma vez que as mesmas podem acarretar sequelas irreversíveis. Para evitar ou minimizar essas complicações, potenciais reações vasculares devem ser imediatamente identificadas e tratadas em curto espaço de tempo (BACHMANN *et al.,* 2009).

Reações de toxicidade podem ser desencadeadas por inúmeros compostos químicos, havendo diferentes formas para que tais eventos sejam avaliados (BORZELLECA, 2000). As mais comumente observadas são a hipersensibilidade, discrasias sanguíneas, hepatotoxicidade, nefrotoxicidade, efeitos teratogênicos e distúrbios gastrointestinais (SHORT, CUMMING, 1999;

BAILLIE, RETTIE, 2011). Devido ao seu importante papel no metabolismo e excreção de drogas e xenobióticos, o fígado é considerado um alvo frequente de manifestações tóxicas destes agentes (STROM *et al.,* 2010; BAILLIE, RETTIE, 2011). A AST ou TGO (transaminase glutâmico oxalacética) está presente em outros órgãos além do fígado, enquanto a ALT ou TGP (transaminase glutâmico pirúvica) é encontrada basicamente neste órgão. A dosagem dos níveis séricos dessas enzimas é utilizada como um indicador de dano hepatocelular (MARINHO *et al.,* 2006).

A creatinina é um produto metabólico formado pela descarboxilação da creatinina-fosfato no músculo e a elevação na sua concentração plasmática é um dos critérios diagnósticos para insuficiêcia renal aguda. Os níveis séricos devem ser dosados para identificar possíveis efeitos tóxicos e são influenciados pelos seguintes fatores: farmacocinética da droga, tolerância à dose, interação medicamentosa, variações fisiológicas, patológicas e genéticas (SHORT, CUMMING, 1999; MARINHO *et al.,* 2006).

Em experimentos toxicológicos, a comparação entre o peso dos órgãos do grupo tratado e controle é utilizada convencionalmente para avaliar o efeito tóxico do produto testado (PETERS, BOYD, 1966; HALEY *et al.*, 2005; MICHAEL *et al.,* 2007). Os dados obtidos com este método devem ser interpretados de forma integrada com achados clínicos e histopatológicos. Mudanças no peso dos órgãos sem correlação macro ou microscópica devem ser interpretadas com devida cautela (TILNEY, 1971; KANERVA *et al.,* 1983; BAILEY *et al.,* 2004; SELLERS *et al.,* 2007).

Segundo alguns autores a pesagem dos linfonodos não é recomendada, pois as medidas variam muito entre os animais, além da dificuldade em isolá-los da gordura adjacente (PETERS, BOYD, 1966; HALEY *et al*., 2005; MICHAEL *et al*., 2007). O exame histopatológico dos linfonodos mais próximos ao local da aplicação é indicado em casos de injeção cutânea,

subcutânea ou intradérmica de xenobióticos. Nestes casos, os padrões de drenagem linfática do rato sempre devem ser levados em consideração (PETERS, BOYD, 1966; TILNEY, 1971).

Os efeitos adversos que podem advir da implantação de PMMA, são passíveis de serem confundidos com lesões estomatológicas de outra natureza, como cistos e neoplasias, sendo fundamental o estabelecimento do diagnóstico diferencial das mesmas através de recursos imaginológicos como a ultrassonografia e tomografia computadorizada, bem como a realização de exame anátomo-patológico (VARGAS *et al.*, 2011). A utilização inadvertida dos materiais de preenchimento facial, bem como a deficiência de estudos científicos sobre possíveis reações de toxicidade sistêmica decorrentes do seu uso, motivaram a realização desta pesquisa.

ARTIGO CIENTÍFICO I

O artigo de revisão de literatura a seguir, intitula-se ***"Complications after polymethylmethacrylate (PMMA) injections in the face: a literature review"*** e foi formatado e submetido de acordo com as normas do periódico ***Gerodontology*** (Anexo 1), o qual apresenta Qualis B2.

Complications after polymethylmethacrylate (PMMA) injections in the face: a literature review

Running title: Complications after PMMA injections in the face

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Conflict of Interest

The authors declare that they have no potential conflict of interest.

Complications after polymethylmethacrylate (PMMA) injections in the face: a literature review

Abstract

Objective: This article is a review of the several types of complications due to facial fillers containing polymethylmethacrylate (PMMA).

Background: PMMA facial fillers are used to soften the results of the aging process and to augment tissue. Although considered safe for the most part by advocates, they have been associated with many adverse reactions such as ecchymosis, hematomas, swelling, itching, erythema, hypertrophic scarring, hypersensitivity, palpable nodules, tissue necrosis, blindness and foreign body granuloma.

Materials and methods: The articles presented in this review are the result of a search and selection of literature from the National Center for Biotechnology Information (NCBI) database, which met the inclusion criteria for the study.

Conclusion: PMMA is widely used because it is inexpensive, readily accessible and simple to apply. However, some complications are severe and permanent and can be confused with other types of stomatological lesions.

Keywords: oral medicine, oral pathology**,** facial fillers, polymethylmethacrylate, adverse effects, foreign-body reaction.

Introduction

This review highlights the clinical complications caused by polymethylmethacrylate (PMMA) facial fillers and provides information about major aspects of the product itself, encouraging dentists to develop a critical point of view by understanding the mechanisms involved in some of the complications and their treatment. PMMA facial fillers have been developed for medical use in order to perform tissue augmentation for aesthetic purposes. Each type of dermal filler has an appropriate technique of implantation, an average duration of its effects, and its own distinct set of possible adverse reactions after injection[1]. Facial aging results from genetic structure, and many extrinsic life-style factors such as exposure to the sun, nicotine and diet all of which may cause loss of dermal elasticity, wrinkles and depigmentation. Facial fillers are used to soften or reverse the effects of age[2-5]. They may be injected into lips, marionette lines, nasolabial folds and neighboring structures,[3, 5-7] and the success of treatment depends on the accuracy of implantation[6-8].

Many dermal defects that are related to the aging process seems to occur due to the loss of collagen and to a decrease of its production[9-11]. Liquid silicon was developed in the 1950's by dermatologists in order to augment soft tissue and correct other dermal imperfections. However silicon was associated with granuloma formation and migration of the filler. During the 1980's, bovine collagen (Zyderm Collagen™) was approved by the Food and Drug Administration (FDA) in the USA for similar use, but became less popular among users due to its high allergy rate [12-14].

New dermal fillers were developed in an attempt to achieve better and longer lasting results such as human collagen, hyaluronic acid, poly-L-lactic acid, autologous fat implant and PMMA[1, 10, 14]. Dermal fillers can be presented as fluids, biological fragments, or suspensions of particles or microspheres[15-17], each with a specific implantation method and possible

complications. Migration of particles or microspheres, for example, can occur if the material is injected into a blood vessel[15, 18] or if it is phagocytized by macrophages or giant cells[17].

Dermal fillers are classified as temporary or biodegradable; permanent or non-biodegradable; and a combination of both materials[18, 19]. Complications associated with temporary fillers usually occur immediately after injection, and are easily treated or they resolve spontaneously. Complications with permanent fillers may occur years after implantation, and typically need more complex treatments [20, 21]. Collagen remains incorporated with the particles in an injected suspension for 1 to 3 months until its replaced with the patient's autologous collagen [21, 22]. Since the microspheres remain in the tissue after the carrier gel has been absorbed inducing an intentional foreign body reaction that results in tissue fibrosis, the composition, microspheric diameter and carrier medium also influence complications[19, 23].

The first generation PMMA filler developed in the early 1990s produced foreign body granulomas associated with its small particle size (<20μm) [22]. Further developments produced fillers that contain larger PMMA microspheres (30-50μm) to avoid phagocytosis [6, 22]. In 2006, the FDA approved for the first time one permanent facial filler that contains PMMA microspheres suspended in bovine collagen, which should be applied strictly for nasolabial folds[7, 23]. It is injected on one or two occasions by tunnelling a 26 or 27 gauge needle into the lower third of the dermis and carefully massaged to distribute the material [15, 22,24].

Facial fillers can cause adverse reactions such as erythema, swelling, itching, bruising and induration that are very common in the first 72 hours after implantation while late onset complications that are likely to arise include persistent erythema, itching, depigmentation, induration, lumpiness, persistent granulomatous foreign body reaction, ulceration, vascular complication and tissue necrosis [6, 14, 17, 25, 26]. Microspheres phagocytosis and local adverse reactions with granulomatous formation are often related to PMMA injections [6, 7, 24 - 29].

In the study by Zielke *et al.*[25] 6 patients received PMMA injections at 21 sites with the highest prevalence in the lip vermilion. The most frequent adverse effects were lumpiness, erythema and swelling. The largest interval between the substance injection and the observed reaction was 6.5 years. The delayed reaction of several years can complicate diagnosis of the problem, especially if patients forget to report the implantation from several years ago.

Immediate complications

Ecchymosis and hematomas can occur if the patient ingests alcohol, anti-platelet-aggregating drugs[14], ticlopidine, vitamin E or nonsteroidal anti-inflammatory drugs days before or after implantation. Such complications are usually spontaneously resolved after a period of 2 to 7 days [30-33]. For about one week after implantation, most fillers will also cause some swelling [30, 34-37], which can be reduced by using ice and compression after implantation [31, 32, 35 - 37].

Itching and erythema are often treated with topical or intradermal applications of corticosteroids, while hypertrophic scarring has been treated with triamcinolone injections and pulsed dye laser [30]. Persistent erythema is associated with several different facial fillers after tissue augmentation[31, 32] and can be prevented by avoiding alcohol consumption, sun exposure and exercise during the first days after the procedure. A protocol to treat severe erythema caused by PMMA fillers is not yet established. In the literature there are reports of using corticosteroids by oral, intralesional or topical administration [6, 38].

Most undesirable events relate to poor surgical technique rather than the composition of the filler, so professionals who are not well trained and qualified should not perform tissue augmentation[22, 30, 39]. Palpable nodules can occur due to an asymmetric or irregular placement of

the filler by agglomeration of the microspheres and also due to superficial implantation [14, 32], and should be removed surgically rather than treated with intralesional injection of corticosteroids, using micro electric dissection, punch excision and dermabrasion as suggested by others[8, 40], since they are not related to granuloma formation [31].

All facial filler, except for autologous fat implants, can induce an allergic reaction, possibly as angioedema or anaphylaxis [32, 39]. The severity of the reaction determines the treatments, which are usually systemic or intralesional corticosteroids and topical tacrolimus[20]. Although rare, hypersensitivity reactions to bovine collagen and PMMA can present as late granulomas [6, 30].

Tissue necrosis is a rare but relevant complication that can happen immediately after an intravascular injection of the filler or due to excessive compression of the blood vessels situated nearby the application site [2, 21, 30, 32]. There is a higher risk of intravascular injection that can be followed by ischemia and tissue necrosis when the filler is injected around the lip vermilion and labial mucosa [2]. In the literature there are 5 case reports on serious tissue necrosis published between 2007 and 2010, one describes an extensive necrosis of the hemiface that occurred after 24 hours from an implantation in the nasolabial fold that presumably damaged the right facial artery [28], while the other 4 cases involved necrosis of the nose and lips after injection in the nasolabial folds[21, 41]. There are also alarming cases of blindness after PMMA injection in the glabellar region that were caused by an embolism in the ophthalmic artery[42, 43]. The causes of tissue necrosis and blindness are not yet fully understood. Currently, intravascular injections and/or compression of many small vessels are hypothesized to be the reason for these complications [2, 26, 30, 32]. Considering the severity of these events, the sale of PMMA fillers should be permitted only for trained professionals. The sequelae of a vascular complication can be

irreversible and should be identified and treated promptly [21, 26].

Late onset complications

It is known that all facial fillers induce at least a mild foreign body reaction but PMMA microspheres although being inert, can produce a granuloma, typically about 6 to 24 months after the PMMA filler implantation[30-32]. The histological appearance of the granuloma is specific for each type of filler and is a challenge even for experienced pathologists. It can be presented as microspheres that are distant from each other and surrounded by macrophages, giant cells, fibroblasts and extensive collagen fibers [30, 44]. This event is affected by chemical and physical properties of the product, volume of the injection, and previous infection or trauma [31, 32]. The indicated treatment for this complication can include intralesional corticosteroid injections in a concentration and interval of time to be determined by the severity of the lesion [8, 20, 45-47]. There are 19 cases of granulomatous reaction due to PMMA fillers reported in the literature from 2003 to 2007 showing different levels of severity and even risk of skin extrusion [18, 21, 22, 38, 42, 44, 46, 48-56]. The patients were mostly women and the time interval between implantation and onset of symptoms ranged from 2 months [46, 49] to 6 years [51]. It is suspected that individuals produce antibodies against the binding proteins of PMMA and that the sudden appearance of the inflammatory reaction is linked to the memory of macrophages that fuse to giant cells in an attempt to phagocytize the larger particles and is triggered by factors such as systemic infection, surgical trauma and pregnancy [21, 22, 57–62].

The migration of the implanted material is another important complication that may be observed years after the PMMA implantation. The cases tend to be difficult to solve because the particles are encapsulated by collagen fibers and can be presented as nodules or indurations that will require surgical removal [31, 37, 60, 63].

Infections are rarely associated with facial fillers and appear as single or multiple erythematous nodules [2]. Many of the complications considered serious can be linked to infectious reactions diagnosed as foreign body granulomas [64]. The presence of biofilms has been also suggested as a possible cause of late foreign body granulomas[17]. As the use of permanent fillers increases so does the frequency of related complications [32]. Since facial fillers can also trigger recurrent herpetic lesions, antiviral prophylaxis is recommended in case of lip augmentation and the procedure is contraindicated in the presence of active lesions [30, 65].

Conclusion

Bioplasty is described as a non-surgical method that does not require patients to interrupt their usual activities making the treatment very attractive to many seeking beauty and the maintenance of youth [1, 66]. Among the materials used, PMMA is a popular product due to its low cost, easy access and simple application technique [16, 47].

Complications arising from the use of PMMA are considered rare by some authors but are usually also severe and permanent and can prove impossible to treat succesfully[21]. In order to prevent and minimize such events the professional must master the application technique and the knowledge of the chosen product besides warning the patient about the risks involved[14].

Faced with several reports of adverse effects resulting from injections of PMMA in the lower face [26, 28], especially in the nasolabial region, it is a major concern to dentists as health professionals to know and identify such complications. These reactions can be confused with other types of stomatological lesions as cysts and tumors and therefore it is essential to establish protocols for proper diagnosis.

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ARTIGO CIENTÍFICO II

O artigo de pesquisa a seguir, intitula-se ***"Polymethylmethacrylate Dermal Fillers: Evaluation of Systemic Toxicity in Rats"*** e foi formatado e submetido de acordo com as normas do periódico ***International Journal of Oral and Maxillofacial Surgery*** (Anexo 3), o qual apresenta Qualis A1.

Polymethylmethacrylate Dermal Fillers: Evaluation of Systemic Toxicity in Rats

Running title: PMMA: Evaluation of Systemic Toxicity

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Polymethylmethacrylate Dermal Fillers: Evaluation of Systemic Toxicity in Rats

ABSTRACT

Objective: This study evaluated local and systemic reaction after an intravascular injection of PMMA in 2 concentrations using a murine model.

Study Design: 30 rats were equally divided into 3 groups (2%PMMA, 30%PMMA and control). The filler was injected at ranine vein. After sedation (7 and 90 days) clinical evaluation was performed. Serum was analyzed for liver and kidney function tests. Lung, liver and kidney were removed after euthanasia, weighed and microscopically analyzed. The submandibular lymph nodes and tongue were removed and examined microscopically.

Results: All groups did not show any clinical alteration. Microspheres were not observed at any distant organs. At day 90, the group injected with 30% PMMA presented higher levels of ALT (P= .047) when compared with the other groups.

Conclusion: The data obtained in this study do not confirm that PMMA fillers have a potential toxic effect, after an intravascular injection, at the concentrations tested and at the times of the study.

INTRODUCTION

The use of facial fillers is one of the options to minimize the signs of facial aging and to correct skin imperfections. They are being increasingly preferred over conventional plastic surgery due to their low financial cost and painless and noninvasive application [1-5].

Each type of soft-tissue filler has its own technique of implantation, period of permanence in tissues and adverse effects that are inherent to its composition [1,2]. Several chemical compounds can trigger toxic reactions, and there are different ways to evaluate the possible outcomes [6].

Facial fillers containing polymethylmethacrylate (PMMA) can be classified as non-biodegradable or permanent, since they consist of non-absorbable microspheres that are suspended in an aqueous carrier or bovine collagen [7]. Permanent fillers must be carefully monitored due to the severity of possible complications that can occur late and can present a difficult or impossible resolution [8-10].

The term "migration" is found in the literature as a definition of the displacement of PMMA microspheres in two different ways: when they are injected into blood vessels [11, 12] or when they are transported through phagocytosis [13].

PMMA has a specific implantation technique and must not be injected into blood vessels, to avoid particle migration and to achieve a satisfactory result. However, there are several published case reports of disastrous local complications due to injections being intravascular or very close to a blood vessel [14-17]. A study regarding systemic manifestations caused by an intravenous injection of PMMA fillers in humans or animals has not been previously published.

The aim of this study was to identify and analyze fast-occurring as well late systemic and local tissue reactions and the occurrence of particle migration to distant organs after an intravascular injection of PMMA at different concentrations using a murine model. It is necessary

to better understand the mechanism of a possible toxicity induced by this material to be able to improve the safety of this treatment. The ventral surface of the rat's tongue was selected as the site of injection owing to the fact that it is less vulnerable to traumatic factors[18] and also for being highly vascularized, comprising a plexus composed of a superficial vascular network and the ranine veins [19].

MATERIALS AND METHODS

This research was initiated after approval from the Scientific and Ethics Committee (protocol 0060/11) and then from the Ethics Committee for Animal Use (protocol 11/00261) of the Pontifical Catholic University of Rio Grande do Sul - PUCRS, Brazil, and the procedures were carried out in accordance with institutional guidelines for animal care and use.

The material used for this experiment was PMMA microspheres suspended in hydroxyethylcellulose gel (Newplastic®; Biomedical Comércio, Porto Alegre, Brazil). Two extreme concentrations (2 and 30%) were chosen to compare the possible systemic toxic effects, when the product is injected into a blood vessel. Since there is a high applicability of this material in the facial region, it is of great interest to oral surgeons to be aware of the possible adverse effects that follow an inadvertent intravascular injection. The materials were injected at the beginning of the experiment. Clinical, histological and serum evaluations were performed at 7 and 90 days.

Animal Model

The sample was composed of 30 female Wistar rats (*Rattus norvegicus)* aged 2 months weighing about 200g at the beginning of the experiment. They were obtained from the animal

facility of the State Foundation of Production and Health Research (FEPPS), Porto Alegre, Brazil. Animals were individually numbered on the tail and housed in plastic cages placed in ventilated racks (Alesco®, Monte Mor, SP, Brazil) at a temperature of 22°C with a 12-hour light-dark cycle. Animals were fed a standard diet of rat chow (Nuvilab®, Colombo, PR, Brazil) and given water *ad libitum*. The animals were randomly allocated to 3 groups, according to the treatment received, i.e., group 1 (n=10): 2% PMMA; group 2 (n=10): 30% PMMA; and group 3 (n=10): control, 0.9% NaCl, with each group equally divided into experimental periods of 7 and 90 days.

Anesthesia

Initially, each rat was weighed on a digital scale (Urano® model UDI 2500/0.5) to calculate the dose of anesthetic. This procedure was performed with an intraperitoneal injection of a mixture of 20 mg/mL xylazine hydrochloride (0.05 mL/100 g; Rompum®; Bayer, SP, Brazil), a sedative, analgesic and muscle relaxant, with 50 mg/mL ketamine hydrochloride (0.1 mL/100 g; Dopalen®; Agribrands do Brasil Ind. e Com., São Paulo, Brazil), an anesthetic for veterinary use. Animals from the respective groups, randomly chosen, were successively anesthetized [4,18].

Material Injection

When sedation was evident, the animal was placed on a surgical table in supine position and its paws tied with the use of elastic strips. The tongue was pulled out with tweezers to expose the ventral tongue region. Using a disposable insulin syringe (1/2 inch 26G; 13 X 0.45 mm), 0.05 mL filling material was injected in groups 1 and 2, into the right ranine vein, also called lingual vein, which is located lateral to the lingual frenulum [19]. The needle was inclined as parallel as possible to the mucosa, with the bevel facing up.

Clinical Evaluation

Before euthanasia, the animals were weighted and sedated in a way tongues could be clinically evaluated. Clinical examination was made to observe possible tissue alterations, such as swelling, nodules, ulceration, necrosis and/or suppuration.

Euthanasia

After injection of the material , animals were sacrificed and terminally bled by cardiac puncture at the respective monitoring periods.

Sample Processing

Immediately before euthanasia, the animals were anesthetized via isoflurane inhalation. After anesthesia, thoracotomy was performed and blood samples collected without hemolysis by cardiac puncture. Samples were centrifuged at 4ºC at 8000 rpm to separate serum for later analysis. The animals were necropsied and their right kidney, lung and liver were removed, weighed and microscopically analyzed. The right submandibular lymph nodes and the tongue were removed and examined microscopically. Sample fixation was carried out with the use of 10% neutral-buffered formalin for a minimum of 24 hours. Samples of the tongue, lymph nodes, right lung, right kidney and liver were sectioned longitudinally into 2 fragments. The inclusion was performed so that the edge of the sample had its long axis parallel to the paraffin block section. For each specimen, there was 1 histologic section of 6 µm per slide, which was stained with hematoxylin and eosin (HE).

Histological Evaluation

In slide analysis, the examiner was previously calibrated and blinded with the use of

masks in all evaluated slides. The analysis of histologic sections was conducted in the Pathology Unit, São Lucas Hospital, Pontifical Catholic University of Rio Grande do Sul, Brazil) using a biological microscope (Zeiss Axioskop 40, Carl Zeiss, Jena, Germany) coupled to a camera (Cool Snap-Pro cf, Media Cybernetics, Bethesda, USA) connected to a Dell® Optiplex GX620 computer, Round Rock, TX) at 100X, 200X and 400X magnifications. Images of the tongue samples were transferred to Image-Pro Plus1, version 4.5.1 (Media Cybernetics, Inc.; 2005). Some slides were analyzed with a fixed stage microscope for electrophysiological research (ECLIPSE FN1, Nikon, Tokyo, Japan) for phase contrast microscopy.

Inflammatory reaction of the tongue. The histological evaluation of the tongue was performed with an analysis of the whole slide to determine the field with the most significant inflammatory reaction. The presence or absence of lymphocytes, plasma cells, macrophages, giant cells, neutrophils, eosinophils, edema, and hyperemia were considered to determine the intensity of the reaction.

Migration. The microscopic evaluation was based on the presence or absence of microspheres in the liver, right kidney, lung and submandibular lymph node of each animal.

Histologic scoring of liver injury. Liver tissue was scored for histologic necrosis and inflammation, according to the modified activity index (HAI) grading [20, 21].

Histologic scoring of kidney and lung injury. Kidney and lung were evaluated based on the presence or absence of inflammatory reaction.

Serum Analysis

Serum was subjected to liver and kidney function tests. Aspartate aminotransferase

(AST) and alanine aminotransferase (ALT) activity in serum was quantitatively measured using VITROS DT60 Chemistry System (Ortho Clinical Diagnostics, Rochester, NY) by a kinetic reaction with multiple measuring points. Serum creatinine level was determined by a two-point kinetic method (Labtest Diagnostics, Lagoa Santa, MG - Brazil). Samples were processed by an automatic biochemical system (Vitros Fusion 5.1® - Ortho Clinical Diagnostics, Rochester, NY), using specific reagents for each test. The tests were performed in the clinical laboratory of São Lucas Hospital (PUCRS- Brazil).

Statistical Analysis

All data were tabulated and analyzed using the SPSS 17 software (SPSS Inc., USA), with a two-way analysis of variance (ANOVA) parametric test, complemented by the Bonferroni correction test, at a significance level of 5%.

RESULTS

Clinical alterations. No group showed any clinical alteration at 7 and 90 days.

Migration. Microspheres were not observed at any distant organs (lung, liver and kidney) or lymph nodes.

Inflammatory reaction of the tongue. Two samples from the 2% PMMA group showed moderate inflammation at 7 days, while another 2 samples from the 30% PMMA group also showed moderate inflammation at 90 days, with infiltrate of mononuclear cells and sparse neutrophils, eosinophils. Mastocytosis was observed during the histologic evaluation of several samples of the tongue, lymph nodes at all times of the study.

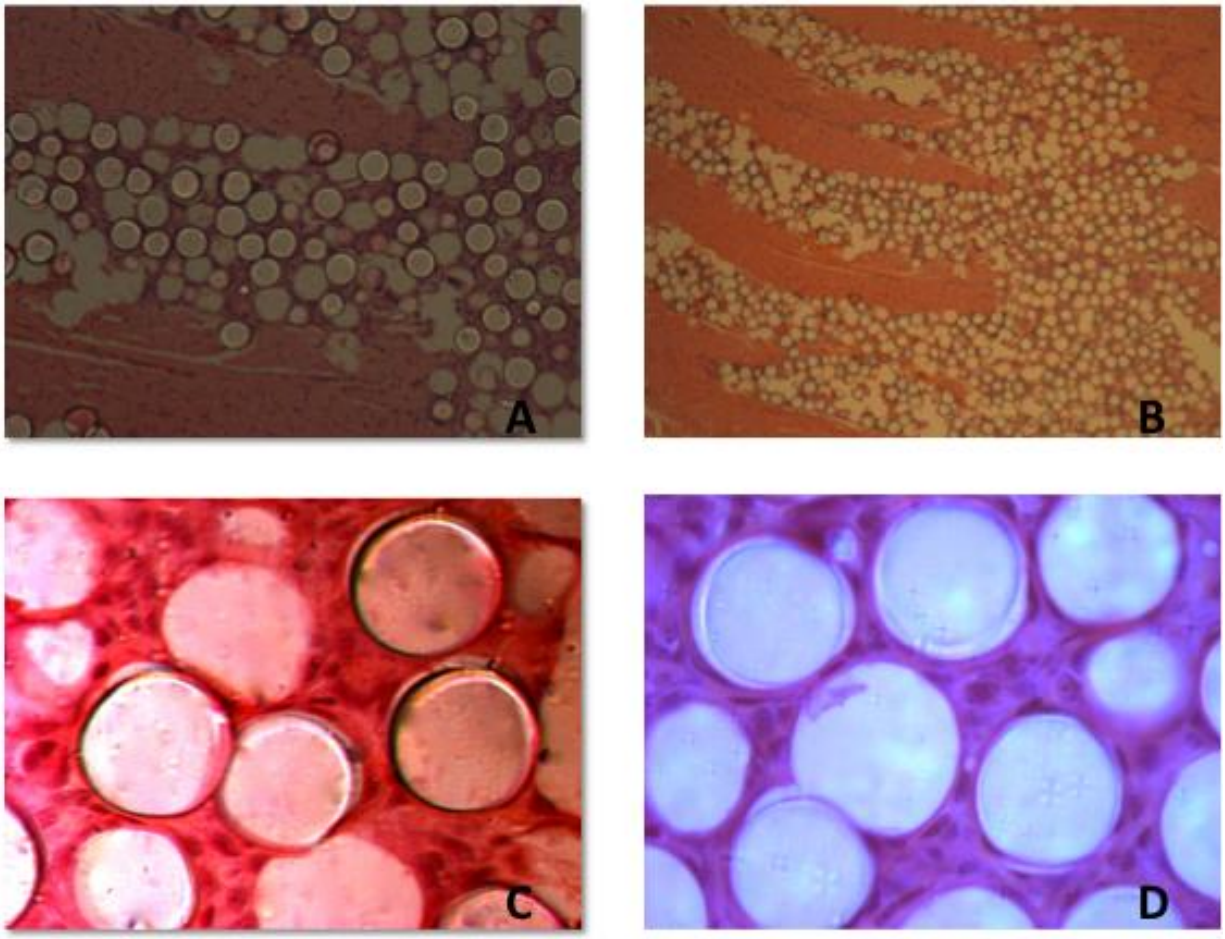

*Fig.1.*Phase contrast microscopy showing PMMA microspheres in the tongue in different manipulations and magnifications - HE (**A**) 200 X, (**B**) 100 X, (**C** and **D**) 400 X.

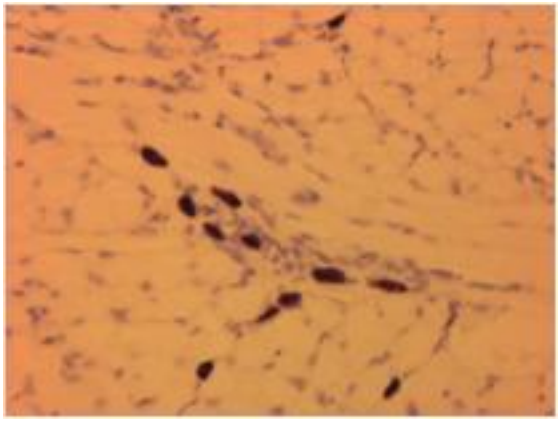

Fig.2. Mastocytosis was evident after staining with toluidine blue - 200 X.

Liver function tests. At 90 days, there was a significant difference in ALT levels (P = .047) between the group injected with 30% PMMA and the other 2 groups (2% PMMA and control) (*Fig. 3).* The levels of AST were not significantly different between groups at any time of the study.

Creatinine. At 90 days, all groups showed a significant increase in creatinine levels (P = .001), but there was no difference between the PMMA groups and the control (*Fig. 3).*

Organ weight evaluation. Changes in liver weight did not differ significantly between groups at any observation time. Lung weight was statistically different between the PMMA groups (P = .047) only at 7 days, while kidney weight increase was associated with the animals body weight gain during the study period (*Fig. 4).*

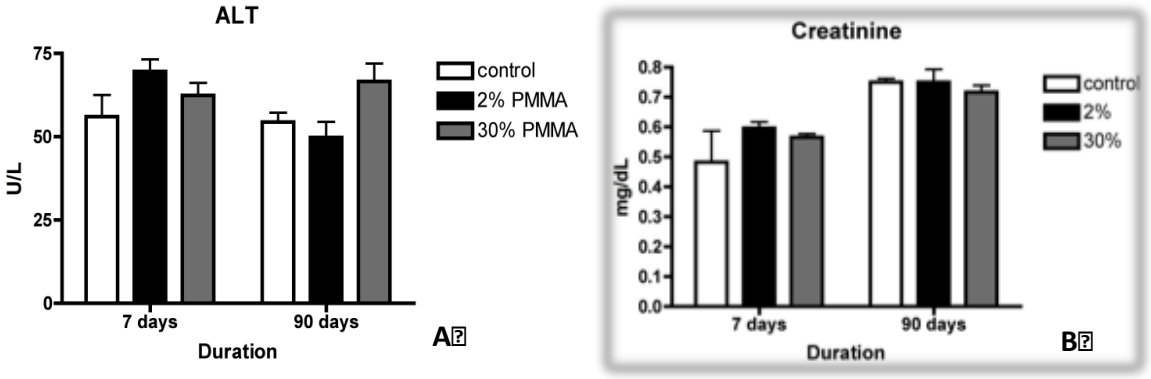

Fig. 3. Serum levels of ALT and creatinine: (**A**) shows a difference between the PMMA groups and control over time, while (**B**) demonstrates that differences were only due to time.

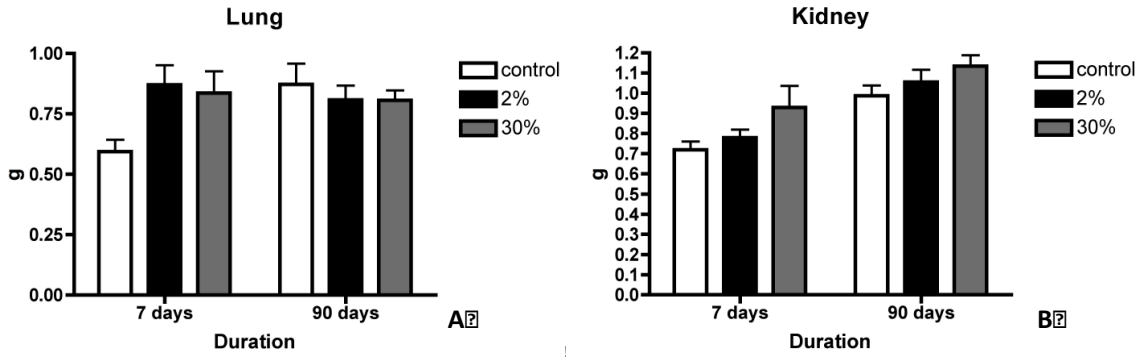

Fig. 4. Lung weight of the samples of the 2 and 30% PMMA groups was statistically higher (P = .048) compared to the control at 7 days (**A**). There was a proportional increase in kidney weight in all groups at 90 days (**B**).

DISCUSSION

The lack of published studies regarding possible toxic effects that could be caused by the systemic distribution of PMMA microspheres prompted this project. Since it has been widely reported that needle misplacement due to professional inexperience could result in an intravascular injection and suggested by some authors that PMMA particles could migrate to distant organs, this study evaluated the aspects involved in a possible systemic reaction to this material using a murine model. The experiments were conducted through the methodology used in most toxicity studies.

Kidney and liver are considered target organs for drug metabolism. Kidney plays an important role in the filtration of the plasma and metabolic homeostasis, and therefore, medications can induce some level of toxicity by interacting directly with the organ's structure or indirectly inducing some damage to the electrolyte balance or blood circulation [22, 23]. Serum

creatinine levels should be measured to identify possible toxic effects on the kidney that are influenced by the following factors: drug pharmacokinetics, dose tolerance, drug interactions, physiological variations, pathological factors and genetic factors [24].

Due to the major role of liver in the metabolism and excretion of drugs and xenobiotics, it will frequently manifest signs of toxicity. In the USA, the principal cause of death due to acute liver failure, is drug-induced liver injury [23, 25]. Serum levels of AST and ALT are considered an important sign of hepatocellular damage [26]. ALT is present basically in the liver, while AST is also involved with other organs.

Although histological signs of liver and kidney damage were not found in this study, the level of ALT activity in serum was higher in the group injected with 2% PMMA than the other 2 groups at 7 days, while at 90 days the 30% PMMA group showed higher levels of ALT. This indicates that according to each group, the effect of PMMA differed from the control group over time. Previous studies have demonstrated that ALT levels higher than 51 U/L could be considered signs of hepatotoxicity [27]. However, no difference was found when the AST levels were assessed, and in comparing creatinine levels, only time seemed to account for increases. This fact could be related to the animals' aging process.

In toxicology experiments, comparison of the organ weights of the treated group and control is conventionally used to evaluate the toxic effect of the test product, and changes may represent a sensitive indicator of chemically induced toxicity. However, organ weight data must be interpreted with caution, as a combination of clinical and histopathological findings, since the changes may not be related to the treatment [28, 29]. Our evaluation of weight changes was not able to determine whether they were signs of toxicity induced by PMMA despite the statistically

different lung weight data between the PMMA groups and control.

The systemic distribution of the injected material was evaluated microscopically in all experimental groups on the basis of the presence or absence of inflammatory response and/or PMMA particles in the right submandibular lymph node, lung, kidney and liver of each animal. Microspheres were not observed in this study at any distant organs and inflammation was not present at any distant organ as well. The submandibular glands of some animals were removed aggregated to the lymph nodes and surprisingly microspheres were found in 2 specimens of the 2% PMMA group after 7 days. Our findings are in line with most previous studies, indicating that PMMA microspheres are not able to migrate to distant organs, in contradiction to the findings of Rosa and De Macedo[30] (2005) who observed hepatic and renal inflammation after PMMA injection in mouse ears. However, the microspheres were observed at the submandibular gland, so the results could indicate a different situation if the organs were entirely examined or if other organs were also assessed, as heart and spleen. Surprisingly thromboembolic complications were not detected at any observation time and also unexpected is the fact that inflammation in the tongue was observed in only 4 animals, 2 in the 2%PMMA group at 7 days and 2 animals in the 30% PMMA group at 90 days. These findings differ from the results obtained by Moure *et al* [4] (2012), where the authors performed a injection at the ventral surface of the tongue of a greater amount of PMMA (0.07mL) found an intense inflammatory reaction with polymorphonuclear neutrophil infiltration after 7 days and chronic inflammation with moderate intensity at 60 and 90 days, represented by the presence of a lymphoplasmacytic infiltrate and giant cells next to the PMMA particles at all observation times. Probably, the greater amount of the material injected as well as the technique of implantation used in the mentioned study contributed for the results to be distinct.

Mastocytosis was observed during the histologic evaluation of several samples of the tongue, lymph nodes at all times of the study. In an attempt to establish a correlation with their presence and a possible foreign-body reaction that could be mediated by this type of cell, mast cells were scored in the tongue samples. However, statistical analysis demonstrated that mast cells decreased in number in the tongue samples until day 90 and that there was no difference between PMMA groups and the control group. It can be assumed that since mast cells are found in larger quantities in acute wounds, participating in the inflammatory reaction, angiogenesis and extracellular-matrix reabsorption and remodeling[31, 32], even the trauma caused by needle insertion could result in an increased presence of mast cells at the injection site. It should also be highlighted that recent studies have demonstrated that plasma estradiol levels increase in the presence of mast cells in several tissues in a dose-dependent way [33], and since female rats were employed in our study, we have to consider the possibility that our findings may be also related to changes in the animals' estrous cycle.

Considering our methods, the data obtained in this study do not confirm that PMMA facial fillers have a potential toxic effect. However, the group injected with 30% PMMA had elevated ALT levels at 90 days and both PMMA groups showed increased lung weight at 7 days, indicating a nonspecific systemic reaction. Due to our present results and the lack of previous studies examining the possible reactions of systemic toxicity and genotoxicity caused by cosmetic fillers containing PMMA, further research on this issue is warranted.

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DISCUSSÃO GERAL

É evidente na literatura que a população está recorrendo cada vez mais ao uso dos diversos materiais de preenchimento dérmico disponíveis no mercado, na tentativa de corrigir rugas, linhas de expressão, cicatrizes de acne ou até mesmo para dar um volume maior a determinadas estruturas anatômicas, tais como lábios, nariz e mento (SANTANA *et al*., 2010; REQUENA *et al*., 2011; HEXSEL *et al*., 2012).

No Brasil, os produtos utilizados para preenchimento facial que contêm PMMA são bastante procurados por possuírem baixo custo, duração permanente, além de serem facilmente aplicados, dispensando anestesia geral e repouso pós-operatório (BORGHETTI *et al*., 2011; VARGAS *et al.,* 2011). Como estes materiais são fabricados e distribuídos em diversos países, existe uma grande divergência nos processos de controle de qualidade realizados pelos fabricantes, bem como na fiscalização executada pelos órgãos de saúde competentes (PICQUADIO *et al*., 2008). Acredita-se que novos parâmetros e normas deveriam ser estabelecidos, visando a garantia da qualidade dos produtos distribuídos e dificultando a comercialização ilegal dos materiais de preenchimento estético a profissionais não habilitados para sua implantação.

No estudo realizado por Picquadio *et al*. (2008), os autores compararam as características das microesferas de PMMA presentes em 5 tipos de materiais de preenchimento disponíveis nos mercados brasileiro, americano, canadense e europeu. Através de uma análise padronizada com microscopia eletrônica de varredura, os autores observaram uma alarmante diversidade na morfologia e no tamanho das esferas contidas em 2 produtos fabricados no Brasil. Quando comparados ao material de última geração produzido nos EUA, tornam-se evidentes as falhas detectadas na confecção das microesferas presentes nos produtos nacionais.

Está disponível no mercado americano apenas um material contendo 20% de microesferas de PMMA em meio de colágeno bovino (LEMPERLE *et al.,* 2003, 2006). Já no Brasil, existem 3 produtos compostos por microesferas de PMMA suspensas em 2 tipos de géis que são liberados pela Agência Nacional de Vigilância Sanitária (ANVISA) em diferentes concentrações (2%, 5%, 10%, 15% e 30%). A diversidade de marcas comerciais justifica-se pela grande procura da população por mudanças na estética corporal e facial, visto que tais produtos são implantados em diversas regiões anatômicas. Considera-se que tais substâncias, principalmente as produzidas no Brasil, possuem baixo custo financeiro quando comparadas à outros tipos de materiais de preenchimento e são facilmente obtidas por profissionais de diversas áreas, que podem ou não possuir treinamento adequado para aplicá-las.

O PMMA deve ser aplicado através da técnica de tunelização, na qual a agulha ou cânula é inserida no 1/3 inferior da derme e o material depositado durante a remoção do instrumento. Para que se obtenha um resultado satisfatório e não ocorra migração das micropartículas orienta-se que este produto não seja injetado indiscriminadamente no interior de vasos sanguíneos. Entretanto, pode ocorrer a injeção intravascular acidental durante a aplicação da substância. Até o momento, inúmeros casos clínicos foram descritos na literatura referindo alterações locais desastrosas decorrentes de prováveis aplicações intravasculares. Segundo alguns autores, esta injeção poderia causar uma embolia do vaso sanguíneo e consequente necrose na área afetada bem como a sua migração para órgãos distantes. A necrose poderia ocorrer ainda pela implantação muito próxima a vasos sanguíneos devido à compressão dos mesmos causada pelo acúmulo do material e por consequência, ocorreria a redução do aporte sanguíneo regional (SILVA, CURI, 2004; KUBOTA, HIROSE, 2005; DE CASTRO, *et al*., 2007; BACHMANN, *et al*., 2009; DE FIGUEIREDO *et al.,* 2010). As possíveis manifestações sistêmicas após a injeção intravascular do PMMA não foram até o momento, documentadas em humanos ou animais.

Optamos por realizar a injeção em superfície ventral da língua devido à sua abundante vascularização, sendo constituída de um plexo vascular composto por uma rede de vasos superficiais e pelas veias raninas (VERLI *et al*., 2008). Essa localização anatômica também é considerada menos vulnerável a fatores traumáticos (BORGHETTI *et al*., 2011; MOURE *et al*., 2012).

Foram identificados e analisados, ao longo do tempo, os efeitos teciduais sistêmicos e a possível migração de partículas para órgãos distantes. Esses podem ser originados a partir da aplicação intravascular de um material de preenchimento composto por microesferas de PMMA suspensas em um gel de hidroxietilcelulose (Newplastic®; Biomedical Comércio, Porto Alegre, Brasil) em 2 concentrações (2% e 30%). Segundo informações provenientes do fabricante, este produto está indicado para correção e aumento do volume corporal e facial. De maneira geral, os preenchedores teciduais que contém PMMA são comumente empregados em variadas estruturas anatômicas tais como, lábio, sulco nasolabial, nariz, mento, dorso da mão, glúteo e bíceps, assim como para preencher rugas nas regiões glabelar, malar, labial e pré-auricular (LEMPERLE *et al.,* 2003; DE FIGUEIREDO, *et al.,* 2010*;* VARGAS *et al.,* 2011). Este estudo buscou contribuir para uma melhor compreensão dos mecanismos de toxicidade dos materiais de preenchimento estético à base de PMMA. Devido à alta e crescente aplicabilidade na região facial é de grande interesse dos cirurgiões-dentistas, conhecer os possíveis efeitos adversos causados por uma inadvertida injeção intravascular.

Algumas manifestações clínicas frequentemente são citadas na literatura, tais como edema, hematoma, nódulo, ulceração, necrose ou supuração (ENGELMAN *et al*., 2005; COHEN *et al*., 2008; SALLES *et al*., 2008; COX, 2009; ROHRICH *et al*., 2010; BAILEY *et al*., 2011). Em nossa pesquisa não observou-se alterações teciduais nas línguas de nenhum grupo estudado. Tais achados diferem dos resultados obtidos no estudo realizado por Moure *et al*. (2012), no qual

realizou-se a injeção submucosa de PMMA à 10% na língua de ratas, onde todos os animais apresentaram ulceração no local injetado após 7 dias de observação.

Em relação a resposta inflamatória, verificou-se que no sétimo dia do experimento, exclusivamente duas amostras de língua, pertencentes ao grupo injetado com PMMA a 2%, apresentaram algum grau de inflamação, sendo esta de intensidade moderada. Aos 90 dias, observou-se resposta inflamatória moderada com infiltrado mononuclear e neutrófilos e eosinófilos esparsos em uma das amostras do grupo 2 (PMMA 30%). No mesmo período os autores citados anteriormente, observaram intensa reação inflamatória na língua de 4 animais, com presença de células gigantes. Aos 60 e 90 dias, 5 amostras apresentaram reação inflamatória moderada. O local de aplicação, técnica de injeção e quantidade de material injetado neste estudo não foram os mesmos adotados em nossa pesquisa. No referido trabalho, os autores realizaram injeção submucosa de 0,07mL de do material, portanto a quantidade menor (0,05mL) adotada em nossa metodologia, associada à injeção intravascular parece ser insuficiente para induzir uma reação inflamatória local de maior expressão bem como alterações clínicas no local onde foi aplicado o produto.

Durante a avaliação histológica das línguas e linfonodos submandibulares, percebemos um grande número de mastócitos em diversas amostras em todos os tempos do estudo. A mastocitose foi detectada e confirmada através da coloração com azul de toluidina. A contagem foi realizada a fim de correlacionar a presença dessas células a uma possível reação de corpo estranho. Após a análise microscópica de todo o corte obtido da língua, foram selecionadas as áreas que apresentavam uma maior quantidade de mastócitos. Os mesmos foram quantificados em 4 campos adjacentes de cada lâmina. Após a análise dos dados obtidos durante a quantificação, não observou-se diferença na contagem dos mastócitos entre os grupos experimentais (PMMA 2%, PMMA 30 % e controle). Verificou-se apenas um decréscimo da

população dos mastócitos, após 90 dias, em todos os grupos de estudo (P= 0.0004). Uma provável justificativa para tais achados é a de que mastócitos podem ser encontrados numa maior quantidade em lesões agudas, participando da reação inflamatória, angiogênese, reabsorção e remodelação da matriz extracelular. Nesse caso, o trauma causado pela inserção da agulha na intimidade do vaso sanguíneo poderia induzir uma mastocitose local na primeira fase do experimento (HEBDA *et al*., 1993; BABEI *et al*., 2012). Também cabe ressaltar que estudos recentes demonstraram existir uma associação entre o aumento dos níveis de estradiol no plasma e a presença de mastócitos em vários tecidos de uma maneira dose-dependente (JING *et al*., 2012). Devido ao emprego de fêmeas em nosso estudo, considera-se que os resultados possam também estar relacionados com alterações no ciclo de estral das ratas (WESTWOOD *et al*., 2008).

Na avaliação da toxicidade sistêmica, não foram observados sinais histológicos de dano renal ou hepático, embora o grupo injetado com PMMA à 30% tenha apresentado aos 90 dias, níveis mais elevados de ALT (P = 0.047) quando comparado aos demais grupos. Estudos prévios demonstraram que valores de ALT superiores a 51U/L podem ser considerados sinais de hepatotoxicidade (LENAERTS, 2005). Em nossa análise, não houve diferença estatisticamente significativa dos níveis de AST entre os grupos em nenhum tempo do estudo. Todos os grupos apresentaram aumento dos níveis séricos de creatinina aos 90 dias do estudo, todavia sem apresentar diferença significativa entre os mesmos. Talvez este fato possa estar exacerbado em decorrência do processo de envelhecimento dos animais.

A comparação do peso dos órgãos entre o grupo tratado e o controle é convencionalmente utilizada em protocolos que buscam avaliar a possível toxicidade do produto testado. Alterações do peso de determinados órgãos podem ser aceitas como um sensível indicador da toxicidade

induzida quimicamente. No entanto, dados obtidos na pesagem dos órgãos devem ser interpretados com cautela, visto que as mudanças podem não estar relacionadas com o tratamento estabelecido (SELLERS *et al.*, 2007; MICHAEL *et al.*, 2007). A partir da avaliação das alterações de peso realizada em nossa pesquisa, não pode-se vincular as mudanças verificadas aos sinais de toxicidade ao PMMA.

A sistematização do material foi avaliada microscopicamente em todos os grupos experimentais com base na presença ou ausência de resposta inflamatória e de partículas do PMMA no fígado, linfonodos submandibulares, rim e pulmão direito de cada animal. Não observou-se microesferas ou reação inflamatória em nenhum dos órgãos distantes. Encontrou-se partículas, especificamente nas glândulas submandibulares de 2 animais do grupo 1 (PMMA 2%) aos 7 dias. Estas foram ocasionalmente analisadas, uma vez que, durante a retirada dos linfonodos submandibulares acabaram por ser removidas de forma conjunta. Nossos resultados corroboram com estudos anteriores (LEMPERLE *et al*., 2004; MOURE *et al*., 2012), os quais constataram que as microesferas de PMMA não são capazes de causar resposta inflamatória em órgãos distantes, contrapondo-se aos resultados obtidos por Rosa e Macedo (2005) que observaram inflamação hepática e renal após injeção de PMMA na cartilagem auricular de ratos. É importante ressaltar a possibilidade de obter-se resultados distintos, caso o órgão fosse analisado histologicamente como um todo. Além disso, o exame de outras estruturas relevantes na área de toxicologia, como o coração e o baço, poderiam dar maiores subsídios para esta avaliação.

Os dados obtidos neste estudo não permitem afirmar que os materiais de preenchimento facial contendo PMMA, são capazes de induzir uma reação de toxicidade sistêmica quando injetados no interior de um vaso sanguíneo. No entanto, uma elevação dos valores de ALT, foi

apresentada pelo grupo que recebeu injeção de PMMA 30%, após 90 dias. Também observou-se que nos animais injetados com PMMA, em ambas concentrações (2 e 30%), ocorreu um aumento maior no peso das amostras de pulmão quando comparadas ao grupo controle (P = .048), aos 7 dias. Nossos achados indicam a ocorrência de uma reação sistêmica que não contempla todos os requisitos necessários para ser caracterizada como tóxica.

O uso indiscriminado dos materiais de preenchimento facial contendo PMMA por profissionais despreparados, a falta de um controle rigoroso na fabricação e distribuição dos mesmos, associados à desinformação da população sobre os riscos envolvidos na injeção desses produtos, fazem com que as reações adversas decorrentes do tratamento sejam mais frequentes e algumas vezes severas (ENGELMAN, 2005; SALLES, 2008; VARGAS, 2011). Em muitos casos, as complicações imediatas ou tardias serão observadas na região oral e perioral e deverão ser identificadas e manejadas apropriadamente pelo cirurgião-dentista.

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ANEXO 1

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The ultimate aim of the subject area of gerodontology is to improve the quality of life and oral health of older people. *Gerodontology* fills the particular place of serving this subject area. The boundaries of most conventional dental specialities must be repeatedly crossed to provide optimal dental care for older people. Furthermore, management of other health problems impacts on their dental care and clinicians need knowledge in these numerous overlapping areas. Bringing together these diverse topics within one journal serves clinicians who have not time to scan many journals and it serves authors whose papers would therefore fail to access their target readership. The juxtaposition of papers from different specialities but sharing this patient-centred interest provides a synergy that serves progress in the subject of gerodontology.

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4. MANUSCRIPT FORMAT AND STRUCTURE
4.1. Format

Language: The language of publication is English. Authors for whom English is a second language may choose to have their manuscript professionally edited by an English speaking person before submission to make sure the English is of high quality. A list of independent suppliers of editing services can be found at

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Font: Manuscripts must be typed double-spaced.

Abbreviations, Symbols and Nomenclature: The symbol % is to be used for percent, h for hour, min for minute, and s for second. In vitro and in vivo are to be italicized. Use only standard abbreviations. Units used must conform to the Système International d'Unités (SI). All units will be metric. Use no roman numerals in the text. In decimals, a decimal point and not a comma will be used. For tooth notation the two digit system of FDI must be used (see Int. Dent. J. 21, 104). Avoid abbreviations in the title. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement. In cases of doubt, the spelling orthodoxy of Webster's Third New International Dictionary will be adhered to.
Scientific Names: Proper names of bacteria should be binomial and should be singly underlined in the typescript. The full proper name (e. g. Streptococcus sanguis) must be given upon first mention. The generic name may be abbreviated thereafter with the first letter of the genus (e. g. S. sanguis). If abbreviation of the generic name could cause confusion, the full name should be used. If the vernacular form of a genus name (e. g. streptococci) is used, the first letter of the vernacular name is not capitalized and the name is not underlined. Use of two letters of the genus (e. g. Ps .for Peptostreptococcus) is incorrect, even though it might avoid ambiguity. With regard to drugs, generic names should be used instead of proprietary names. If a proprietary name is used, ® must be attached when the term is first used.

4.2. Structure

Original Articles submitted to *Gerodontology* should include: Title Page, Abstract, Introduction, Material and Methods, Results, Discussion, Conclusions, References, and Acknowledgements, Tables, Figures and Figure Legends were appropriate.

Title Page: should contain the title of the article, name(s) of the author(s), initials, and institutional affiliation(s), a running title not to exceed 40 letters and spaces, and the name and complete mailing and email address of the author responsible for correspondence. The author must list 4 keywords for indexing purposes.
The Title Page should be uploaded as a separate document, using the File designation ‘Title Page’ which is available from the drop down menu.

Abstract: A separate structured abstract should not exceed 250 words. The abstract should consist of 1) the objective 2) the background data discussing the present status of the field 3) materials and methods 4) results 5) conclusion.

Introduction: Summarize the rationale and purpose of the study, giving only strictly pertinent references. Do not review existing literature extensively.

Material and Methods: Materials and methods should be presented in sufficient detail to allow confirmation of the observations. Published methods should be referenced and discussed only briefly, unless modifications have been made.

Results: Present your results in a logical sequence in the text, tables, and illustrations. Do not repeat in the text all of the data in the tables and illustrations. Important observations should be emphasized.

Discussion: Summarize the findings without repeating in detail the data given in the Results section. Relate your observations to other relevant studies and point out the implications of the findings and their limitations. Cite other relevant studies.

Conclusion: Conclude the findings in brief. If authors cannot conclude with any punch line, the referee will question who would want to read the paper and why.

Acknowledgements: Acknowledge only persons who have made substantive contributions to the study. Authors are responsible for obtaining written permission from everyone acknowledged by name because readers may infer their endorsement of the data and conclusions. Sources of financial support may be acknowledged.

Research in Brief/Short Reports: These should include the aims and objectives of the work reported, methods used, findings, and the implications for the practise, management or education of the older adult and further research. Research in brief submissions should be no more than 1000 words in length, with a clear and concise title and no more than five subheadings. These may take the format of a mini paper. A maximum of 10 references may be included but these must be clearly related to the work reported. A limited number of figures and tables can be included but they must be essential to the understanding of the research.

4.3. References

References should be numbered consecutively in the order in which they appear in the text, and should be kept to a pertinent minimum. Only references which are cited in the text may be included. References should include the beginning and ending page numbers. Identify references in the text, tables, and figure legends by Arabic numerals in parentheses. References cited only in the tables or figure legends should be numbered in accordance with a sequence established by the first notation of that figure or table in the text. Use the style of the examples below, which is based on Index Medicus. Manuscripts accepted but not published may be cited in the reference list by placing 'in press" after the abbreviated title of the journal - all such references should be submitted to the Editor for approval. References must be verified by the author(s) against the original documents.

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Examples:

(1) Standard journal article

(List all authors up to 3; for 3 or more list the first 3 and add 'et al.")

Dockrell H, Greenspan JS. Histochemical identification of T- cells in oral lichen planus. Oral Surg 1979; 48: 42-49.

Thomas Y, Sosman J, Yrigoyen O, et al. Functional analysis of human T- cell subsets defined by monoclonal antibodies. I. Collaborative T-T interactions in the immunoregulation of B-cell differentiation. J Immunol 1980; 125: 2402-2405.

(2) Corporate author

The Royal Marsden Hospital Bone- Marrow Transplantation Team. Failure of syngeneic bone- marrow graft without preconditioning in post- hepatitis marrow aplasia. Lancet 1977; 2: 628-630.

(3) No author given

Anonymous. Coffee drinking and cancer of the pancreas [Editorial]. Br Med J 1981; 283: 628-635.

(4) Journal supplement

Mastri AR. Neuropathology of diabetic neurogenic bladder. Ann Intern Med 1980; 92 (2 pt 2): 316- 324.

Frumin AM, Nussbaum J, Esposito M. Functional asplenia: demonstration of splenic activity by bone marrow scan. Blood 1979; 54 (suppl 1): 26- 28.

(5) Journal paginated by issue

Seaman WB. The case of the pancreatic pseudocyst. Hosp Pract 1981; 16 (Sep): 24-29.

(6) Personal author(s)

Eisen HN. Immunology: an introduction to molecular and cellular principles of the immune response, 5th edn. New York: Harper Row, 1984:406-420.

(7) Editor, compiler, chairman as author

Dausset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973: 12-18.

(8) Chapter in a book

Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: WB Saunders, 1974: 457-480.

(9) Published proceedings paper

DePont B. Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of 3rd Annual Meeting of the International Society for Experimental Hematology. Houston: International Society for Experimental Hematology, 1974: 44-50.

(10) Agency publication

Ranofsky AL. Surgical operations in short-stay hospitals: United States - 1975.

Hyattsville, Maryland: National Center for Health Statistics, 1978; DHEW publication no. (PHS) 78-1785. (Vital and health statistics; series 13; no. 34.)
(11) Dissertation or thesis
Cairns RB. Infrared spectroscopic studies of solid oxygen. Berkeley, CA: University of California, 1965. 156pp. Dissertation.

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For instructions, see Gerodontology Guide to Tables and Figures (http://blackwellpublishing.com/pdf/Gerodontology-Authors%27-Guide-to-Tables-and-Figures.pdf)

Figures: At the Editor's discretion clinical photographs, photomicrographs, line drawings and graphs will be published as figures. All figures should clarify the text and their number should be kept to a minimum. Details must be large enough to retain their clarity after reduction in size. Illustrations should preferably fill a single column width (54 mm) after reduction, although in some cases 113 mm (double column) and 171 mm (full page) widths will be accepted. Micrographs should be designed to be reproduced without reduction, and they should be dressed directly on the micrograph with a linear size scale, arrows, and other designators as needed. The inclusion of colour illustrations is at the discretion of the Editor. The author may pay for the cost of additional colour illustrations.

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Figure Legends: Figure legends must be typed double-spaced on a separate page at the end of the manuscript.

4.5 Submission of a revision

When uploading a revision, please take care to respond to the concerns raised by the Reviewers. Please upload a copy of the revised document which shows easily what changes have been made to the original. This could include using red font or highlighting. However, please note that you should NOT use Track Changes. The revised manuscript should be uploaded with the file designation 'Main Document'.
The online submission form will prompt you to respond to the Reviewers' comments in writing. Please provide as far as possible, a clear, point by point response to the comments of each Reviewer in which you describe for each of the points raised exactly how you have dealt with them. Please complete the online submission form and upload your response as a separate manuscript file, using the designation 'Supplementary File for Review'.
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5. AFTER ACCEPTANCE

5.1 Proof Corrections

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ANEXO 3

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Assunto:	Submission Confirmation for Polymethylmethacrylate Dermal Fillers: Evaluation of the Systemic Toxicity in Rats
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Para:	clarissacgmedeiros@yahoo.com.br; clarissa.medeiros@acad.pucrs.br;
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ANEXO 4

Normas para publicação de artigos no periódico ***International Journal of Oral and Maxillofacial Surgery.***

Guide for Authors

Would authors please note that the reference style for the journal has now changed. Please pay special attention to the guidelines under the heading "References" below

Authors wishing to submit their work to the journal are urged to read this detailed guide for authors and comply with all the requirements, particularly those relating to manuscript length and format. This will speed up the reviewing process and reduce the time taken to publish a paper following acceptance.

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Upon submission you will be required to complete and upload this form (pdf version or word version) to declare funding, conflict of interest and to indicate whether ethical approval was sought. This information must also be inserted into your manuscript under the acknowledgements section with the headings below. If you have no declaration to make please insert the following statements into your manuscript:

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PLEASE NOTE that all funding must be declared at first submission, as the addition of funding at acceptance stage may invalidate the acceptance of your manuscript.

Authorship

All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data (2) drafting the article or revising it critically for important intellectual content (3) final approval of the version to be submitted.

Normally one or two, and no more than three, authors should appear on a short communication, technical note or interesting case/lesson learnt. Full length articles may contain as many authors as appropriate. Minor contributors and

non-contributory clinicians who have allowed their patients to be used in the paper should be acknowledged at the end of the text and before the references.

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Patients have a right to privacy. Therefore identifying information, including patients' images, names, initials, or hospital numbers, should not be included in videos, recordings, written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and you have obtained written informed consent for

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Format

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200 words maximum. Do not use subheadings or abbreviations; write as a continuous paragraph. Must contain all relevant information, including results and conclusion.

T ext

Please ensure that the text of your paper conforms to the following structure: Introduction, Materials and Methods, Results, Discussion. There is no separate Conclusion section. There should be no mention of the institution where the work was carried out, especially in the Materials and Methods section.

Introduction

• Present first the nature and scope of the problem investigated • Review briefly the pertinent literature • State the rationale for the study • Explain the purpose in writing the paper

• State the method of investigation and the reasons for the choice of a particular method •; Should be written in the present tense

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• Give the full details, limit references • Should be written in the past tense • Include exact technical specifications, quantities and generic names • Limit the number of subheadings, and use the same in the results section • Mention statistical method • Do not include results in this section

Results

• Do not describe methods • Present results in the past tense • Present representations rather than endlessly repetitive data • Use tables where appropriate, and do not repeat information in the text

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T ables

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Instructions for Letters to the Editor

The IJOMS welcomes Letters to the Editor. To facilitate submission of the highest quality of Letters to the Editor, the following guidelines should be followed:

1. Letters are meant to be focus pieces and, therefore, are limited to no more than 600 words, 6 references and a maximum of 2 figures. One reference should include a reference to the IJOMS article being addressed.

2. It is recommended that you limit your letter to one or two important and critical points to which you wish to provide a clear and precise discussion regarding the previously published article.

3. One should support all assertion by peer review literature which should be a primary research or large clinical studies rather than a case report.

4. Please include any financial disclosures at the end of the letter. This would include the potential conflicts of interest not just related to the specific content of your letter but also the content of the IJOMS article and other related areas.

5. Please recognize that letters that are essentially in agreement with the author's findings and offer no additional insights provide little new information for publication. Likewise, letters that highlight the writer's own research or are otherwise self promotional will receive a low publication priority.

6. There may be a need for additional editing. Should editing be required the letter will be sent back to the author for final approval of the edited version.

7. It is important to use civil and professional discourse. It is not advisable that one adopt a tone that may be misconstrued to be in anyway insulting.

8. Finally, it is not advisable to provide a letter that is anecdotal. While personal experiences can have great value in patient care, it is generally not strong evidence to be placed in a letter to the editor.

ANEXO 5

Parecer consubstanciado emitido em 21 de setembro de 2011 pela Comissão Científica e de Ética da Faculdade de Odontologia da PUCRS.

Comissão Científica e de Ética
Faculdade da Odontologia da PUCRS

Porto Alegre 21 de Setembro de 2011

O Projeto de: Dissertação

Protocolado sob nº:	0060/11
Intitulado:	Estudo experimental em ratos submetidos à injeção intravascular de polimetilmetacrilato: avaliação da toxicidade sistêmica.
Pesquisador Responsável:	Profa. Dra. Maria Antonia Z. de Figueiredo
Pesquisadores Associados:	Clarissa Castro Galvão Medeiros
Nível:	Dissertação / Mestrado

Foi ***aprovado*** pela Comissão Científica e de Ética da Faculdade de Odontologia da PUCRS em *21 de Setembro de 2011.*

Este projeto deverá ser imediatamente encaminhado ao CEUA/PUCRS.

Profa. Dra. Ana Maria Spohr
Presidente da Comissão Científica e de Ética da
Faculdade de Odontologia da PUCRS

Av. Ipiranga, 6681, Prédio 06 sala 210
Porto Alegre /RS – Brasil – Cx. Postal:1429
90619-900
Fone/Fax: (51) 3320-3538
e-mail: odontologia-pg@pucrs.br

ANEXO 6

Parecer consubstanciado emitido em 01 de novembro de 2011 pela Comissão de Ética no

Uso de Animais da PUCRS.

Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Ofício 142/11 – CEUA Porto Alegre, 01 de novembro de 2011.

Senhora Pesquisadora:

A Comissão de Ética no Uso de Animais da PUCRS apreciou e aprovou, seu protocolo de pesquisa, registro CEUA 11/00261 intitulado: **"Estudo experimental em ratos submetidos à injeção intravascular de polimetilmetacrilato: avaliação da toxicidade sistêmica".**

Sua investigação está autorizada a partir da presente data.

Atenciosamente,

Prof. Dr. Paulo Márcio Condessa Pitrez
Coordenador-Adjunto da CEUA/PUCRS

Ilma. Sra.
Profa. Maria Antonia Figueiredo
Faculdade de Odontologia
Nesta Universidade

PUCRS | Campus Central
Av. Ipiranga, 6690 – Prédio 60, sala 314
CEP: 90610-000
Fone/Fax: (51) 3320-3345
E-mail: ceua@pucrs.br

ANEXO 7

Parecer da Banca Examinadora no Exame de Qualificação do projeto de pesquisa

realizado em 09 de setembro de

Pontifícia Universidade Católica do Rio Grande do Sul
FACULDADE DE ODONTOLOGIA
PÓS-GRADUAÇÃO

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO: ESTOMATOLOGIA
NÍVEL: MESTRADO
EXAME DE QUALIFICAÇÃO – ATA 36/11

Data: 09 /setembro/2011 – 9h30min

Candidata: CLARISSA CASTRO GALVÃO MEDEIROS

Orientadora: Profa. Dra. Maria Antonia Zancanaro de Figueiredo

Título da pesquisa: "Estudo Experimental em ratos submetidos à injeção intravascular de polimetilmetacrilato: avaliação da toxicidade sistêmica."

Comissão Examinadora: Profa. Dra. Maria Martha Campos
Profa. Dra. Fernanda Gonçalves Salum

Parecer:
(X) Aprovado

( ) Aprovado com projeto pendente

( ) Reprovado

Ass.: _____
Clarissa Castro Galvão Medeiros
Aluna

Ass.: _____
Profa. Dra. Maria Antonia Zancanaro de Figueiredo
Orientadora

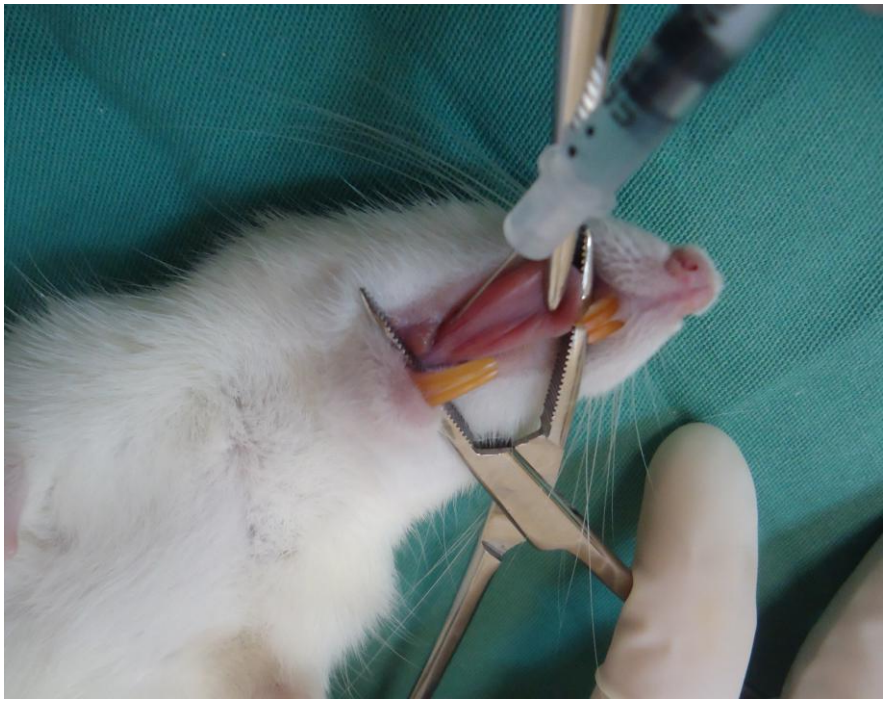
Ass.: _____
Profa. Dra. Maria Martha Campos
Professora Avaliadora

Ass.: _____
Profa. Dra. Fernanda Gonçalves Salum
Professora Avaliadora

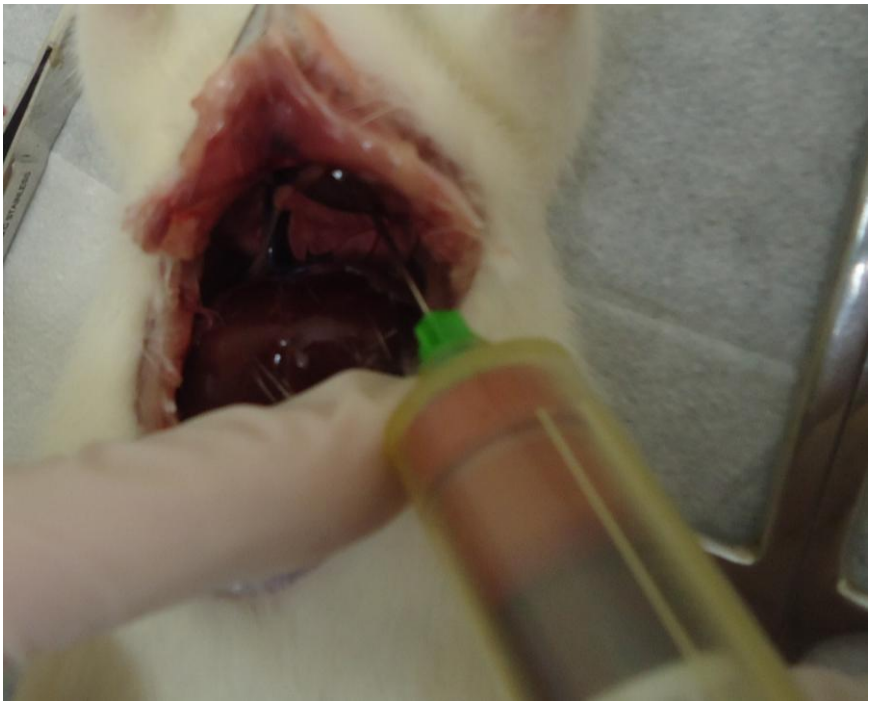
Ass.: _____
Prof. Dr. José Antonio Poli de Figueiredo
Coordenador do Programa de Pós-Graduação em Odontologia

2011.

APÊNDICE A

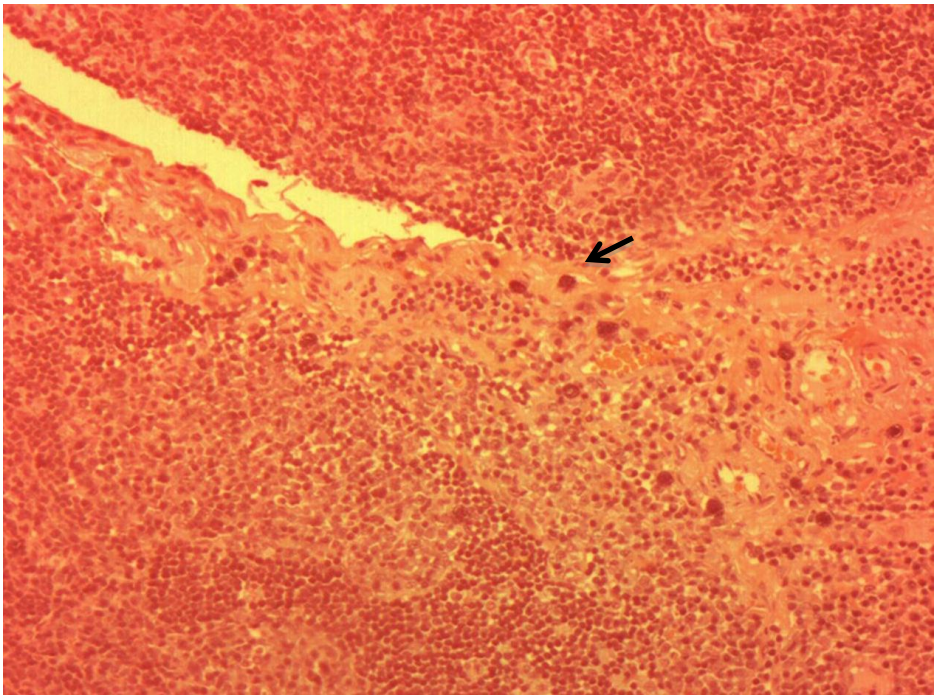


Injeção do material na veia ranina direita, localizada no ventre lingual.

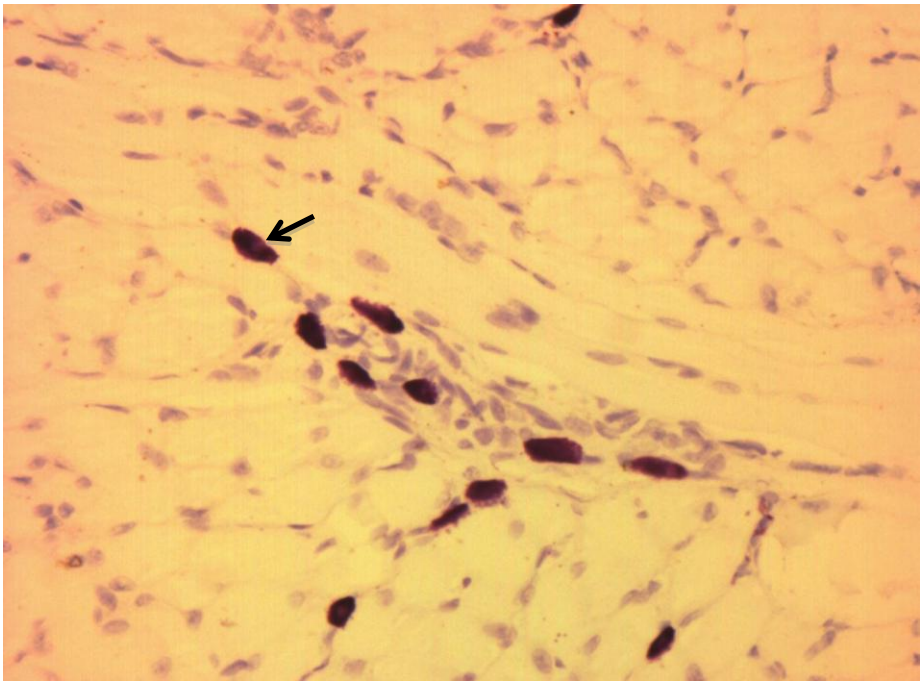


Punção cardíaca.

APÊNDICE B

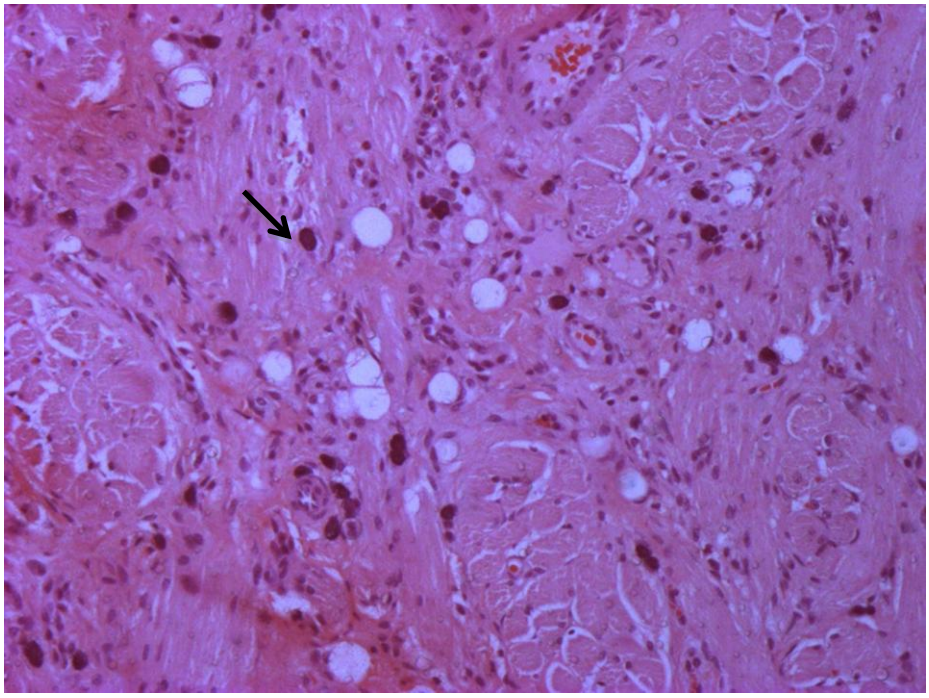


Fotomicrografia demonstrando mastocitose em linfonodo submandibular direito (PMMA 30%) aos 7 dias (HE, aumento aproximado100x).

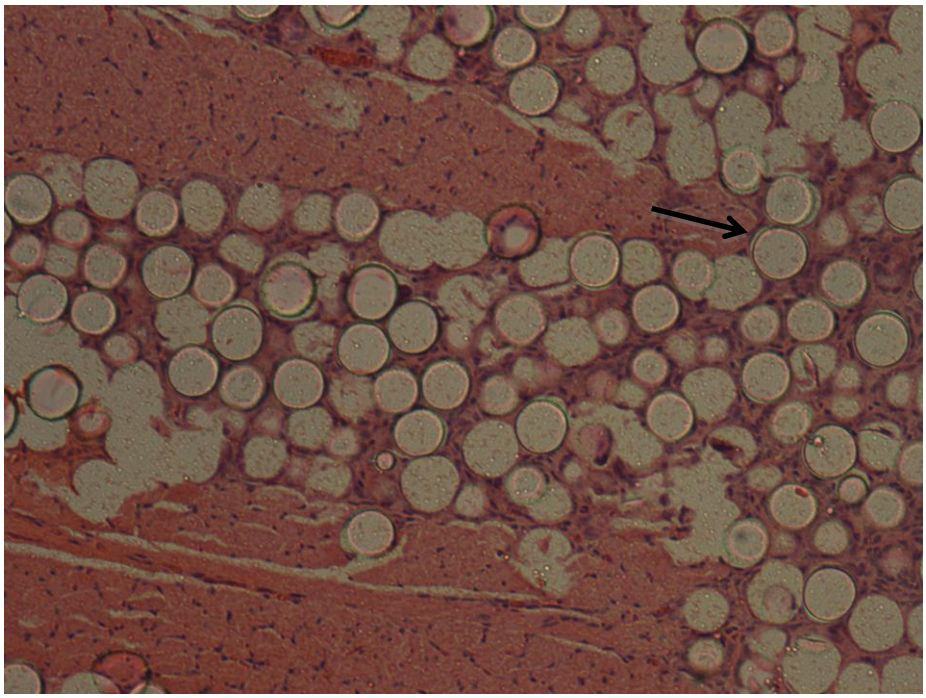


Fotomicrografia demonstrando mastocitose em língua (PMMA 2%, aos 7 dias) confirmada através da coloração com azul de toluidina (aumento aproximado 200x).

APÊNDICE C

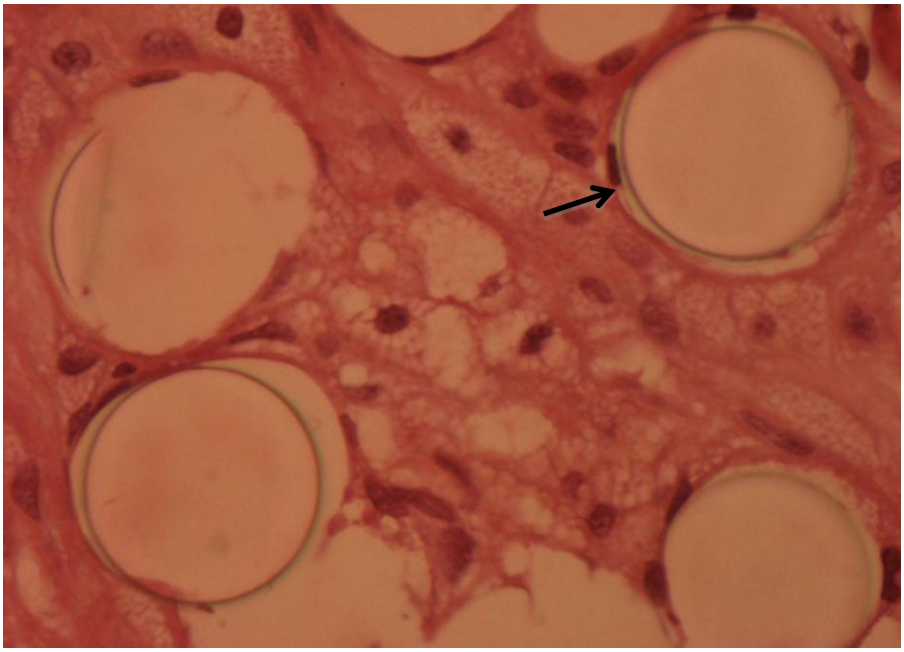


Mastocitose em língua de amostra pertencente ao grupo 1 (PMMA 2%), aos 7 dias (HE 100x).

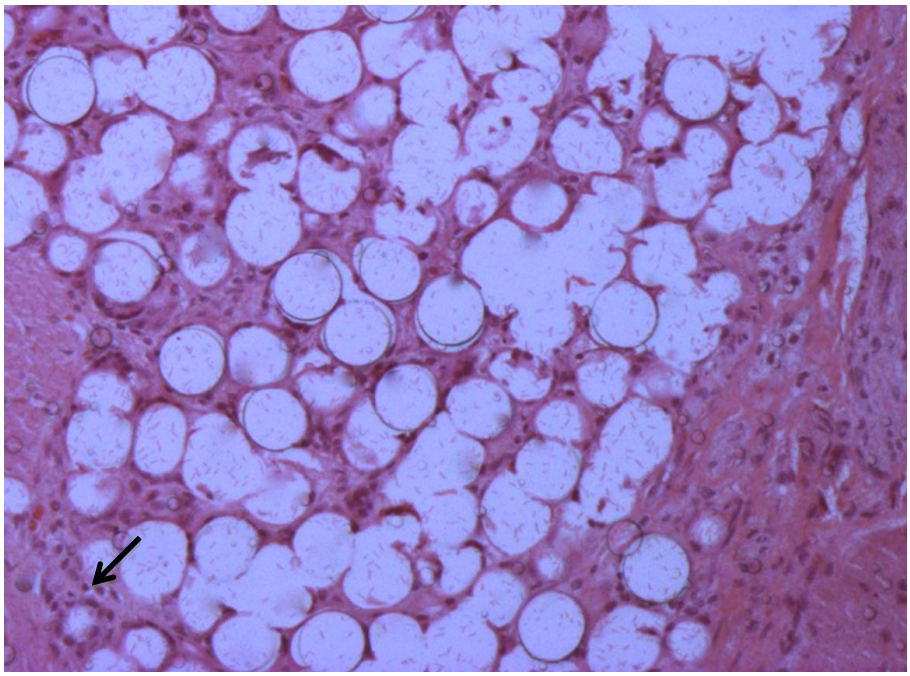


Microscopia com contraste de fase. Presença de microesferas de PMMA na língua evidenciadas pela luz polarizada (HE, aumento aproximado 200x).

APÊNDICE D

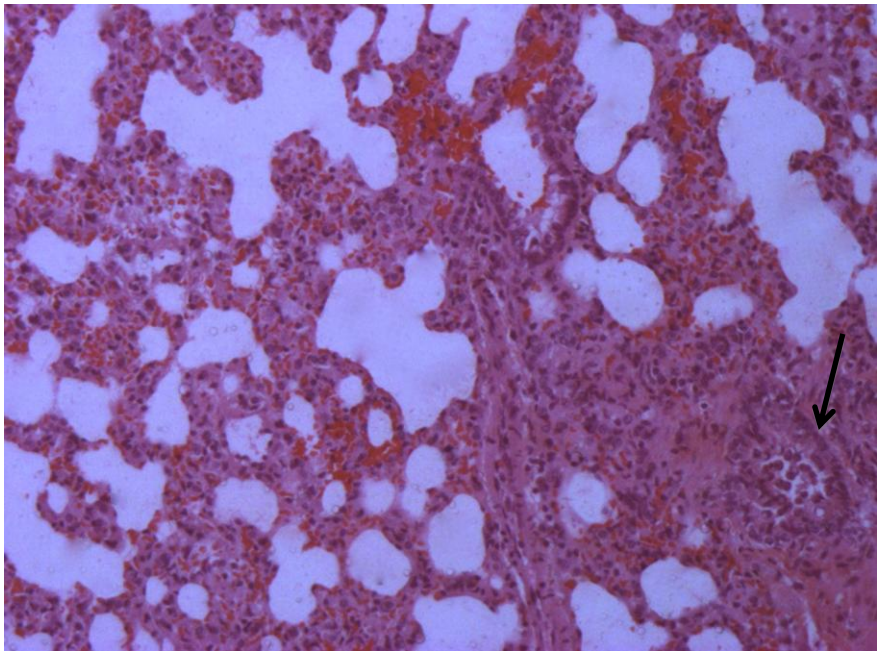


Fotomicrografia demonstrando a presença de microesferas de PMMA na língua (PMMA 2% aos 90 dias) evidenciadas pela luz polarizada (HE, aumento aproximado 400x).

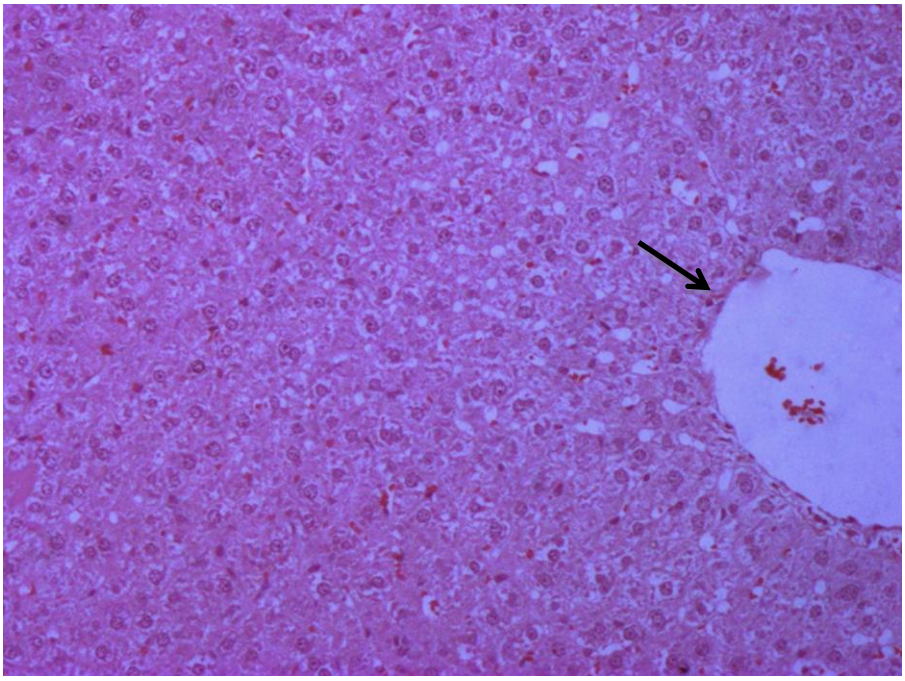


Fotomicrografia demonstrando reação inflamatória moderada em amostra de língua (PMMA 30%), aos 90 dias (HE, aumento aproximado 200x). Seta indicando a presença de célula gigante.

APÊNDICE E

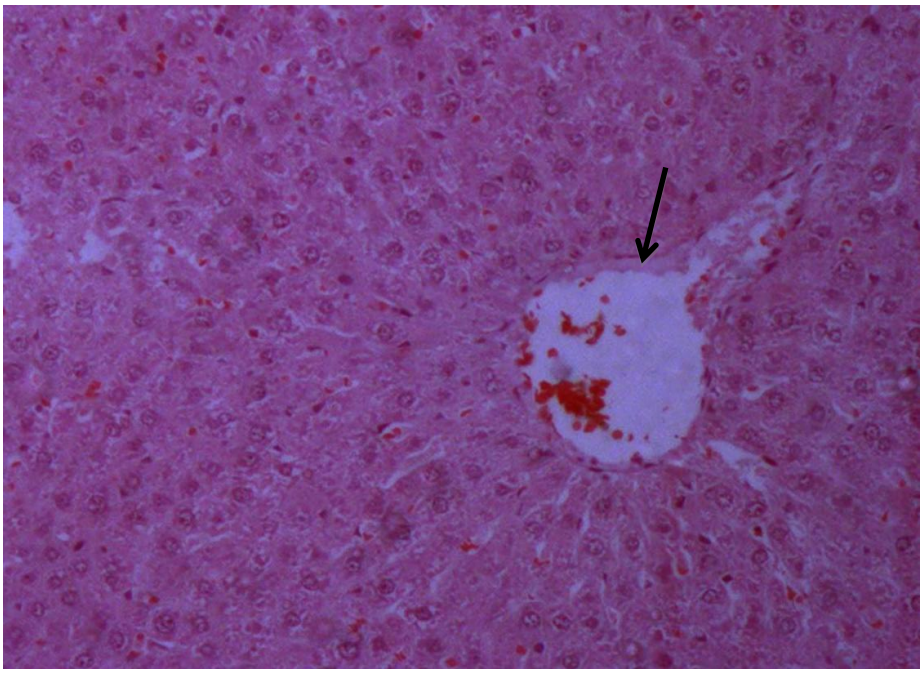


Fotomicrografia demonstrando a ausência de microesferas em pulmão (PMMA 30%) aos 90 dias (HE, aumento aproximado 100x). Bronquíolo indicado pela seta.

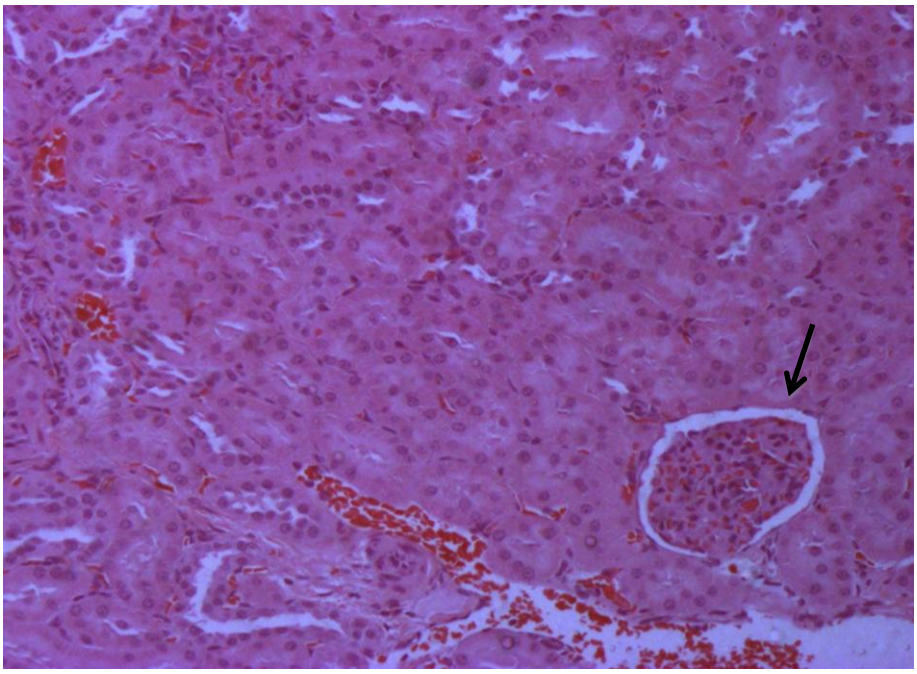


Fotomicrografia demonstrando a ausência de microesferas em fígado (PMMA 30%) aos 7 dias (HE, aumento aproximado 100x). Seta indicando veia centro lobular.

APÊNDICE F

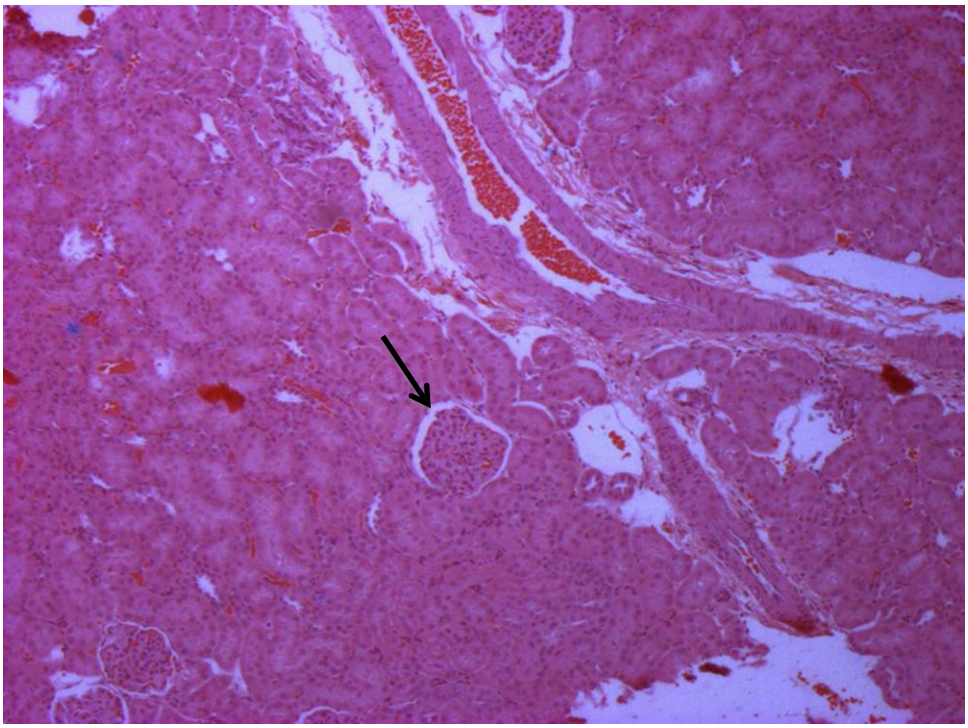


Fotomicrografia demonstrando a ausência de microesferas em fígado (PMMA 30%) aos 90 dias (HE, aumento aproximado 100x). Seta indicando veia centro lobular.



Fotomicrografia demonstrando a ausência de microesferas e/ou inflamação renal (PMMA 30%) aos 7 dias (HE, aumento aproximado 100x). Glomérulo indicado pela seta.

APÊNDICE G



Fotomicrografia demonstrando a ausência de microesferas e/ ou inflamação renal (PMMA 30%) aos 90 dias (HE, aumento aproximado 50x).

APÊNDICE H

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

FACULDADE DE ODONTOLOGIA

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

ÁREA DE CONCENTRAÇÃO EM ESTOMATOLOGIA CLÍNICA

FICHA DE AVALIAÇÃO CLÍNICA

IDENTIFICAÇÃO Rata nº___ Peso inicial:____kg Peso final:____kg Material de preenchimento: Grupo 1 (PMMA 2%) Grupo 2 (PMMA 30%) Grupo 3 (NaCl 0,9%)	Lâmina nº___ Tempo: Subgrupo A (7 dias) Subgrupo B (90 dias)

AVALIAÇÃO CLÍNICA LOCAL

Sem alterações clínicas
Edema
Nódulo
Ulceração
Necrose/ Supuração
Fibrose

Sinais secundários: Sim Não

Especifique:

_____
_____
_____
_____
_____
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_____
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_____

Fotos: Data da avaliação: / /

APÊNDICE I

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

FACULDADE DE ODONTOLOGIA

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

ÁREA DE CONCENTRAÇÃO EM ESTOMATOLOGIA CLÍNICA

FICHA DE AVALIAÇÃO HISTOLÓGICA

IDENTIFICAÇÃO **Rata nº___ Peso inicial:____kg** **Peso final:____kg** **Material de preenchimento:** **Grupo 1 (PMMA 2%)** **Grupo 2 (PMMA 30%)** **Grupo 3 (NaCl 0,9%)**	**Lâmina nº___** **Tempo:** **Subgrupo A (7 dias)** **Subgrupo B (90 dias)**
AVALIAÇÃO HISTOLÓGICA HE: **LÍNGUA** **VARIÁVEL** Microesferas: Presença Ausência Linfócitos Plasmócitos Macrófagos Eosinófilos Neutrófilos Células Gigantes Fibroplasia Edema Hiperemia	Sem inflamação Células mononucleares esparsas Infiltrado mononuclear e/ou neutrófilos e eosinófilos esparsos Infiltrado polimorfonucleares de neutrófilos e eosinófilos

LINFONODOS SUBMANDIBULARES

Presença de microesferas
Ausência de microesferas
Resposta inflamatória
Observações:

Fotos: **Data da avaliação: / /**

RIM DIREITO

Peso:____

Presença de microesferas
Ausência de microesferas
Resposta inflamatória
Observações:

Fotos: **Data da avaliação: / /**

FÍGADO

Peso:____

Presença de microesferas
Ausência de microesferas
Resposta inflamatória
Observações:

Fotos: **Data da avaliação: / /**

PULMÃO DIREITO

Peso:____

Presença de microesferas
Ausência de microesferas
Resposta inflamatória
Observações:

Fotos: **Data da avaliação: / /**

APÊNDICE J

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FACULDADE DE ODONTOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO EM ESTOMATOLOGIA CLÍNICA
FICHA DOS TESTES LABORATORIAIS</td></tr>
<tr><td>IDENTIFICAÇÃO

Rata nº___ Peso inicial:____kg
Peso final:____kg

Material de preenchimento:

Grupo 1 (PMMA 2%)

Grupo 2 (PMMA 30%)

Grupo 3 (NaCl 0,9%)</td><td>

Tubo nº___

Tempo:

Subgrupo A (7 dias)

Subgrupo B (90 dias)</td></tr>
<tr><td>RESULTADO AST:

RESULTADO ALT:

RESULTADO CREATININA:</td><td>

Data da avaliação: / /</td></tr>
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