FACULDADE DE ODONTOLOGIA

RESPOSTA TECIDUAL EM RATAS SUBMETIDAS À INJEÇÃO NA LÍNGUA DE POLIMETILMETACRILATO EM DISTINTAS CONCENTRAÇÕES - AVALIAÇÃO CLÍNICA E HISTOLÓGICA

KARLON FRÓES DE VARGAS

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Orientadora: Profa. Maria Antonia Zancanaro de Figueiredo

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“UM PASSO A FRENTE, E VOCÊ NÃO ESTÁ MAIS NO MESMO LUGAR.”

CHICO SCIENCE (1966-1997)

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RESUMO

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O Polimetilmetacrilato (PMMA) é um dos materiais utilizados no preenchimento facial, sendo atualmente o produto de maior emprego nesta área. Sua forma injetável é composta por microesferas dispersas em meio colóide de carboximetilcelulose, empregado, dentre outras aplicações cirúrgicas, para atenuar rugas, aumentar o volume e melhorar o contorno facial. As microesferas do material permanecem nos tecidos após o colágeno ser reabsorvido e tornam-se encapsuladas pelo tecido conjuntivo, o que contribui para formar o volume efetivo do material. O aparecimento de granulomas ou lesões nodulares após as injeções desse material tem sido descritas até 6 anos após sua aplicação, permanecendo desconhecida até o momento, a patogênese destes nódulos. O presente experimento teve como objetivo analisar clínica e histologicamente a reação tecidual local e sistêmica frente a injeção de PMMA, em diferentes concentrações e tempos experimentais. Foram utilizadas 54 ratas Wistar (Rattus norvegicus) divididas aleatoriamente em 3 grupos de acordo com o material utilizado (Grupo 1: PMMA 2%, Grupo 2: PMMA 30% e Grupo 3: NaCl 0,9%) e após em 3 subgrupos com distintos tempos de acompanhamento previstos (A: 7, B: 60 e C: 90 dias). Os animais foram previamente sedados e submetidos a injeção no ventre lingual de 0,07ml das respectivas substâncias. Clinicamente foram observadas nos grupos PMMA a 2% e PMMA a 30%, lesões ulceradas no período de 7 dias. Aos 60 dias do experimento, nos mesmos grupos presenciou-se lesões nodulares e placas esbranquiçadas. Após a eutanásia das ratas em seus respectivos períodos experimentais, foram removidos para análise microscópica, a língua e o rim direito. As peças operatórias das línguas foram coradas por hematoxilina/eosina (HE) e picrosírius, enquanto as dos rins, exclusivamente pela técnica de rotina (HE). Nos cortes da

estrutura da língua corados por HE, foram mensurados parâmetros de ausência ou presença de reação inflamatória, sendo esta classificada como ausente, leve, moderada ou intensa. Nos cortes corados com picrosírius foi estabelecida a quantificação de fibras colágenas presentes. Já nas amostras obtidas do rim, foram avaliadas a presença ou ausência de resposta inflamatória em toda a extensão da lâmina. Histologicamente notou-se reação inflamatória nos grupos testes, sendo que, no período de 7 dias, foi observada resposta tecidual intensa no grupo PMMA a 30%. No tempo de observação de 60 dias, nos grupos teste, a resposta inflamatória foi moderada, com presença de células gigantes do tipo corpo estranho. O mesmo quadro foi evidenciado aos 90 dias de experimento nos grupos PMMA a 2% e PMMA a 30%. A fibroplasia foi observada nos mesmos grupos em todos os períodos do estudo, sendo mais evidente no grupo PMMA a 30% e não sendo notada no grupo controle. Não houve resposta tecidual nas lâminas dos rins em todos os grupos da pesquisa, sugerindo a ausência de migração ou sistematização do material de preenchimento. As duas concentrações de PMMA produziram uma resposta inflamatória com presença marcante de células gigantes do tipo corpo estranho e uma maior fibroplasia na concentração de PMMA a 30%.

Palavras-chaves: materiais de preenchimento facial, polimetilmetacrilato, efeitos adversos, reação de corpo estranho.

SUMMARY

SUMMARY

Polymethylmethacrylate (PMMA) is one of the most commonly used facial filling material, being largely applied in this area. Its injectable form is composed of microspheres dispersed in a colloid medium of carboxymethylcellulose, applied for minimizing wrinkles, increasing volume and improving facial contour, among other surgical applications, and is also used as a type of bone cement. The material microspheres remain in the tissues after collagen is resorbed, becoming encapsulated by connective tissue, which contributes to the formation of the effective volume of material. The occurrence of granulomas or nodular lesions has been described until 6 years after its injection and nodule pathogenesis remains unknown. The present experiment had the objective of analyzing, both clinically and histologically, local and systemic tissue reaction to PMMA injections, in different concentrations and monitoring periods. Fifty-four Wistar female rats (Rattus norvegicus) were used. They were randomically divided into 3 groups according to the material used (Group 1: 2% PMMA, Group 2: 30% PMMA and Group 3: 0.9% NaCl) and then subdivided into 3 groups with different monitoring periods (A: 7, B: 60 and C: 90 days). In test groups 2% PMMA and 30% PMMA, ulcerations were clinically observed in the 7-day period. In the 60-day period, nodules and white plates were detected in groups 2% PMMA and 30% PMMA. After being euthanized, the rats had their tongue and right kidney removed for microscopic analysis. The tongue samples were stained with hematoxylin/eosin (HE) and picrosirius, while the kidneys received only HE. In the tongue sections stained with HE, absence or presence of inflammatory reaction was measured, being classified as absence, mild, moderate or severe. In the sections stained with picrosirius, collagen fiber density was established. In the kidney samples, presence or absence of inflammatory response was evaluated in the entire slide. Histologically,

there was presence of inflammatory reactions in test groups. In the 7-day period in group 30% PMMA there was severe tissue response. In the 60-day period, inflammatory responses in groups 2% PMMA and 30%PMMA were moderate, with presence of foreign body giant cells. The same could be seen in the 90-day period in test groups. Fibroplasia was observed in test groups from all monitoring periods, being more evident in 30% PMMA and totally absent in the control group. The microscopic evaluation of tissue response in kidney slides did not show alterations in all research groups, suggesting there was no migration or systematization of filling material. Both PMMA concentrations produced inflammatory response with strong presence of foreign body giant cells and more fibroplasia in the 30% PMMA concentration.

Key words: facial filling materials, polymethylmethacrylate, adverse effects, foreign body reaction.

SUMÁRIO

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INTRODUÇÃO

1. INTRODUÇÃO

A busca do homem por alternativas que auxiliem na recuperação ou na manutenção da eterna juventude tem sido, ao longo da história, assunto de grande interesse para diversas pesquisas. Nas últimas décadas, os materiais de preenchimento facial são alvos de inúmeros estudos, especialmente na área médica. No entanto, a principal dificuldade encontrada pelos pesquisadores é de conciliar as propriedades físico-químicas e mecânicas requeridas para a funcionalidade dos materiais utilizados no preenchimento facial atingindo um nível aceitável de reação tecidual. Esta é geralmente causada pela presença de moléculas solúveis ou íons liberados pelos materiais utilizados, quando em contato com os tecidos do organismo (ROMAGNOLI; BELMONTESI, 2008; SARNOFF et al., 2008; BUCK; ALAM; KIM, et al., 2009). Tais substâncias consistem de fluidos com alta viscosidade ou suspensão de micropartículas que são injetadas nos tecidos moles da face com objetivo estético e funcional. O referido procedimento visa reduzir os efeitos do envelhecimento, bem como a correção de defeitos congênitos ou traumáticos dos tecidos moles e duros da face. Dentre suas indicações, estão os portadores de esclerodermia, paralisias faciais unilaterais, cicatrizes de acne e, ainda, pacientes fissurados (LEMPERLE MORHENN; CHARRIER, 2003; LOMBARDI et al., 2004; NARINS; BOWMAN, 2005). Na grande maioria dos casos, as substâncias de preenchimento também podem ser associadas a procedimentos cirúrgicos, buscando a melhora dos seus resultados finais (NARINS; BOWMAN, 2005).

De acordo com alguns autores, estes materiais podem ser classificados conforme o grau de permanência nos tecidos após a sua injeção (LEMPERLE; ROMANO; BUSSO, 2003; ZIMMERMANN; CLERICI, 2004; NARINS; BOWMAN,

2005; LEMPERLE; FAZIO; NICOLAU, 2006; ATHRE, 2007; DAYAN; BASSICHIS, 2008; KARAGOZOGLU; VAN DER WAAL, 2008; SMITH, 2008; THIOLY-BENSOUSSAN, 2008; BUCK; ALAM; KIM, 2009). Os materiais temporários ou absorvíveis são aqueles que produzem resultados com tempo limitado de duração, ou seja, que sofrem um processo de reabsorção até 6 meses após a sua aplicação. Estes requerem injeções repetidas para que se consiga obter resultados prolongados.

Os materiais de preenchimento semi-permanentes são aqueles que sofrem reabsorção após um determinado período, variando este de 6 a 24 meses. Já os permanentes são descritos como substâncias que produzem resultados por longo tempo, uma vez que permanecerão indefinidamente no interior dos tecidos.

Para que seja considerado ideal, o mesmo deverá apresentar as seguintes características: ser biologicamente inerte (OWENS, 2005); não ser carcinogênico ou teratogênico (NARINS; BOWMAN, 2005; BUCK; ALAM; KIM, 2009); não sofrer migração no sítio da infiltração (NARINS; BOWMAN, 2005); ter consistência que permita seu uso com agulhas de pequeno calibre (NARINS; BOWMAN, 2005; EPPLEY; DADVAND, 2006; DHALIWAL; FRIEDMAN, 2008); não produzir reações alérgicas nem requerer teste dérmico (NARINS; BOWMAN, 2005); ter facilidade de inserção e remoção, quando requerida ou desejada (EPPLEY; DADVAND, 2006; BUCK; ALAM; KIM, 2009); não produzir resposta inflamatória persistente (EPPLEY; DADVAND, 2006; NAIR; LAURENCIN, 2007; DHALIWAL; FRIEDMAN, 2008); ter longo período de benefício (OWENS, 2005; EPPLEY; DADVAND, 2006; BUCK; ALAM; KIM, 2009); apresentar similaridade com o tecido normal, ou seja, textura semelhante, para que não seja palpável ou perceptível no exame físico (OWENS, 2005; BUCK; ALAM; KIM, 2009).

As primeiras substâncias de preenchimento facial a serem desenvolvidas foram o óleo de parafina e o silicone líquido, classificadas como permanentes ou não-biodegradáveis. Entretanto, o silicone líquido injetável foi removido do mercado devido a inúmeras complicações resultantes do seu uso, tais como, celulite, deformação no sítio da infiltração, além de intensa reação inflamatória local com consequente fibrose e migração de suas partículas, provocando o deslocamento do material (KALANTAR-HORMOZI et al., 2008; BUCK; ALAM; KIM, 2009).

As constantes pesquisas com utilização de substâncias naturais ou sintéticas vêm sendo amplamente estimuladas em razão da grande procura deste tipo de procedimento por parte dos pacientes, especialmente no que se refere aos polímeros (NAIR; LAURENCIN, 2007; DAYAN; BASSICHIS, 2008; ROMAGNOLI; BELMONTESI, 2008; BUCK; ALAM; KIM, 2009). Os polímeros no formato rígido, como por exemplo, o metacrilato de metila, são frequentemente utilizados na reconstrução da calvária. Na forma líquida, temos o polimetilmetacrilato (PMMA), formado por microesferas (30 a 40µm de diâmetro) de superfície lisa e 3,5% de colágeno bovino, desenvolvido para implantação subdérmica e classificado como implante sintético permanente. Existem ainda outras formas de polímeros, tais como o poliuretano, polietileno, teflon e o dácron, sendo estes utilizados para cobrir as próteses mamárias de silicone, reforço na parede abdominal, correção de fraturas do soalho da órbita e do nariz (ACHILLES, 2004).

Mais recentemente, diversas outras substâncias absorvíveis de preenchimento têm sido disponibilizadas no mercado, incluindo o colágeno humano, o ácido hialurônico (AH) e os polímeros biossintéticos (BUCK; ALAM; KIM, 2009).

Atualmente encontra-se disponível no mercado uma grande diversidade de substâncias de preenchimento de tecidos moles, cada uma com seus distintos constituintes químicos, indicações e efetividade. É fundamental que os profissionais habilitados tenham pleno conhecimento das propriedades dos materiais utilizados, proporcionando, consequentemente, o tratamento cosmético mais adequado para cada paciente (BUCK; ALAM; KIM, 2009).

A segurança na utilização dos materiais de preenchimento injetáveis é ainda um assunto bastante discutido por diversos autores. Embora, os biomateriais conduzam a um menor risco de complicações do que os aloplásticos, a redução dos efeitos colaterais decorre da rápida degradação dos mesmos, resultando numa perda de efeito clínico e na necessidade de repetição do procedimento. A literatura descreve que a maioria das reações adversas foram causadas pelo uso de silicone, seguidas da injeção de colágeno e do AH. Dentre os materiais eventualmente associados a efeitos indesejáveis estão as suspensões de hidrogel acrílico, ácido poliláctico, polimetilmetacrilato e o politetrafluoretileno (JHAM et al., 2009).

A patogênese das reações adversas destes produtos é ainda desconhecida. Quando os biomateriais são injetados nos tecidos, observa-se uma reação granulomatosa com presença de histiócitos e formação de novo colágeno circundando a área. Alguns pacientes desenvolverão uma reação tecidual mais severa resultando clinicamente em nódulos visíveis. Outros estudos sugerem o aparecimento de lesões envolvendo uma reação imunológica, na qual a substância de preenchimento pode agir como um estímulo para infecção ou contaminação cruzada via técnica injetável. Além disso, alguns pesquisadores consideraram que os efeitos indesejáveis também podem ser causados por impurezas presentes nas preparações, técnicas e pacientes

inadequadamente selecionados, ou, ainda, devido a inexperiência dos profissionais (BUCK; ALAM; KIM, 2009; JHAM et al., 2009). Embora muitos desses efeitos colaterais sejam temporários, torna-se importante comunicar previamente aos pacientes a possível ocorrência dos mesmos.

A presença de hemorragia, a partir da utilização dos materiais de preenchimento, é comumente associada a pacientes que utilizam terapias anticoagulantes (GLADSTONE; COHEN, 2007; BUCK; ALAM; KIM, 2009). As complicações infecciosas são raras, entretanto, pacientes com suscetibilidade às mesmas ou com história de herpes simples, por exemplo, deverão realizar previamente terapia antibacteriana e/ou antiviral (BUCK; ALAM; KIM, 2009).

A reação alérgica aguda é considerada uma séria complicação, quando utilizados preenchimentos que contenham componentes bovinos e xenogênicos. Para minimizar estes riscos, é sempre recomendável a realização de testes dérmicos prévios ao tratamento (ATHRE, 2007; PRICE; BERRY; NAVSARIA, 2007; DHALIWAL; FRIEDMAN, 2008; BUCK; ALAM; KIM, 2009; JHAM et al., 2009; KUSIN; LIPPITZ, 2009). Comumente o paciente refere a presença de dor após a injeção do material, podendo esta ser reduzida com o uso de agulhas de menores calibres (LOMBARDI et al., 2004; BUCK; ALAM; KIM, 2009; JHAM et al., 2009). A anestesia tópica ou regional, incluindo bloqueio nervoso, também pode ser utilizada (BUCK; ALAM; KIM, 2009). A persistência de reação inflamatória inespecífica para preenchimentos semi-permanentes e permanentes conduz o tecido a um processo inflamatório crônico (ZIMMERMANN; CLERICI, 2004; PRICE; BERRY; NAVSARIA, 2007; JHAM et al., 2009). A formação de abscesso é rara, e pode estar presente no período de 7 dias até 12 meses após a infiltração (ZIMMERMANN; CLERICI, 2004). Mesmo com o uso de

biomateriais, como o AH, as reações granulomatosas severas ainda têm sido descritas. Tais alterações podem surgir no período de 6 meses até alguns anos após o procedimento, sendo esta manifestação frequentemente tratada através da excisão cirúrgica (LOMBARDI et al., 2004; GLADSTONE; COHEN, 2007; DHALIWAL; FRIEDMAN, 2008; KALANTAR-HORMOZI et al., 2008; BUCK; ALAM; KIM, 2009; DA COSTA et al., 2009; JHAM et al., 2009). A ocorrência de adversidades menores, tais como hematoma, edema, descoloração e sensibilidade da epiderme também foram descritas por alguns autores (GLADSTONE; COHEN, 2007; PRICE; BERRY; NAVSARIA, 2007; DHALIWAL; FRIEDMAN, 2008; KALANTAR-HORMOZI et al., 2008). Entretanto, estas complicações são ditas temporárias, tendo sua resolução usualmente no prazo de duas semanas (DHALIWAL; FRIEDMAN, 2008). O edema transitório pode persistir de 24 a 72 horas sendo o mesmo resultado da irritação local pelo implante do produto ou ainda pela utilização de técnica inadequada (ATHRE, 2007; GLADSTONE; COHEN, 2007; JHAM et al., 2009).

Muitos pacientes buscam os profissionais para atendimento especializado referindo como queixa principal uma assimetria facial. Portanto, sugere-se que sempre seja feita uma documentação fotográfica dos casos clínicos nos períodos pré e pós-procedimento evitando transtornos posteriores (GLADSTONE; COHEN, 2007; MATARASSO, 2008).

POLIMETILMETACRILATO

Introduzido comercialmente na Alemanha a partir de 1991, o Polimetilmetacrilato (PMMA) é a combinação de colágeno líquido e microesferas de

polimetilmetacrilato (sólido), sendo muito utilizado para o preenchimento de partes moles em cirurgias estéticas. As microesferas do material permanecem nos tecidos após o colágeno ser reabsorvido e tornam-se encapsuladas pelo tecido conjuntivo, o que contribui para a obtenção do resultado final. O aparecimento de granulomas ou lesões nodulares após as injeções desse material tem sido descritas até 6 anos após sua injeção. A patogênese destes nódulos é desconhecida, entretanto em alguns casos, os tratamentos realizados com esteróides têm apresentado êxito (CHRISTENSEN et al. 2005). O PMMA representa no momento, a opção de maior escolha utilizada para preenchimento cosmético com materiais permanentes. Sabe-se que durante o período inicial, de 4 a 6 semanas após a injeção do produto, as esferas de PMMA provocam uma reação de corpo estranho, sendo, posteriormente, encapsuladas pelas fibras colágenas do tecido, às quais previnem a migração do material (KUSIN; LIPPITZ, 2009).

O PMMA encontra-se disponível para uso em distintas concentrações, variando estas de acordo com a sua indicação. O PMMA 2% é utilizado na região intradérmica para minimizar rugas finas especialmente na área labial. O PMMA 10% é indicado para áreas móveis visando diminuir o aspecto de cansaço provocado pela flacidez. Já o PMMA 30% é indicado para injeção onde exista uma estrutura óssea abaixo, sempre a nível intramuscular ou justaperiostal. Nesta concentração é utilizado para aumentar o volume da região de interesse.

Inúmeras pesquisas recentes têm sugerido o uso de distintas modalidades estéticas alternativas visando restaurar os tecidos faciais perdidos ou alterados com o avanço da idade. Entretanto, os efeitos adversos do preenchimento de determinadas regiões da face com materiais disponíveis no mercado, no que se refere à biocompatibilidade, permanece como uma das mais importantes complicações do seu

uso, tendo impacto significativo no resultado esperado pelos pacientes. Assim, a busca contínua de alternativas que proporcionem melhores condições de bem-estar e saúde àquelas pessoas que desejam realizar procedimentos rejuvenescedores, bem como, a investigação de possíveis efeitos indesejáveis constitui, sem dúvidas, uma área de grande interesse para pesquisas.

Através deste experimento buscou-se avaliar as respostas clínicas e histológicas em língua e rim de ratas submetidas a injeção de PMMA, um material de preenchimento estético amplamente utilizado na atualidade. Por meio de um estudo padronizado, objetivou-se identificar, analisar e comparar tais reações, visando um maior conhecimento científico dentro do tema proposto.

ARTIGO 1

2. ARTIGO 1

O artigo a seguir intitula-se “**USE OF POLYMETHYLMETHACRYLATE AS PERMANENT FILLING AGENT IN THE JAW, MOUTH AND FACE REGIONS – IMPLICATIONS ON DENTAL PRACTICE”** o mesmo foi submetido e aceito pelo periódico Gerodontology (Anexo A).

USE OF POLYMETHYLMETHACRYLATE AS PERMANENT FILLING AGENT IN THE JAW, MOUTH AND FACE REGIONS – IMPLICATIONS ON DENTAL PRACTICE

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2.1. SUMMARY

The search for becoming young is a constant attitude amongst Brazilians. The low cost of filling agents have been stimulating its indiscriminate use, especially the bioplasty (non-incisional method). The polymethylmethacrylate (PMMA) is the cosmetic filling agent of choice, due to its low cost, easy access and simple application technique. The impact on Dentistry and Oral Medicine needs further awareness. This paper is a literature review of PMMA focusing its properties, indications, contraindications and adverse effects. The article aims at calling the attention of odontology and medical professionals to the product's applicability and its possible complications.

Key Words: facial filling agents, polymethylmethacrylate, adverse effects, polymers, foreign body reaction

2.2. INTRODUCTION

Search for alternatives which can assist in the recovery or conservation of human youth has been subject of great interest in several researches across the years. During the past decades, the facial filling agents are target of numerous studies, especially in the medical field. The procedure aims at retarding the aging effects, as well as correcting congenital or traumatic defects of both soft and hard facial tissues. However, there are very few studies relating the long-term physical, chemical and mechanical properties required for the functionality of these materials (1); (2); (3).

Although there are several filling agents available in the market, the ideal product is yet to be created. A filling agent can be qualified as biocompatible when it does not initiate immunologic reaction, is not cancerigenous, does not produce infectious response or migrate, and brings long-term benefits. Other desirable characteristics are effective cost, simple handling and, whenever necessary, easy insertion and remotion (4); (5); (6); (7).

According to some authors (3); (8); (9); (10); (11) any material injected in tissues must not alter their functional characteristics so that the natural aspect of the organ can be maintained.

The facial filling agents can be classified according to their degree of permanence in the tissues after application. Temporary and absorbable materials are the ones which produce a limited time effect, meaning they are absorbed up to 6 months following application. These materials require repeated injections so that long-term clinical results can be achieved. The permanent materials, on the other hand, are

described as substances which produce long-term results once they shall indefinitely remain inside the tissues (1); (3); (12); (13); (14); (15); (16); (17); (18).

2.3. BACKGROUND

The polymethylmethacrylate (PMMA) is a synthetic polymer produced for the first time in 1902 by the German chemist Rahm and patented in 1928 as Plexiglass®. It was first used in 1938 to reconstruct monkey skulls, being later used in orthopedic surgical procedures as bone cement (19); (20); (21); (22). The substance was commercially introduced in Germany in 1991. This product is obtained through the combination of liquid collagen and solid PMMA microspheres, being used for filling soft tissues in aesthetic surgery, known as bioplasty, non-incisional and minimally invasive method (23); (24); (25). The injectable form is composed of disperse microspheres in carboxymethylcellulose colloid, applied for wrinkles reduction, volume increase and improvement of the facial contour, among other surgical applications (17); (19); (20); (21); (25); (26); (27); (28); (29); (30); (31); (32); (33); (34).

The first experimental study using PMMA in soft tissues occurred in Germany. Lemperle et al. (1991) did the first tests implanting nonabsorbable particles in rat skin. In these studies, a smaller number of reactions was achieved with the PMMA microspheres, then used mainly as bone cement. Only in 1994 there was the idea of mixing PMMA michrospheres to bovine collagen creating a pasty vehicle which was easy to apply by using thin needles on the subdermis.

From then on, a longer permanance of the implant, collagen compounds mixed to PMMA michrosphere, was noticed, causing great expectations for researchers and the medical community.

In 2001, PMMA started being applied to the gastroesophageal reflux therapy through the implant in the esophageal submucosa via transoral endoscopy (20); (21); (22). It is also used in the correction of body deformities, in intraocular lens, in preventive haemostasis and in urinary bladder dysfunction (19); (23); (24); (35); (36); (37); (38); (39); (40).

2.4. CHEMICAL CHARACTERISTICS

PMMA is considered a rigid thermoplastic; it is transparent in visible light and presents great resistance in ambient conditions. It is considered the most important of the thermoplastic group designated acrylic (41); (42).

Several companies regularly commercialize PMMA compounds. They are available in the market under the following trade names: Artecoll®, Artefill®, Metacrill® and Newplastic®. The products vary in terms of carrier substance and microspheres size and they can be found already prepared in syringes or in glass vials (23); (24); (25); (40); (43); (44); (45).

According to Lemperle et al. (2003), the injectable PMMA (ArteFill, Artes Medical, San Diego, California) is a suspension containing 20% of smooth PMMA microspheres and 80% of bovine collagen and it was approved by the FDA in 2006 for the correction of nasolabial folds. It represents a product containing few nanoparticles

(smaller than 20 microns) and such particles are frequently associated with granulomatous reactions (1).

2.5. PMMA INDICATIONS

PMMA has been usually recommended for lipodystrophy correction due to the use of antiretrovirals in HIV positive patients (46), facial contour (1); (17); (34); (47), glabellar lines (47); (48), nasolabial folds (1); (17); (34); (47), filling of the malar region (48); (49); wrinkles correction (17); (34); (48); (50) and lip filling (17); (34); (48); (49); (50).

PMMA is available for use in three distinct concentrations (2%, 10% and 30%), varying according to its respective indication.

- 2% PMMA - The only concentration that can be applied with a needle, in depth, in the intradermal region, indicated for minimizing thin wrinkles, especially in the lip region.

- 10% PMMA - Used in moving areas aiming at reducing the aspect of fatigue provoked by flaccidity (nasolabial folds, expression marks, lips and hand dorsum), being invariably injected in the subcutaneous tissue.

-30% PMMA - Indicated for intramuscular or periosteal areas. In this concentration, the aim is to increase the volume of the region of interest, such as: chin, malar, mandibule line, nose correction, nasolabial folds, gluteal augmentation, chest, biceps and calves.

Due to technology advances, it has been possible to create personalized PMMA implants by using high-resolution computed tomography modeling. Customized implants are made to meet the patient's specific needs. In a study developed by Groth and collaborators (2006) significant complications have not been found and the results obtained proved to be satisfactory in long term for the correction of orbitofacial defects. This technique can be applied to other alloplastic implants, promoting a better contour to the implant and reducing surgery time (51).

2.6. CONTRAINDICATIONS

The PMMA application is contraindicated in the presence of inflammatory processes near the region of interest (52); (53); and for patients who are allergic to xenogenic compounds (bovine collagen).

Lemperle et al. (1995) contraindicate the PMMA injection to breast augmentation, tendons, ligaments and bones, during pregnancy or breast-feeding and to patients with platelet or coagulation disorders (50).

2.7. TISSUE RESPONSE

The PMMA microspheres remain in the tissues after collagen is absorbed and are encapsulated by connective tissue, which contributes to the formation of effective volume of the material. The defect correction occurs due to the sum of the volume of the injected product and the cellular and fiber growth around area (32).

After the injection of the substance in the organism, there are intercellular reactions through chemical mediators. The macrophages, not being able to phagocytize large and smooth spheres, group up in giant cells which enwrap the spheres for a period of approximately 14 days (54); (55).

Allen (1992) described in a longitudinal study the cellular reactions after injections of inert materials in the human subdermis. In the first 24 hours, neutrophils and small round cells were predominant. Monocytes were present in a larger number in the first 48 hours; and the foreign-body giant cells in approximately 7 days. After 14 days, cellular response was already considered moderate. After 30 days, monocytes differentiated into epithelioid cells and the first fibroblasts appeared. After 6 weeks, foreign-body giant cells and collagen fibers were noticed. In 60 days, there were chronic inflammatory cells spread throughout the massive collagen concentration. Afterwards, the foreign-body cellular reaction stabilizes and in 6 months, giant cells, low intensity of cellular response and low quantity of dense collagen and fibroblasts converting into fibrocytes are observed (56).

Some authors report the occurrence of dermal granulomas in the subcutaneous tissue and the skeletal muscle. In a thorough evaluation, epithelioid cells, foreign-body giant cells and limphocytes are noticed. According to the same researchers, the histological pattern remained the same, though they underlined the fact that there is a lack of appropriate studies (57).

In a histopathological study after the implantation of PMMA in humans, noted that the predominant inflammatory infiltrate was lymphohistiocytic, adnexal and perivascular. In 9 days, the gaps between the microspheres presented fibroblasts. After a month, a thin fibrous capsule was noticed involving the particles. After 60 days, there

was an increase in collagen density and they concluded that the fibrosis and neovascularization phases ended after 4 months (12).

Particle migration, according to some researchers, was detected in small number of cases due to the diameter and the completely smooth surfaces of the particles, making phagocytosis difficult. Histologic experiments proved a low rate of macrophages with microspheres inside, assuring the permanence of the implant at its place (58); (59); (60).

Morhenn et al. (2002) reported that particles smaller than 20 μm are easily phagocytized by macrophages. According to the authors, the implant displacement is avoided by the fast individual encapsulation of spheres (54).

PMMA represents, at the moment, the most chosen option for cosmetic filling with permanent materials. It is known that during the initial period, from 4 to 6 weeks after the product's injection, the PMMA spheres provoke a foreign-body reaction, being later encapsulated by the collagen fibers in the tissue, which prevent material migration (34).

2.8. ADVERSE EFFECTS AND COMPLICATIONS

After PMMA application, short-term adverse reactions are observed such as: pain, edema, itching, allergic reactions and hematomas. The presence of hemorrhage after the use of filling materials is associated with patients who use anticoagulant therapies (11); (61). Infectious complications are rare, though patients susceptible to infections or the ones who have history of herpes simplex, for instance, should undergo antibacterial and/or antiviral therapy (13). Acute allergic reactions are considered a

severe complication when fillings contain xenogenic or bovine compounds. In order to minimize those risks, it is always recommended to perform skin tests before the treatment (11); (34); (61); (62); (63); (64); (65).

In the literature, there is a report of a case of blindness due to the implantation of several materials, including PMMA, in glabellar lines. This complication is due to the intercommunication between cutaneous, subcutaneous and ocular vessels (46). No severe illness, trauma, death or carcinogenesis is associated with PMMA implants (20).

There are complications related to the application technique, for instance the prolonged erythema or visible granules due to a rather superficial implantation (25). This abscess formation is extremely rare though it can be present in a period of 7 days to 12 months after infiltration (12).

Pollack (1999) reported that a larger percentage of complications is related to the implantation in the superficial dermis under the form of a foreign-body granulomas (66).

In an experimental study, the following materials were compared: PMMA with bovine collagen (Artecoll® - Artes Medical Inc., San Diego, California, USA), Dimethylsiloxane (DMS® - Vikomed, Meinerzhagen, Germany), Hyaluronic Acid (Restylane® - Q-Med AB, Uppsala, Sweden) and PMMA (Metacrill® - Nutricel, São Bernardo do Campo, Brazil), injected in mice ears with a 6-month follow-up. There were different histologic patterns for each tested substance in comparison with the controls. In the PMMA case, there was the development of an intense foreign-body granulomatous reaction, followed by the absorption of PMMA spheres. Some mice implanted with PMMA and dimethylsiloxane presented severe complications with the

migration of the substance used to the liver, producing a hepatic abscess and to the kidneys, producing interstitial nephritis and chronic pyelonephritis (67).

According to Laeschke (2004), 5 individuals reported 6 late events that occurred from 2 to 5 years after the initial injection. The total number of adverse reactions occurred in 272 cases of treatment of wrinkles was 6, equivalent to 2.2%. Granulomatous reactions can be treated with intralesional cortisone combined with an antibiotics therapy (68).

The importance of the microparticles chemical composition is due to the possibility that the material can be biodegradable. Depending on its chemical structure and surface characteristics, biological and synthetic materials such as the polymethylmethacrylate and polylactic acid start a foreign-body reaction which can last up to several months (66).

For some decades, PMMA has been used as bone cement. The occurrence of granulomes or nodular lesions after injections of this material has been described up to 6 years after injection. The pathogenesis of these nodules is unknown. However, in some cases, the treatment of these complications has been successful when steroids are used (69).

There is the description of 2 types of granulomatous complications: the ones produced by defect or lack of technique by the applier and the ones caused by the product itself. The most frequent defects after application are the overcorrection. The nodules formation and the inflammatory responses are cyclic. In part of the cases, these complications disappear spontaneously or with the use of systemic or intralesional corticosteroids (70).

The adverse effects and the complications from PMMA as a facial filling agent can be acute as edema, ecchymosis, erythema, dyschromia or they can be late, such as embolism, granulomatous reactions, idiosyncratic reactions to the injectable liquid, as well as the migration of the injected product (70).

Complications such as granulomas vary between 0.01 a 2.5 % of applications, taking into consideration the different PMMA filling agent manufacturers. The presence of small and painless nodules is common after PMMA application but there are hardly ever formal complaints by the patients. In Brazil, there are no studies which are completely trustworthy regarding the frequency of those small nodules present after PMMA applications (71).

With the use of PMMA in areas such as nasolabial folds, lips and chin, the dentists must improve their knowledge on facial filling agents adverse reactions, once it is of great diagnostic interest to those professionals. The usual complications presentation of these materials clinically occurs as nodules in the oral mucosa or submucosa, making it hard for professionals to identify them once they are similar to salivary gland neoplasms and liposarcomas.

2.9. FINAL CONSIDERATIONS

The demand to restore facial volumetric loss which occurs due to the aging process has stimulated the appearance of several filling agents for aesthetic reasons. Although patients desire long-lasting results, the use of permanent materials must be observed, aiming at a safe way which avoids long and short-term complications. There is a higher level of concern regarding the use of PMMA due to past experiences of misuse of liquid silicone. PMMA is being indiscriminately applied due to its low cost

and easy access. Sales control of the product is not restricted; therefore those who are not medical professionals or medical professionals without proper training qualification have access to the product and apply the substance.

As described in the literature, several precepts are necessary to the correct indication and application of the product. Besides, the granulomatous reactions can occur regardless of the used technique, varying individually from a patient to another. The clinical treatment is always the first option, for both local and systemic complications and surgical therapy should only be chosen for carefully selected cases. The treatment for late complications is long and complex, demanding patience from the patient and great skills from the plastic surgeon.

Studies with critical analysis of results and a longer follow-up of patients are necessary in order to obtain definitive conclusions. Permanent filling agents can provide long-lasting corrections, though this type of substance might not be ideal, once the product remains static while the overlying dermis suffers dynamic changes resulting from the aging process.

In order to avoid incorrect uses responsible for a nonexistent therapeutical result or the worsening of a situation causing a permanent defect in the patient, it is necessary to study and precisely know the anatomy, the filling agents, the possibilities of integration of tissue and injected product and the compatibility between the agents and the patient. Although there is not a material that can be universally applied, there are several trustable products which minimize risks, considering they are still available. It is expected that science and biology industry may provide other biocompatible materials in a near future so that this branch of aesthetic medicine can continue to progress.

Thus, this literature review is justified with the aim of stimulating the constant search for alternatives which can improve better well-being and health conditions to those who want to undergo rejuvenating procedures, as well as investigating possible undesirable effects aiming at the safe use of these products on patients.

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ARTIGO 2

3. ARTIGO 2

O artigo a seguir intitula-se **“TISSUE RESPONSE IN FEMALE RATS SUBMITTED TO PMMA INJECTIONS IN TONGUE IN DIFFERENT CONCENTRATIONS”** e foi formatado e submetido conforme as normas do periódico Oral Diseases (Anexo B)

TISSUE RESPONSE IN FEMALE RATS SUBMITTED TO PMMA INJECTIONS IN TONGUE IN DIFFERENT CONCENTRATIONS

TISSUE RESPONSE TO PMMA IN FEMALE RATS

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3.1. SUMMARY

This study evaluated clinically and histologically local and systemic inflammatory reactions in 54 female rats, submitted to tongue injection of 2% polymethylmethacrylate, 30% polymethylmethacrylate and 0.9% NaCl in periods of 7, 60 and 90 days. They were clinically evaluated and then euthanized, having their tongue and right kidney removed. The specimens were fixed in 10% formalin for optical microscopy analysis and the histologic sections were stained with hematoxylin/eosin, the tongue slides also being stained with picrosirius. Clinically 33.3% of group 2% PMMA samples presented ulcers in the ventral tongue after 7 days. After 60 days, nodules could be noticed in 60% of the animals in group 30% PMMA. After 90 days, however, no group presented clinical alterations. Histologically, in 88.8% of samples there was presence of inflammatory infiltrate, with strong presence of foreign body giant cells. The systemic evaluation performed by the renal histological analysis did not show any migration or systematization of the material with absence of long distance inflammatory response. It was possible to conclude that, regardless of the PMMA concentrations used, they induced an intense reaction (7 days), followed by gradual reduction during the study, favoring the presence of fibroplasia adjacent to the material.

Key words: facial filling materials, polymethylmethacrylate, adverse effects, foreign body reaction, rats

3.2. INTRODUCTION

Several types of substances can be used as facial filling material with aesthetic purposes. Among them, there are collagen (autologous, bovine, cadaveric and derived from fibroblasts), hyaluronic acid (biological and synthetic), polylactic acid, autologous fat, silicone, botulinum toxin and polymethylmethacrylate (PMMA). For each material there are different indications for use varying according to concentration levels.

PMMA is a synthetic polymer commercially introduced in Germany in 1991. This product is composed by the combination of liquid collagen and (solid) PMMA microspheres, being largely used for soft tissue fillings in aesthetic surgeries (Achilles, 2004, Dayan, Bassichis, 2008, Kusin, Lippittz, 2009, Lemperle et al., 2003a, Nacul, 2005). It currently represents the most commonly used option for cosmetic filling with permanent materials. Its injectable form is composed of microspheres dispersed in a colloid medium of carboxymethylcellulose, applied for minimizing wrinkles, increasing volume and improving facial contour, among other surgical applications, and is also used as a type of bone cement.

PMMA is available for use in several concentrations, ranging accordingly to its respective indication. PMMA in 2% concentration is indicated for minimizing thin wrinkles especially in the lip region while 10% PMMA is used for motile areas aiming at reducing the aspect of fatigue caused by flaccidity (nasolabial folds, expression marks, lips, back of hands). A concentration of 30% is indicated for increasing the volume of the region of interest, such as: chin, malar, jaw line, nasal corrections, nasolabial folds, gluteus augmentation, chest, biceps and calves.

The material microspheres remain in the tissues after collagen is resorbed, becoming encapsulated by connective tissue, which contributes to the formation of the effective volume of material (Morhenn et al., 2002). The occurrence of granulomas or nodular lesions after injecting this material has been described until 6 years after its injection. The nodules pathogeny is unknown, though in some cases, treatments with steroids have been successful (Christensen et al., 2005). It is known that, during the initial period (4 to 6 months after injection), the PMMA spheres provoke a foreign body reaction and are later encapsulated by the collagen fibers of the tissue, which prevents material migration (Kusin, Lippitz, 2009).

Safety while using injectable filling materials is still debated by several authors (Achilles, 2004, Quatela, Chow, 2008, Sabatovich, Kede, 2004). Although biomaterials lead to smaller risks of complications then the alloplastic ones, the reduction of side effects is due to the rapid degradation of materials, resulting in loss of clinical effect and in the necessity of repeating the procedure. Scientific literature describes that most adverse reactions were caused by the use of silicone, followed by injections of collagen and hyaluronic acid (HA). Among the materials occasionally associated to undesirable effects are the following suspensions: acrylic hydrogel, polylactic acid, PMMA and polytetrafluoroethylene (Jham et al., 2009).

The pathogenesis of adverse reactions for these products is still unknown. When exogenous materials are injected in the tissues, a granulomatous reaction with presence of histiocytes and the formation of new collagen surrounding the area can be observed (Pistóia, Figueiredo, 2002). Dayan and Bassichis (2008) mention that some patients may develop a more severe tissue reaction resulting in clinically visible nodules. Besides, they have considered that undesirable effects can also be caused by

impurity present in preparations, techniques, and inappropriately selected patients or even by inexperienced professionals. Although many of those side effects can be temporary, it is essential to communicate to the patients their possible occurrence, before they undergo any procedure (Vargas et al., 2011).

Several recent researches have suggested the use of distinct alternative aesthetics modalities aiming at restoring lost or altered facial tissues due to aging. However, the adverse effects of filling determined facial regions with available materials in the market, with regards to biocompatibility, remains as one of the most important complications of their use in organic tissues, having significant impact on the result expected by the patient. Thus, the continuous search for alternatives which can provide better well-being and health to those who wish to go through rejuvenating procedures, as well as, the investigation of possible undesirable effects constitute, an area of great interest for scientific research in Dentistry, since many facial procedures, for other purposes, reflect on the anatomic region directly related to dental surgeons.

3.3. MATERIAL AND METHODS

This research was done after approval from the Scientific and Ethic Committee for Animal. This investigation was through a randomized longitudinal experimental study using 54 2-month-old female Wistar rats (Rattus norveggicus) which were healthy, weighing approximately 200g, from the same animal facility. PMMA at 2% and 30% (*NewPlastic*®; *Lebon Produtos Químicos e Farmacêuticos Ltda, Brasil*) were respectively used in 2 distinct test groups, 1 and 2. The rats were kept in an appropriate place, with ventilation, air filtration and 22ºC temperature, in 12-hour light-dark cycles, fed with balanced rat chow and filtered water *ad libitum*.

3.3.1. Study groups

The animals were randomly divided into 3 groups, according to the material used: Group 1: 2% Polymethylmethacrylate (18 animals); Group 2: 30% Polymethylmethacrylate (18 animals); Group 3 - control - Saline (0.9% Na Cl) (18 animals). Each group was subdivided into subgroups A, B and C respectively according to the estimated monitoring time until the euthanasia of the animals (7, 60 and 90 days).

3.3.2. Procedure for anesthesia of animals

The animals were manipulated in accordance with the Brazilian College of Animal Experimentation (COBEA). Initially the female rat number 1 was weighed in a digital scale so that the dosage of anesthetic could be calculated. This procedure was performed with an intraperitoneal injection of a mixture of Xylazine Hidrochloride (20mg/mL) 0,05mL/100g, with Ketamine Hydrochloride (50mg/mL) 0,1mL/100g. Animals from the respective groups (1, 2 e 3), randomly chosen, were successively anesthetized as they were submitted to the standard injection procedures for each material.

3.3.3. Procedure for 2% and 30% PMMA and 0.9% NaCl injections

As sedation could be observed, the animal was placed on a surgical table, in supine position and having their paws tied using elastic strips. The tongue of rat 1 was pulled out with a tweezer exposing the ventral tongue region. Using a disposable insulin syringe , 0,07 mL of the filling material (2% PMMA) was injected in the middle third of the ventral tongue, 7mm ahead of the frenum. The needle was inclined as parallel as possible to the mucosa, with the bevel facing up, 7mm deep, and this measure being standardized by an endodontic silicone stop. The infiltration of substances used in

animals from all groups of this study followed the same administration protocols established for rat 1, varying exclusively the material to be injected according to the respective group.

3.3.4. Clinical analysis

After 7 days, preceding the euthanasia of the animals from 2% PMMA group , there was sedation and subsequent clinical evaluation of each rat's tongue. The same procedure was performed in subgroups B (60 days) and C (90 days) according to the respective monitoring periods. In the clinical analysis, possible tissue alterations such as edema, ulcer, nodule or fibrosis were observed.

3.3.5. Euthanasia

At the end of clinical analysis, euthanasia was performed through isoflurane inhalation. After necropsy, the animals were treated as biohazard waste, being frozen and collected in accordance with the regulations of the PUCRS animal facility.

3.3.6. Sample processing

After being euthanized, all animals were necropsied, having their tongue and right kidney removed for microscopic analysis. Sample fixation was carried out with the use of 10% neutral buffered formalin for a minimum of 24 hours and samples were sent to the pathological analysis laboratory. Samples of the tongue were sectioned longitudinally into equal parts for later inclusion in paraffin block. The inclusion was done so that the edge of the tongue sample had its long axis parallel to the paraffin block section plan. From each specimen, 2 histological sections of 6µm each in order to obtain 2 slides, later stained with hematoxylin and eosin (HE) and picrosirius (specific coloration for collagen fiber analysis) (Vier-Pelisser et al., 2007).

3.3.7. Histological analysis

The tongue slides were validated for histopathological analysis through the identification of the filling agent in the Oral Medicine Service Unit (São Lucas Hospital) through light microscopy in approximate magnifications of 40, 100, 200 and x400. So that there could be a standard criteria judgment, there was a training session with an experienced pathologist. The intra-examiner calibration was performed with the reanalysis of each slide in a 7-day interval between observations. For the slides analysis, the examiner was previously calibrated and blinded with the use of slide masks in all procedures.

3.3.7.1. Inflammatory reaction

For groups 2% PMMA and 30% PMMA, the histological evaluation was performed with an analysis of the microscopic fields adjacent to the filling material, choosing for analysis the ones which showed a higher intensity of inflammatory response. Thus, the absence or presence of lymphocytes, plasma cells, macrophages, neutrophils, eosinophils, giant cells, fibroblastic condensation and hyperemia was analyzed with x200 magnification. For 0.9% NaCl group, field selection and analysis were carried out according to the anatomical references where 0.9% NaCl was injected. In order to standardize inflammatory scores, the parameters described by Figueiredo et al., (2001) and Gomes et al., (2007) were adapted as follows: **1**: absence of inflammation; **2**: sparse mononuclear cells; **3**: infiltrate of mononuclear cells and/or sparse neutrophils and eosinophils; **4**: infiltrate of neutrophils and eosinophils.

3.3.7.2. Connective tissue reaction

Tongue slides stained by picrosirius were selected through the identification of the injected filling material. In the control group, the selection was made by using the anatomical reference and the applied methodology. Three to four areas (μm^2) which contained most connective tissue were selected using x100 magnification. Those images were exported to the *Image Pro Plus*® version 4,5.1 software (Media Cybernetics, Inc.; 2005), in which images obtained with polarized light are recognized and converted into shades of red (collagen representative area). Thus, the proportion of collagen fibers was determined by the calculation of the areas occupied by them in comparison to the total area of each field (Vier-Pelisser et al., 2007).

3.3.7.3. Migration

Migration or material systematization was evaluated microscopically in all experiment groups, based on the presence or absence of inflammatory response in the right kidney of each female rat.

3.3.8. Statistical analysis

In order to perform the statistical analysis, the following softwares were used: SPSS 17 (*SPSS Inc.*) and SYSTAT 13 (*Systat Software Inc.*). Kruskal-Wallis tests with Dwass-Steel-Chritchlow-Fligner Post Hoc tests were used for all pairwise comparisons, considering the differences with significance levels set at 5% ($p<0,05$). To analyze the picrosirius (which has a numeric variable), Mixed Models with Fisher Post Hoc analysis were used with significance levels set at 5%. The fixed effects of the statistical model were group, time and time and group interaction whereas the random effect was the animal whose histological slide had multiple reading fields.

For the examiner calibration (re-analysis of each slide every 7 days), the concordance values for the 52 observation pairs (Kappa value ± standard deviation) were 0,936 ± 0,044 ($p<0,001$).

3.4. RESULTS

During a 90-day period experiment, 2 animals died in control group, resulting in the latter group n= 4. Clinical analysis test groups 2% PMMA and 30% PMMA displayed clinical alterations such as ulcers (Figure1), white plates and nodules (Figure 2). Control groups, on the other hand, had no such features in any of the animals.

In the 7-day clinical evaluation, two rats subjected to application of 2% PMMA and one rat to 30% PMMA exhibited ulcerations. Within 60 days, 2 animals from the 2% PMMA group and 1 from the 30% PMMA group showed white plaques on the injection site. There were nodular lesions with firm consistency in 4 animals from the 30% PMMA group after 60 days. After 90 days, no clinical changes were observed in the anatomical region where the material was injected.

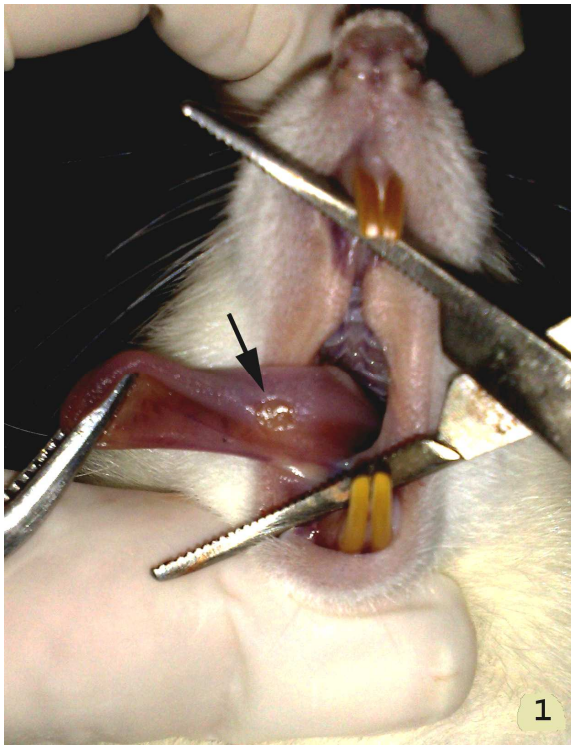


Figure 1. Presence of ulcer in the ventral tongue, near the 2% PMMA injection site (7 days).

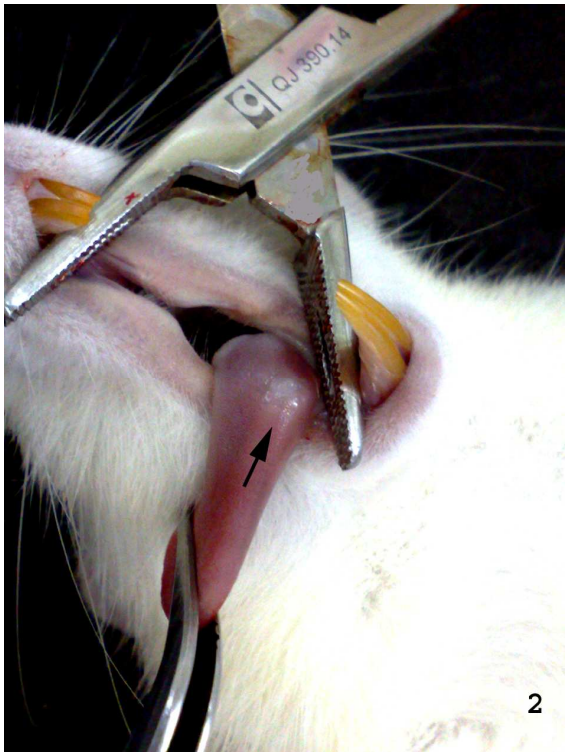


Figure 2. Presence of white nodule in the 30% PMMA implantation site (60 days).

Histological Evaluation

In figure 3, the photomicrograph illustrates PMMA histological pattern which is composed of transparent microspheres, variable in size, distributed within the tissue.

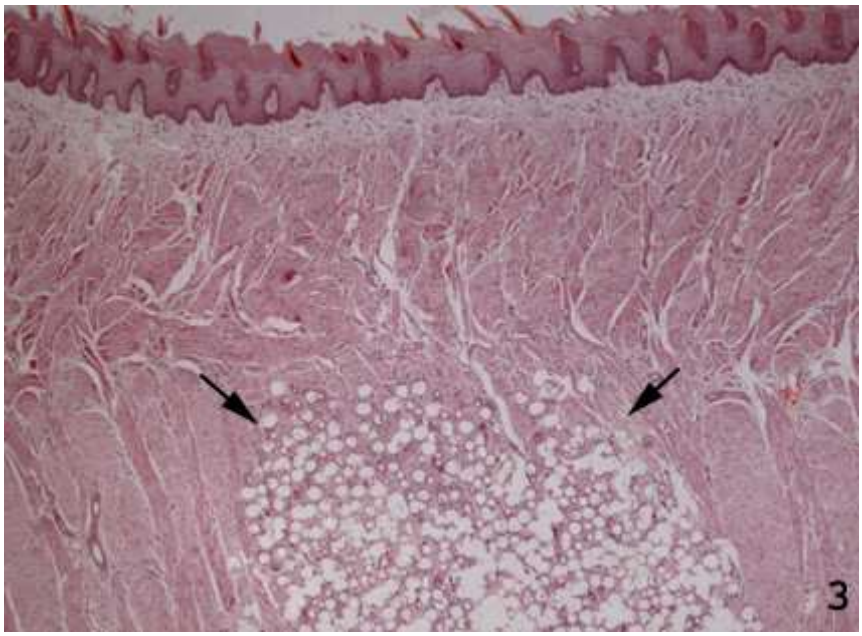
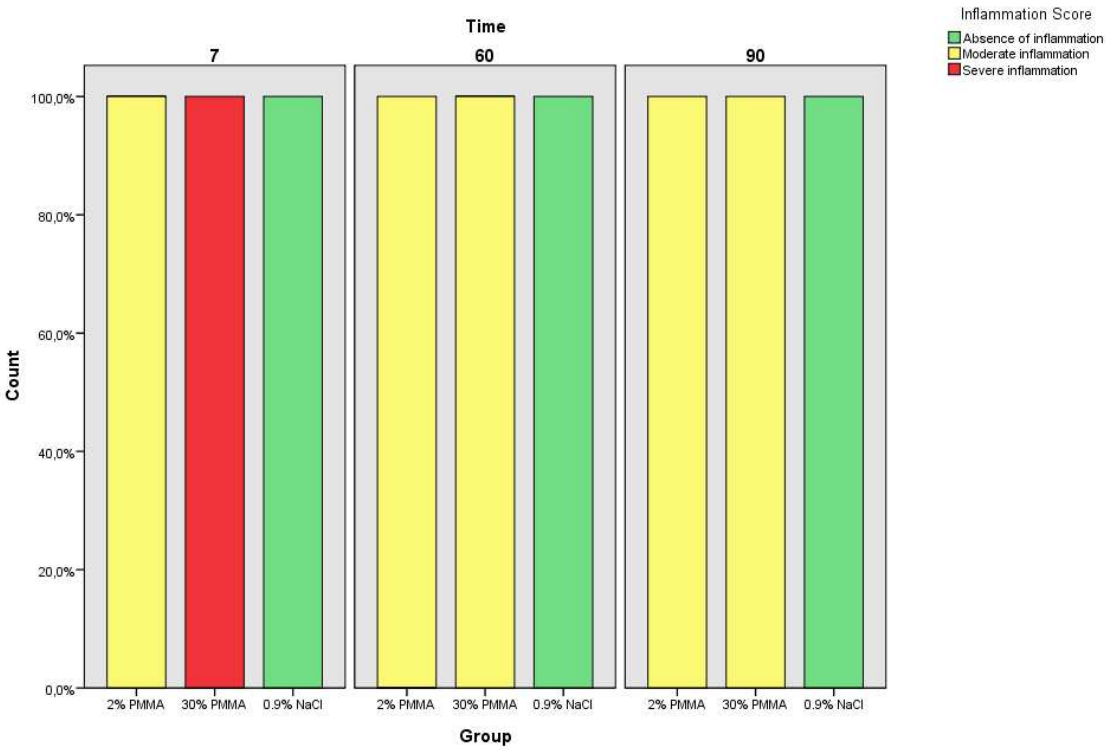
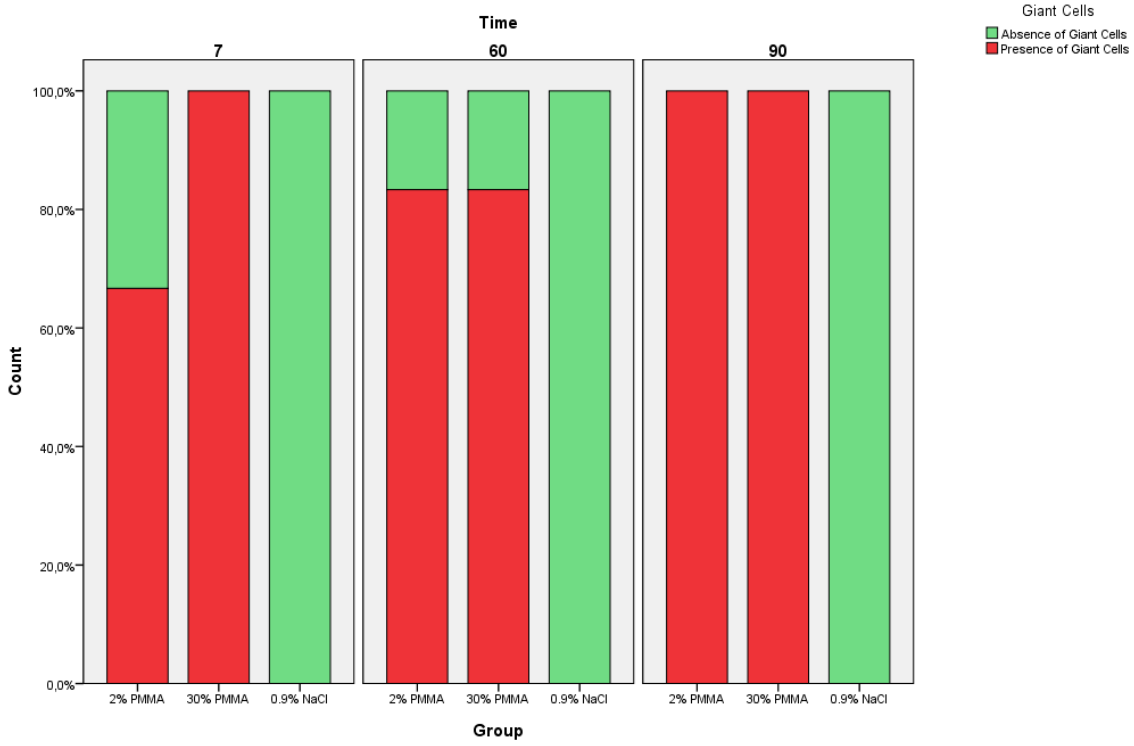


Figure 3: PMMA photomicrograph within the tissue.
(HE, x40 magnification).



Graph 1. Illustrates the score distribution of the inflammatory response evaluation of groups according to each monitoring period. It can be noticed that all rats from control groups showed absence of inflammatory response. In a general way, there was predominance of score 3 (moderate response - Figure 4) in the different PMMA concentrations and monitoring periods. However, in group 2, after 7 days, there was an evidently severe inflammatory response.



Graph 2. Shows strong presence of foreign body giant cells in test groups 1 and 2, regardless of the monitoring period analyzed. In the control groups there was total absence of this type of cell.

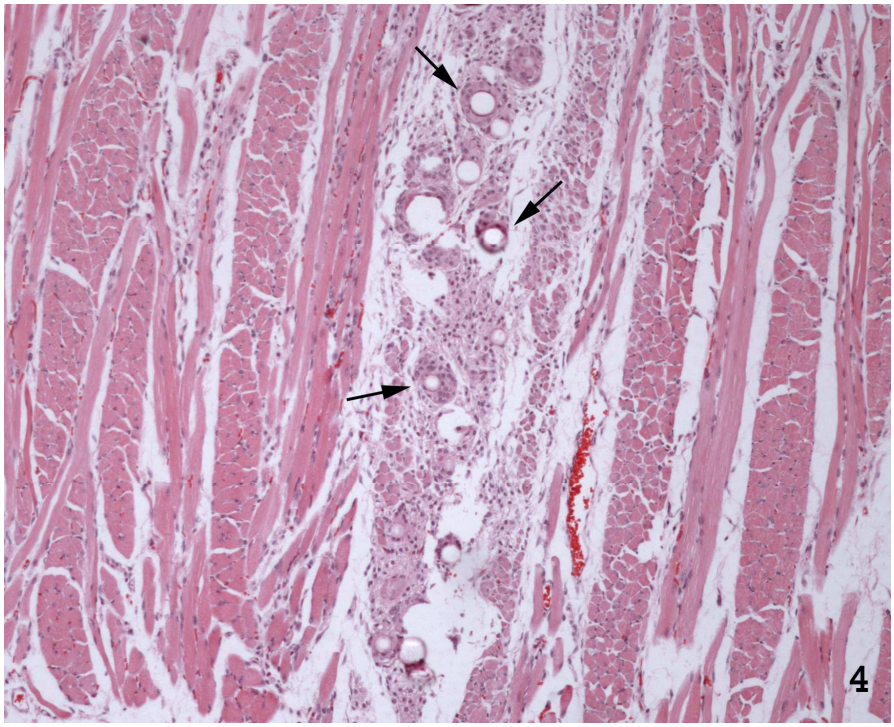


Figure 4. Photomicrograph illustrating moderate inflammatory reaction, with presence of giant cells (arrows), after application of 2% PMMA (90 days). (HE, x100 magnification).

In the test group samples, there was presence of lymphocytes in 97%, plasma cells in 77% and macrophages in 100%.

On the other hand, eosinophils were present in 16.6% of the 2% PMMA group (60 days), 100% of the 30% PMMA group (7 days) and 33.3% of samples of the 30% PMMA group (60 days). At 90 days of monitoring test groups and at all periods of the control group there was absence of this cell type. In the 2% PMMA group, neutrophils were observed in 50% of the tests at 7 days and in 16.6% in other periods. In the 30% PMMA group, there was a gradual decrease in the number of neutrophils at different monitoring periods, ranging from 100% (7 days) to 83.3% (60 days) and 33.3% (90days).

In this experiment, the presence of neovascularization was clearly evident in all test groups (Figure 5).

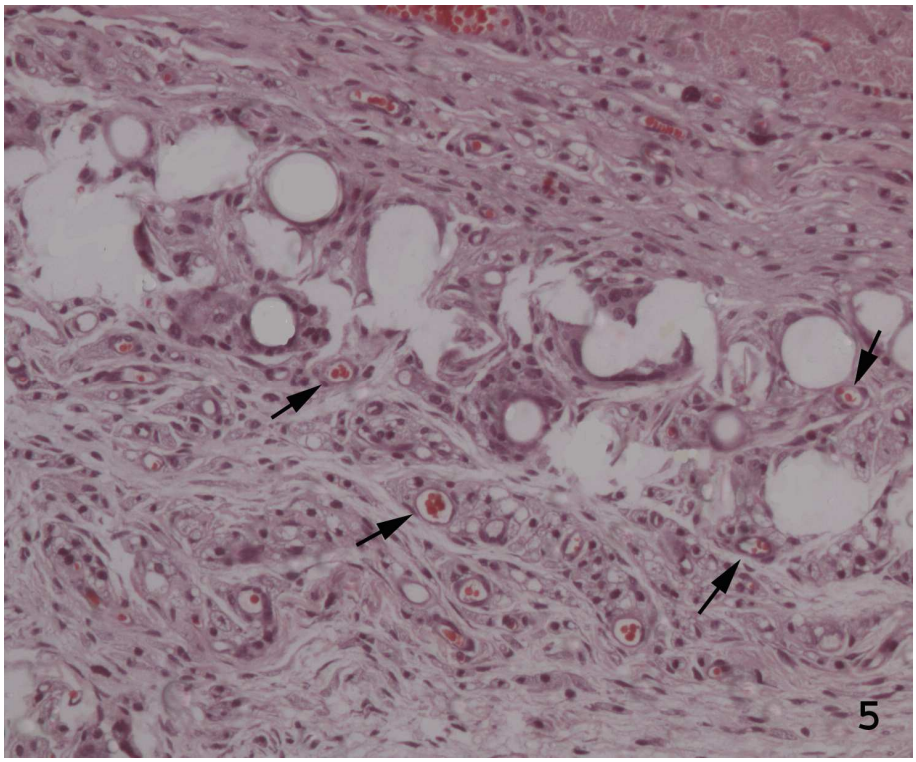


Figure 5. Photomicrograph showing newly formed blood vessels (arrows) near the 30% PMMA spheres (90 days).
(HE, x200 magnification).

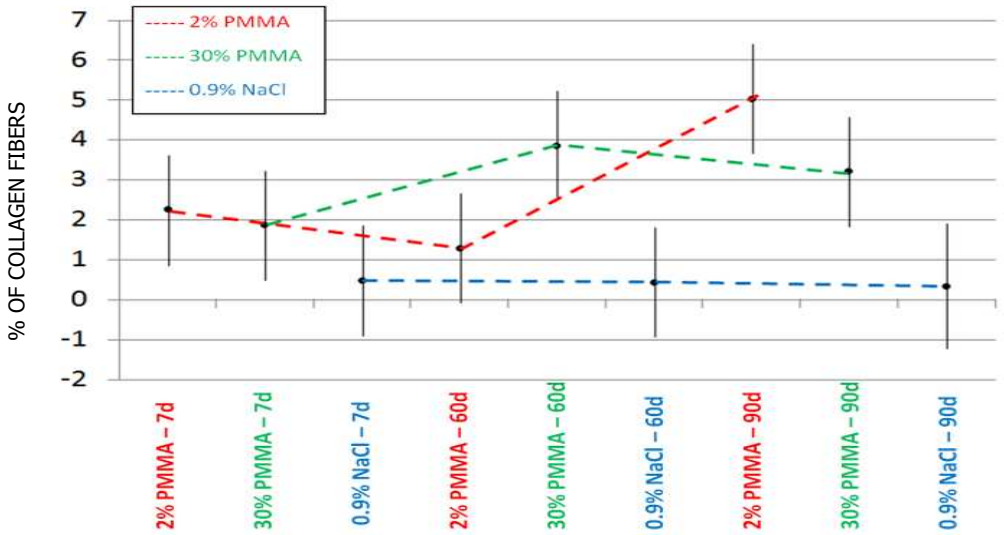


Figure 6. Distribution of the presence of fibroplasia in groups and its variation in different monitoring periods. There is slow and gradual newly formed collagen fibers in group 2% PMMA (Figure7). Concerning group 30% PMMA, there is immediate and severe fibroplasia followed by process stabilization. Group 0.9% NaCl remained stable in all monitoring periods, with total absence of this event.

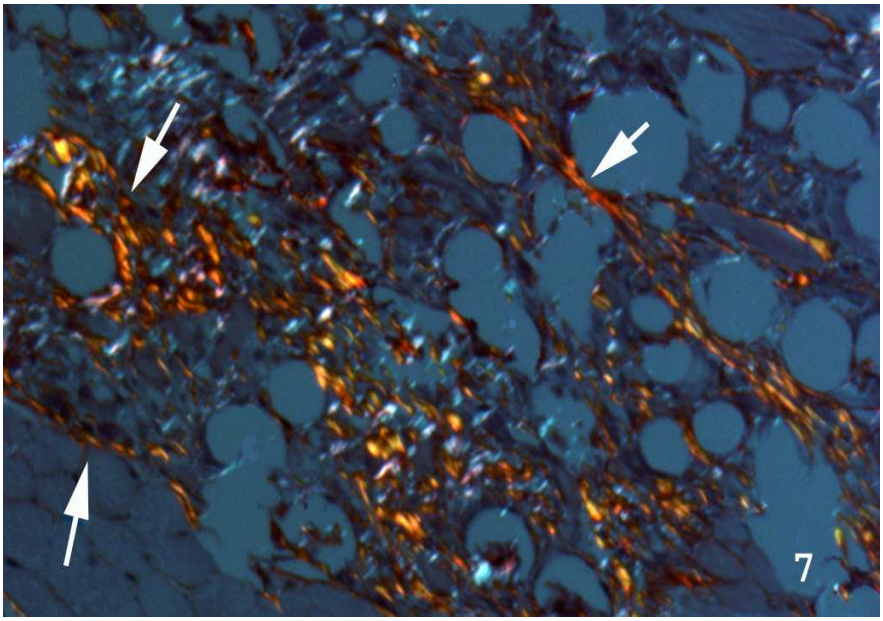


Figure 7. Photomicrograph showing the characteristic collagen formation (arrows) around 2% PMMA (90 days) microspheres. (Picrosirius, x100 magnification).

3.5. DISCUSSION

This research was encouraged due to the identification of oral lesions in patients, associated to adverse effects of filling materials with aesthetic purpose. It is believed that a higher number of cases occur for different reasons; among them there is the excessive valuation of facial aesthetics with the purpose of preserving a young aspect. In addition to that, there is also the fact that these procedures are less invasive if compared to traditional surgical interventions, they are low-cost and can be easily accessed.

The intention of this experiment was to evaluate clinical and histological responses of PMMA, considered the most commonly used aesthetic filling material nowadays. Extreme concentrations (2% and 30%) of this material were applied with the objective of assessing possible variations of tissue inflammatory response degree using the same product in different ways.

In this study, presence of clinical alterations was observed in 27.7% of 36 groups test samples, all in monitoring periods of 7 and 60 days. In the first period (7 days), there was presence of ulcerations in 16.6% of animals (Figure 1). In group 30% PMMA, there were nodular regions in 22.2% of samples, probably justified by PMMA injection (Figure 2). White plates were also described in 16.6% animals in the 60-day period. Zimmermann and Clerici (2004) and Borghetti et al. (2011) highlighted that the most likely distinct clinical complications to occur in humans could come from filling material injections. They also made reference to hardening of tissue in the injected region, besides the appearance of nodules in the application sites ranging from 3 to 24 months after the procedure. Christensen et al. (2005) have mentioned that granulomas and nodules can appear up to 6 years after material injection.

PMMA is microscopically constituted of transparent microspheres variable in size, distributed within the tissue (Figure 3). Histologically, in this experiment, in the 7-day monitoring period it was possible to observe that the entire test group 30% PMMA presented intense inflammatory response with presence of infiltrate of neutrophils and eosinophils. Lemperle et al (1991) emphasized in a study that neutrophil infiltration reaches its peak on the first 24 hours, increasing inflammatory reaction and assisting in infection control. After 72 hours, there was the end of neutrophil migration and macrophages started to accumulate in the implantation site therefore becoming the predominant type of cell. These findings coincide with the ones in the present study, reinforcing the results obtained in the experiments. Moderate alterations, on the other hand, occurred in all samples of test group 2% PMMA and in 66% of test group 30% PMMA (Figure 4). It was noticed that more than 77% of samples (groups 1 and 2) showed presence of lymphocytes, plasma cells, macrophages and giant cells. Corroborating this data, Lemperle et al. (2004) reported that after a period of 1 to 9 months, the PMMA injection promoted a moderate inflammatory response, with presence of macrophages, foreign body giant cells and newly formed collagen fiber. In the present study, foreign body giant cells were evident in 88.8% of samples of test groups. These results disagree with the ones reported by McClelland et al. (1997), since the authors have mentioned that there is a constant presence of giant cells when dealing with PMMA implants.

The present study showed presence of fibroplasia, with the respective percentages of 55.5% and 100% of samples of groups 2% PMMA and 30% PMMA (Figure 7). According to Lemperle et al. (2006), in 4 weeks, all microspheres had been individually encapsulated by fibroblasts and collagen fibers. The amount of present macrophages was very small; however, capillary growth was evident. Zimmermann and

Clereci, in 2004, reported that the monocyte invasion occurred 3 days after material implantation, fibroblast differentiation happened in 6 days and the microsphere interstitial space was filled in 9 days. After 2 months, each microsphere was surrounded by a thin fibrous capsule with the reduction of monocytes and histiocytes. After 3 months, all the injected collagen had been phagocytized by macrophages and the fibrous phase seemed to end in 4 months, remaining stable. Christensen et al. (2005) have described that, between 8 and 10 weeks after facial filling material application, they have found not only filling material, but also macrophages, lymphocytes and giant cells in the analyzed histological slides. In no case there was presence of polymorphonuclear. Alster and West (2000) have reported that after 12 weeks collagen continued suffering alterations, becoming denser and more resistant, reaching stability in collagen formation and in the inflammatory process.

In the present study, presence of hyperemia could be observed in 100% of samples of groups 2% PMMA and 30% PMMA (Figure 5). In agreement with these results, several authors have reported that the presence of PMMA microspheres stimulates neovascularization (Allen, 1992, Alster, West, 2000, Menezes, 2009).

Regarding material migration or its systemic response, findings obtained from microscopic analysis of rats' kidneys in this research did not show any alteration that could be related to this process. On the other hand, in scientific literature, systemic migration cases have already been mentioned by Rosa et al. (2008), who observed the presence of hepatic and renal inflammatory infiltrates in rats submitted to PMMA injections of 0.05ml in the ear. Constant presence of periportal and intralobular infiltrates in the liver has been reported, as well as chronic pyelonephritis and interstitial nephritis in the kidney. These alterations have been justified by drugs systematization,

which could act at long distance, in a metabolism or excretion organ, as a chemotactic substance.

In our experiment, it was concluded that regardless of the PMMA concentration, there were inflammatory reactions with strong presence of foreign body giant cells. The experiment has shown fibroplasia was more often prevalent in the group 30% PMMA.

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DISCUSSÃO GERAL

4. DISCUSSÃO GERAL

A realização desta pesquisa foi estimulada a partir da detecção de lesões orais em pacientes, associadas aos efeitos adversos da utilização dos materiais de preenchimento facial com finalidade estética. Proporcionalmente ao fácil acesso e aumento da utilização destes produtos no Brasil, está o relato de complicações inerentes ao seu uso.

Outro aspecto que favoreceu a execução deste experimento, foi a dificuldade em estabelecer o diagnóstico diferencial, tanto clínica quanto histologicamente, das lesões causadas por estas substâncias. De um modo geral, durante a anamnese, os pacientes costumam omitir do cirurgião-dentista, procedimentos estéticos faciais prévios e quando mencionam, com frequência, desconhecem o tipo de produto utilizado e o exato local onde foi aplicada a substância de preenchimento. Quando a lesão encontra-se presente na face, caracteriza-se como um nódulo único ou múltiplo, de evolução crônica e tamanho variável, consistência firme, sem limites definidos, indolor, distribuído costumeiramente na região perioral ou labial. Enfermidades envolvendo glândulas salivares acessórias (cistos de retenção, neoplasias benignas e malignas), patologias associadas ao tecido adiposo, como o lipossarcoma, ou ainda reações de corpo estranho, podem suscitar lesões semelhantes, promovendo dúvidas no momento de estabelecer o diagnóstico clínico conclusivo. Outro fator que dificulta o manejo dos pacientes nestes casos é que o produto, por ser injetado, apresenta-se sem limites precisos, ficando o mesmo distribuído na intimidade tecidual, impedindo usualmente a sua total remoção.

O PMMA, objeto de estudo desta pesquisa, é um dos materiais permanentes mais utilizados na medicina estética atual, sendo um dos produtos com esta finalidade

de menor custo no mercado. Sabe-se ainda que, microscopicamente, as esferas de PMMA podem simular o aspecto das células adiposas, diferenciando-se, principalmente, por estas últimas apresentarem um discreto núcleo na periferia.

Acredita-se que o maior número de casos encontrados atualmente nos indivíduos, seja oriundo da excessiva valorização estética corporal, especialmente da face, que, no intuito de preservar o seu aspecto jovial, favorece por vezes a indicação inadvertida destas substâncias. O fato destes procedimentos serem menos invasivos, se comparados as intervenções cirúrgicas tradicionais, além do seu baixo custo e fácil acesso, faz com que estas terapias sejam amplamente aplicadas. No entanto, a reduzida quantidade de estudos e experimentos científicos publicados sobre o PMMA, quase restrita a relato de casos, não segue a velocidade da sua popularização de uso em todo mundo. Isto reforça a necessidade de disponibilizar aos profissionais da área de saúde, o maior número de informações sobre o tema, proporcionando o conhecimento, não só dos benefícios, mas também de eventuais efeitos adversos deste tipo de terapia.

Através deste experimento procurou-se avaliar a resposta clínica e histológica de diferentes concentrações de PMMA. As microesferas usadas neste estudo, segundo Lemperle et al. (1991), apresentam as características do implante cutâneo ideal, uma vez que são de custo acessível, fácil obtenção, quimicamente inertes, longa duração e consistência física semelhante a do tecido não tratado. Foram empregadas concentrações extremas do PMMA com indicação de uso (2% e 30%), buscando ponderar sobre possíveis variações no grau de resposta inflamatória tecidual a partir de distintos empregos do mesmo produto. Ressalta-se que as concentrações selecionadas tem múltiplas aplicabilidades clínicas, entre elas na região facial.

Lemperle et al. (1991) foram os primeiros a utilizar ratos em estudos experimentais, com implantação cutânea de PMMA. Segundo Menezes (2009), este

animal é uma espécie que facilita seu uso em laboratório, uma vez que é capaz de viver em ambiente variável, consumir dieta padronizada, ser de fácil obtenção, manutenção e observação. Além disso, tem-se a possibilidade de manusear um maior número de animais, com padrão genético estabelecido, curtos ciclos vitais e suficiente número de informações a respeito da sua anatomia e fisiologia disponíveis na literatura.

A região de escolha para injeção da substância foi o ventre lingual, por este mostrar-se anatomicamente menos vulnerável a fatores extrínsecos evitando assim, maior probabilidade de traumas locais. Tais estímulos poderiam mascarar a resposta inflamatória presente e por consequência a fidedignidade dos resultados obtidos neste experimento, uma vez que os animais são roedores e permaneceriam acomodados em grupos dentro de uma mesma gaiola. Havia a opção de injetar o material na região do filtro, que apresenta melhor acesso, facilitando a manipulação local. Entretanto, a expressiva presença de anexos cutâneos e folículos pilosos da região, dificultaria a posterior análise microscópica, durante a leitura e interpretação dos resultados.

Neste estudo, foram detectadas alterações clínicas em 27,7% das 36 amostras dos grupos teste, sendo todas, nos tempos experimentais de 7 e 60 dias. No primeiro período (7 dias) dos grupos PMMA a 2% e PMMA a 30% notou-se a presença de ulcerações em 25% dos animais justificada pela própria introdução da agulha no momento da injeção, associada a eventual aumento de volume e/ou edema na área, uma vez que estes materiais são mais densos, favorecendo algum tipo de traumatismo secundário.

No grupo PMMA a 30%, foram observadas áreas nodulares em 22,2% da amostra, provavelmente decorrentes da injeção de PMMA. Na inspeção digital destes nódulos observou-se uma consistência firme dos tecidos adjacentes a área da aplicação

do produto. Também foram descritas placas esbranquiçadas em 16,6% dos animais no período de 60 dias, correspondendo provavelmente a um espessamento da camada de ceratina na superfície tecidual. Zimmermann e Clerici (2004) ressaltaram que as distintas complicações clínicas passíveis de ocorrer em humanos, poderiam advir da injeção de substâncias de preenchimento. Referiram a possibilidade de endurecimento dos tecidos na área, além do surgimento de nódulos nos locais das aplicações variando de 3 a 24 meses após o procedimento. Pollack (1999) e Vargas, Amorin e Pitanguy (2009) relataram em pesquisas conduzidas junto a pacientes, que as formações de granulomas ocorreram quando a aplicação do material foi feita superficialmente.

Christensen et al. (2005) mencionaram que granulomas e nódulos podem surgir até 6 anos após a injeção do produto. Riesberger et al. (2003) constataram a presença de granulomas de corpo estranho mesmo após 6 meses da injeção subcutânea de PMMA. Lemperle, Fazio e Nicolau (2006) divulgaram seus estudos em humanos, referindo que os granulomas são considerados efeitos adversos raros, uma vez que ocorreram em 0,02% dos casos entre 6 e 24 meses após a injeção do PMMA (1:5000 pacientes). Tal apreciação foi inviabilizada neste experimento, uma vez que o último período de observação previsto na metodologia foi de 90 dias. Os distintos resultados encontrados pelos pesquisadores, reforçam a importância de executar estudos longitudinais padronizados, com tempos de observação mais extensos, buscando com isto, um maior número de informações sobre o comportamento tecidual destas substâncias a longo prazo.

O PMMA apresenta-se microscopicamente como microesferas transparentes de tamanhos variáveis, distribuídos na intimidade tissular. Histologicamente, neste experimento, no período de 7 dias de acompanhamento observou-se que todo o grupo

PMMA a 30% apresentou resposta inflamatória intensa, ou seja, presença de infiltrado de neutrófilos e eosinófilos. Lemperle et al. (1991) enfatizaram em seus estudos, que o pico de infiltração de neutrófilos ocorreu nas primeiras 24 horas, ampliando a resposta inflamatória e auxiliando no controle de infecções. Após 72 horas, descreveram o fim da migração de neutrófilos, momento em que os linfócitos e macrófagos passaram a se acumular no sítio do implante, sendo estes últimos, o tipo celular predominante. Estes achados coincidem com os encontrados no presente estudo. Já as alterações moderadas ocorreram na totalidade dos animais do grupo PMMA a 2% e nos grupos PMMA a 30% aos 60 e 90 dias. Notou-se que mais de 77% das amostras dos grupos teste apresentou linfócitos, plasmócitos, macrófagos e células gigantes. Corroborando com esses dados, Lemperle et al. (2004), relataram que, após intervalo de 1 a 9 meses, a injeção de PMMA promoveu uma resposta inflamatória moderada, com presença de macrófagos, células gigantes do tipo corpo estranho e fibras colágenas neoformadas. No presente estudo, evidenciou-se células gigantes do tipo corpo estranho em 88,8% das amostras nos grupos teste. Os achados de McClelland et al. (1997) vão de encontro a esse resultado, uma vez que os autores mencionaram a constante presença de células gigantes em se tratando de implantes de PMMA.

O colágeno é uma proteína de importância fundamental na constituição da matriz extracelular do tecido conjuntivo, sendo responsável por grande parte de suas propriedades físicas. Tem um importante papel na manutenção da integridade estrutural dos tecidos, tornando de grande valia, a utilização de métodos que consigam identificar, quantificar e analisar o colágeno presente. Desta forma, escolhemos neste estudo, o uso do picrosírius, visando detectar e quantificar fibras colágenas que se depositaram nas regiões onde foi injetado o PMMA, bem como ao redor das microesferas do produto. Associou-se para esse fim a coloração pelo picrosírius, a luz polarizada e um *software*

para análise das imagens, calculando posteriormente a proporção de fibras colágenas neoformadas durante os tempos de acompanhamento do estudo.

Neste estudo 53,8% de todas as avaliações demonstraram a presença de fibroplasia, com percentuais de 55,5 e 100% respectivamente nos grupos PMMA a 2% e PMMA a 30%.

Segundo os achados de Lemperle, Fazio e Nicolau. (2006) em 4 semanas, todas as microesferas haviam sido individualmente encapsuladas, sendo envolvidas por fibroblastos e fibras colágenas. A quantidade de macrófagos presentes era rara, entretanto a neoformação capilar observada foi evidente. No mesmo estudo, os autores referiram a deposição de proteína sérica na superfície das microesferas, como sendo o primeiro evento que ocorre após a aplicação do material. O segundo seria a invasão de neutrófilos e monócitos, sendo que estes últimos, rapidamente se diferenciam em macrófagos. Os macrófagos, na tentativa de fagocitar as partículas maiores, se unem formando as células gigantes. O terceiro evento descrito é a formação de um tecido granulomatoso composto por macrófagos, fibroblastos, capilares e colágeno, preenchendo assim, os espaços intersticiais das microesferas. Já Zimmermann e Clereci em 2004, relataram que a invasão de monócitos ocorreu 3 dias após a implantação do material, a diferenciação dos fibroblastos se deu em 6 dias e os espaços intersticiais das microesferas foram preenchidos em 9 dias. Após 2 meses, cada microesfera estava envolvida por uma fina cápsula fibrosa havendo uma redução do número de monócitos e histiócitos. Após 3 meses, todo o colágeno injetado tinha sido fagocitado pelos macrófagos e a fase fibrosa pareceu encerrar em 4 meses, permanecendo, assim, estável. Christensen et al. (2005) descreveram que, entre 8 e 10 semanas após a aplicação de materiais permanentes de preenchimento facial, encontraram na totalidade das lâminas

histológicas analisadas, além das substâncias preenchedoras, macrófagos, linfócitos e células gigantes. Em nenhum dos casos observaram a presença de polimorfonucleares. Alster e West (2000) relataram que após 12 semanas, o colágeno continuou sofrendo alterações, no sentido de tornar-se mais denso e resistente, alcançando desta forma, estabilidade na sua formação e na do processo inflamatório.

No presente estudo, foi constatada a presença de hiperemia em 100% das amostras dos grupos teste. Concordando com estes resultados, diversos autores relataram que a presença das microesferas de PMMA estimulam o processo de neovascularização da área envolvida (ALLEN, 1992, ALSTER; WEST, 2000, MENEZES, 2009).

Lemperle et al. em 2004, descreveram 3 vias pelas quais as microesferas de PMMA poderiam migrar. As vias hematogênicas e linfáticas, estarão envolvidas, caso a injeção do material atinja algum vaso de maior calibre, podendo alcançar os capilares pulmonares e mais especificamente na via linfática, os linfonodos regionais. A terceira via citada é a fagocitose, uma vez que as microesferas de PMMA, fagocitadas pelos macrófagos, podem ser absorvidas no local do implante, que migram posteriormente para os linfonodos regionais.

Quanto à migração do produto ou da sua resposta sistêmica, os achados obtidos nesta pesquisa, a partir da análise microscópica renal das ratas, não demonstraram qualquer alteração que pudesse ser vinculada a este processo. Em contrapartida, casos de migração sistêmica já foram referidos por Rosa, de Magalhães e de Macedo, em 2008, onde observou a presença de infiltrado inflamatório hepático e renal em ratos submetidos à injeção de 0,05ml de PMMA em suas orelhas. Foi relatada a presença constante de infiltrado intralobular e periportal no fígado, assim como pielonefrite

crônica e nefrite intersticial. Estas alterações foram justificadas em consequência da sistematização das drogas, que eventualmente poderiam agir à distância, em algum órgão de metabolismo e excreção, como uma substância quimiotática.

Através desta pesquisa buscou-se, por meio de um estudo padronizado, avaliar a resposta clínica e histológica de um material de preenchimento estético amplamente utilizado na atualidade. Objetivou-se identificar, analisar e comparar tais reações, visando um maior conhecimento dentro do tema proposto, ampliando-se as linhas de pesquisas vinculadas e oportunizando, dentro da aplicabilidade clínica, fornecer informações que, de alguma forma, possam contribuir para o uso responsável destes produtos junto aos pacientes. Em nosso estudo concluiu-se que independentemente da concentração do PMMA, ambos estimularam uma reação inflamatória, com marcada presença de células gigantes do tipo corpo estranho. O experimento mostrou também neoformação vascular sanguínea presente em todos os tempos dos grupos teste, bem como fibroplasia, com maior prevalência no grupo cuja concentração de PMMA era de 30%.

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ANEXOS

ANEXO A

Gmail by Google

karlon froes de vargas <karlon001@gmail.com>

Gerodontology - Decision on Manuscript ID GER-10-RV-0286

j.newton@abdn.ac.uk <j.newton@abdn.ac.uk> **14 de novembro de 2010 18:58**
Para: karlon001@gmail.com

14-Nov-2010

Dear Mr. Vargas:

It is a pleasure to accept your manuscript for publication in Gerodontology. The comments, if any, of the reviewer(s) who refereed your manuscript are included at the foot of this letter.

The manuscript now becomes the copyright of this journal. In accordance with accepted practice, the research findings reported in this paper must not appear in another publication before its publication in this journal.

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We will then edit your manuscript for style and send it to press; proofs should be with you in due course.

Thank you for this manuscript. We look forward to your continued contributions to the Journal.

Sincerely,
Prof. James Newton
Editor in Chief, Gerodontology
j.newton@abdn.ac.uk

ANEXO B

ORAL DISEASES

SCHOLARONE Manuscripts

Main Menu ➡ Corresponding Author Dashboard ➡ Submission Confirmation

You are logged in as Karlon Vargas

Submission Confirmation

Thank you for submitting your manuscript to *Oral Diseases*.

Manuscript ID:	ODI-02-11-OM-1980
Title:	TISSUE RESPONSE IN FEMALE RATS SUBMITTED TO PMMA INJECTIONS IN TONGUE IN DIFFERENT CONCENTRATIONS
Authors:	Vargas, Karlon Figueiredo, Maria Borghetti, Ruchielli Moure, Sabrina Cherubini, Karen
Date Submitted:	27-Feb-2011

Print Return to Dashboard

ANEXO C

Comissão Científica e de Ética
Faculdade da Odontologia da PUCRS

Porto Alegre 09 de março de 2010

O Projeto de: Dissertação

Protocolado sob nº:	0008/10
Intitulado:	Respostas tecidual em ratas submetidas à injeção submoca de polimetilmetacrilato em distintas concentrações - avaliação clínica e histológica
Pesquisador Responsável:	Profa. Maria Antonia Zancanaro de Figueiredo
Pesquisadores Associados	Karlon Fróes de Vargas; Ruchielli Loureiro Borghetti e Sabrina Ponzatti Moure
Nível:	Mestrado

Foi ***aprovado*** pela Comissão Científica e de Ética da Faculdade de Odontologia da PUCRS em *09 de março de 2010.*

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 D

Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMITÊ DE ÉTICA PARA O USO DE ANIMAIS

Ofício 028/10 – CEUA

Porto Alegre, 18 de março de 2010.

Senhora Pesquisadora:

O Comitê de Ética para o Uso de Animais apreciou e aprovou seu protocolo de pesquisa, registro CEUA 10/00150, intitulado: **"Resposta tecidual em ratas submetidas à injeção submucosa de polimetilmetacrilato em distintas concentrações - Avaliação Clínica e histológica".**

Sua investigação está autorizada a partir da presente data, com recomendação.

Atenciosamente,

Profa. Dra. Anamaria Gonçalves Feijó
Coordenadora do CEUA – PUCRS

Ilma. Sra.
Profa. Dra. Maria Antonia de Figueiredo
N/Universidade

PUCRS

Campus Central
Av. Ipiranga, 6690 – Prédio 60, sala 314
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APÊNDICES

APÊNDICE A

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. ____ **Data da aplicação: __/__/____.**

Substância injetada

□ Grupo 1 (PMMA 2%)

□ Grupo 2 (PMMA 30%)

□ Grupo 3 (NaCl 0,9%)

Tempo:

□ Subgrupo A (7 dias)

□ Subgrupo B (60 dias)

□ Subgrupo C (90 dias)

AVALIAÇÃO CLÍNICA LOCAL

□ Sem alterações clínicas

□ Edema

□ Nódulo

□ Ulceração

□ Necrose/supuração

□ Fibrose

Sinais significativos: □ sim □ não

Especifique (sangramento, abscesso...):

_____
_____
_____
_____

Fotografia: _____

Data da avaliação: __/__/____.

Examinador:

_____

APÊNDICE B

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

Rato nº:_____ Peso inicial: _____ Lâmina nº: _____

Substância injetada:

□ Grupo 1 (PMMA 2%)

□ Grupo 2 (PMMA 30%)

□ Grupo 3 (Solução Salina- NaCl 0,9%)

Tempo:

□ Subgrupo A (7 dias)

□ Subgrupo B (60 dias)

□ Subgrupo C (90 dias)

AVALIAÇÃO HISTOLÓGICA HE:

VARIÁVEL	**ESCORE**
□ Linfócitos	□ 1- Sem inflamação
□ Plasmócitos	□ 2- Células mononucleares esparsas
□ Macrófagos	□ 3- Infiltrado mononuclear e/ou neutrófilos-eosinófilos esparsos
□ Eosinófilos	
□ Neutrófilos	□ 4- Infiltrado polimorfonucleares de neutrófilos-eosinófilos
□ Células Gigantes	
□ Fibroplasia	
□ Edema	
□ Hiperemia	
□ Vasos sanguíneos (capilares)	

MIGRAÇÃO RIM DIREITO

□ Presença do material

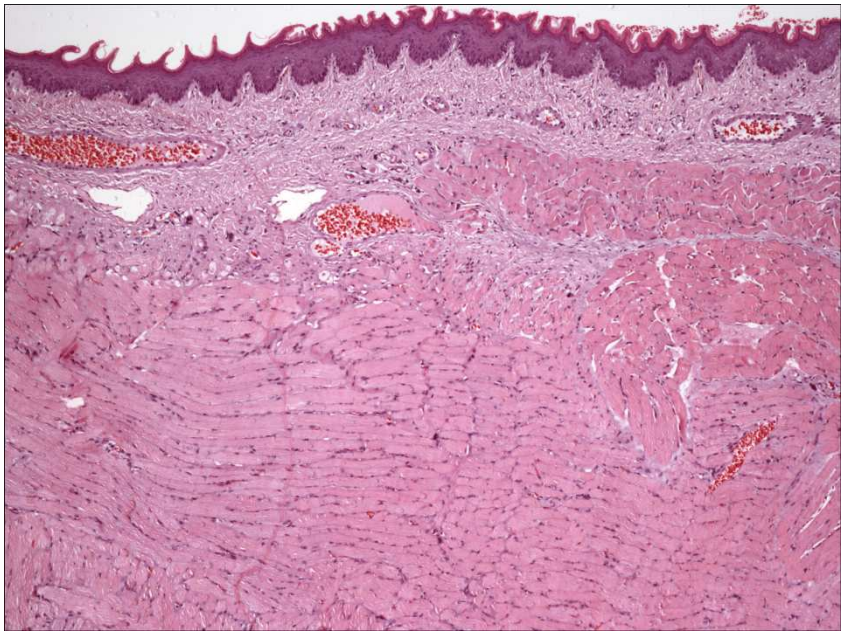
□ Ausência do material

□ Resposta inflamatória

Observações: _____

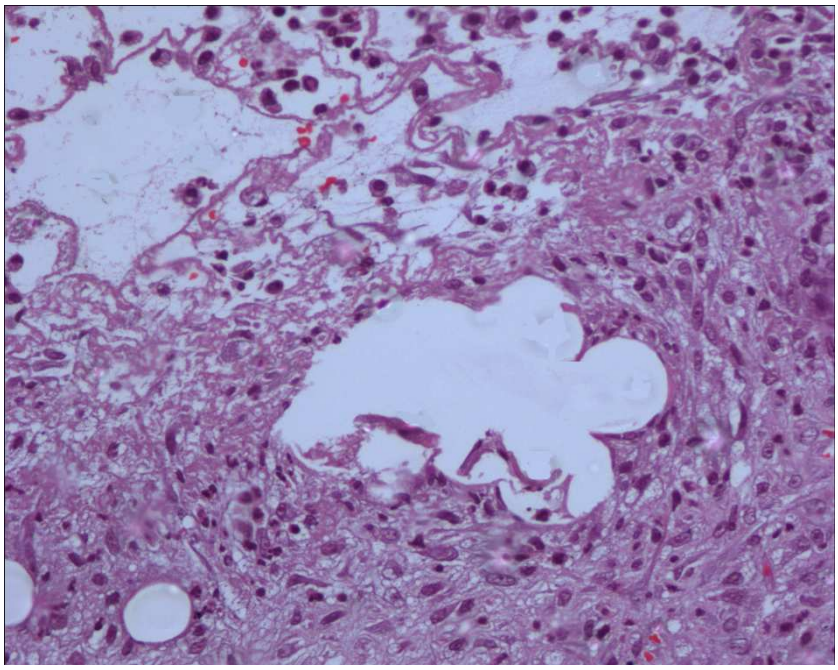
Fotos:_____ Data da avaliação: __/__/__.

APÊNDICE C



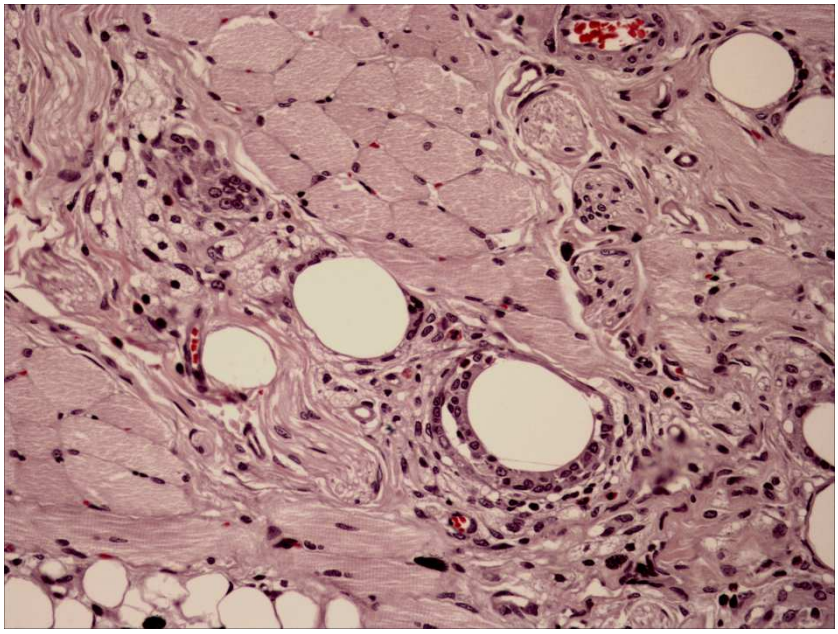
Fotomicrografia ilustrando ausência de resposta inflamatória. Grupo controle NaCl 0,9% após 7 dias de aplicação. (HE, aumento aproximado 40x).

APÊNDICE D



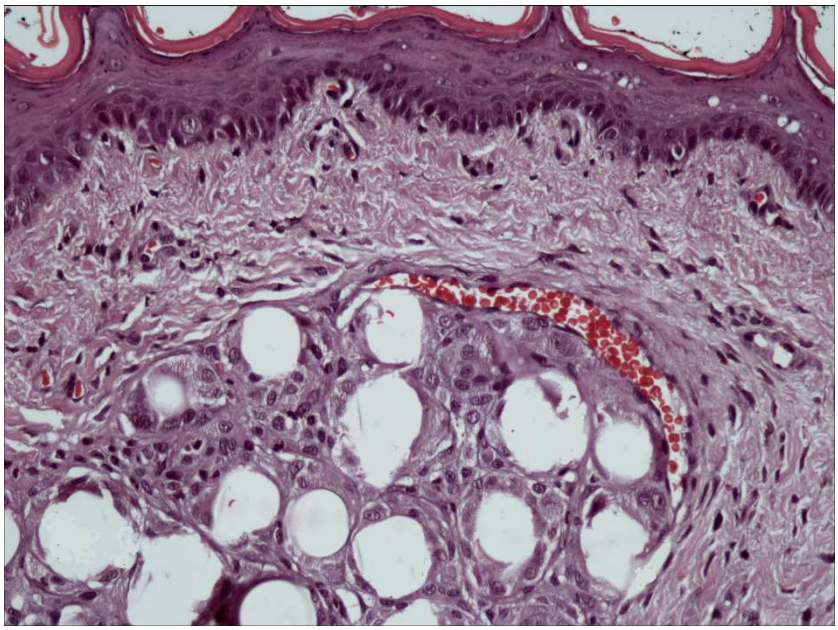
Fotomicrografia mostrando reação inflamatória intensa, com infiltrado de células polimorfonucleares e edema, após 7 dias da aplicação de PMMA 30% (HE, aumento aproximado de 400x).

APÊNDICE E



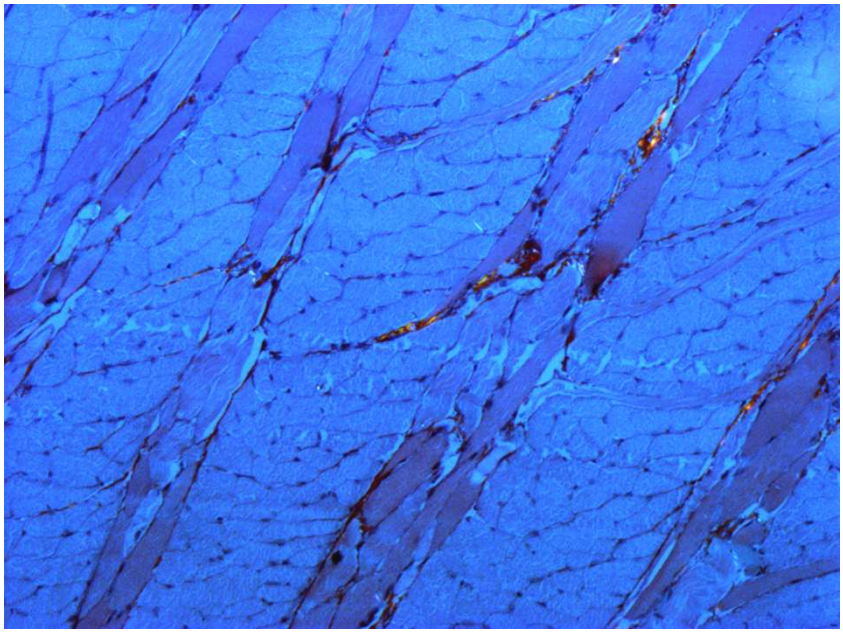
Fotomicrografia ilustrando resposta inflamatória moderada com presença de células gigantes do tipo corpo estranho e esparsos neutrófilos, 60 dias após injeção de PMMA a 2%. (HE, aumento aproximado 400x).

APÊNDICE F



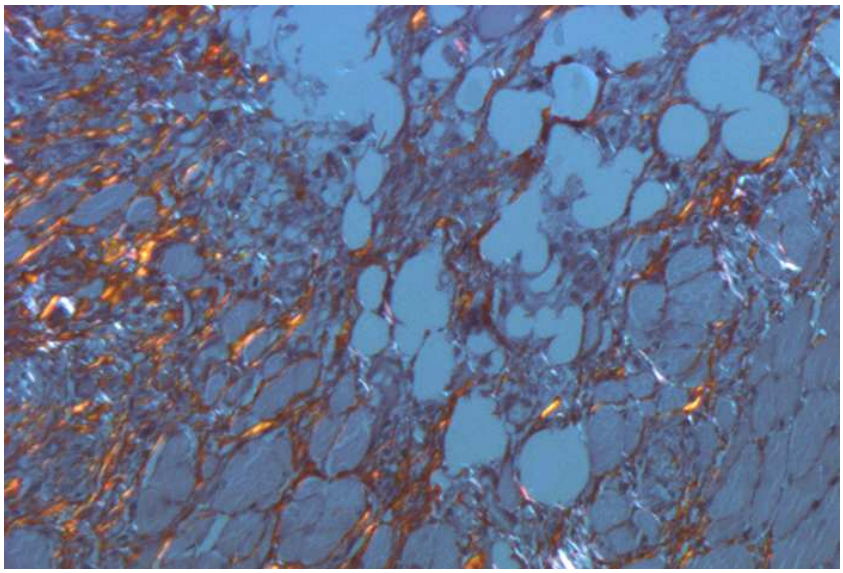
Fotomicrografia ilustrando resposta inflamatória moderada com presença de células gigantes do tipo corpo estranho, 7 dias após injeção de PMMA a 30%. (HE, aumento aproximado 400x).

APÊNDICE G



Fotomicrografia ilustrando fibras colágenas, presente no grupo controle (3C). (Picrosírius, aumento aproximado 100x).

APÊNDICE H



Fotomicrografia ilustrando a formação de fibras colágenas, 90 dias após injeção de PMMA a 2%. (Picrosírius, aumento aproximado 100x).