FACULDADE DE ODONTOLOGIA

RESPOSTA TECIDUAL EM RATOS SUBMETIDOS À INJEÇÃO SUBMUCOSA DE DOIS MATERIAIS DE PREENCHIMENTO COM FINALIDADE ESTÉTICA: ANÁLISES CLÍNICA E HISTOLÓGICA

SABRINA POZATTI MOURE

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Tese apresentada como parte dos requisitos para obtenção do título de **Doutor em Odontologia**, área de concentração em **Estomatologia Clínica**.

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Epígrafe

“*Se eu vi mais longe, foi por estar de pé sobre ombros de gigantes.*”

Isaac Newton

Dedicatória

Aos meus pais, **Telmo** (*in memoriam*) e **Arléte**
que com sabedoria me instigaram a ver mais longe
e por vidas de trabalho, esforço e amor
me possibilitaram chegar até aqui.

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RESUMO

Os materiais de preenchimento são produtos injetáveis utilizados frequentemente na medicina estética com a intenção de atenuar as rugas da face e aumentar o volume dos lábios. Essa modalidade de tratamento, que muitas vezes substitui procedimentos cirúrgicos tradicionais, como a ritidoplastia, apresenta resultados estéticos satisfatórios, embora se saiba da possibilidade de alguns efeitos indesejados ocorrerem no local da implantação do produto ou mesmo à distância. A literatura odontológica relata inúmeros casos de lesões buco faciais decorrentes do uso de materiais de preenchimento, refletindo uma nova realidade na prática do cirurgião dentista. Nessa pesquisa, 2 dos materiais com finalidade estética mais utilizados por dermatologistas e cirurgiões plásticos foram injetados em línguas de ratos: polimetilmetacrilato 10% (n=16) e ácido hialurônico 20mg/mL (n=18), além de uma solução inerte para controle (n=16). Após 7, 60 e 90 dias, avaliaram-se as alterações locais clínicas e histológicas de cada produto. Foi verificada a intensidade da resposta inflamatória na área da injeção (hematoxilina e eosina), a quantidade de vasos sanguíneos neoformados e de macrófagos (imunoistoquímica) e a densidade de fibras colágenas (picrosírius). Nos mesmos tempos experimentais, a migração sistêmica das substâncias injetadas foi verificada por meio do exame microscópico de órgãos de metabolismo e excreção (fígado e rim) pela coloração de hematoxilina e eosina. Os resultados mostraram que os 2 materiais de preenchimento desencadearam, em maior ou menor grau, algum tipo de resposta inflamatória local. Na análise comparativa, constatou-se que o polimetilmetacrilato demonstrou casos de reação a corpo estranho, enquanto o ácido hialurônico apresentou características que sugerem biocompatibilidade. Essa pesquisa faz parte de um conjunto de experimentos vinculados ao uso de materiais de preenchimento facial com finalidade estética e reforça a necessidade do cirurgião dentista identificar e manejar as reações adversas que podem decorrer desse tipo de tratamento amplamente utilizado na atualidade.

Palavras-chave: Biocompatibilidade. Polimetil Metacrilato. Ácido Hialurônico. Histologia.

Abstract

ABSTRACT

Dermal fillers are injectable products commonly used in aesthetic medicine with the intention of alleviating face wrinkles and increasing lip volume. This type of treatment, which often replaces traditional surgical procedures such as rhytidectomies, provides satisfactory cosmetic results. It is known, however, that there is a risk of undesired effects at the site of injection of the product or even at a distance. Dental literature reports numerous cases of orofacial injuries caused by the use of dermal fillers, reflecting a new reality in dental surgeons' practice. In this research, 2 of the most commonly used materials for aesthetic purposes by dermatologists and plastic surgeons were injected in rat tongues: 10% polymethylmethacrylate (n=16) and 20mg/mL hyaluronic acid (n=18), compared to an inert solution for control (n=16). After 7, 60 and 90 days, local clinical and histological alterations of each product were analyzed. The following factors were verified: intensity of the inflammatory response in the injection area (hematoxylin and eosin), the amount of newly formed blood vessels and macrophages (immunohistochemistry) and the density of collagen fibers (picrosirius). At the same experimental times, systemic migration of injected substances was observed through microscopic examination of organs of metabolism and excretion (liver and kidney) stained by hematoxylin and eosin. Results showed that both filling materials triggered some kind of local inflammatory response to a greater or lesser degree. In the comparative analysis, it was found that polymethylmethacrylate showed cases of foreign body reaction, while hyaluronic acid demonstrated characteristics suggesting biocompatibility. This research is part of a set of experiments related to the use of facial dermal fillers for aesthetic purposes and it reinforces the need for dental surgeons to identify and manage adverse reactions which may result from such currently widely used treatment.

Key-words: Biocompatibility. Polymethyl Methacrylate. Hyaluronic Acid. Histology.

SUMÁRIO

1 INTRODUÇÃO

O processo de envelhecimento da face causa alterações estruturais e funcionais nos tecidos orgânicos que independem dos fatores ambientais. A epiderme se torna mais fina e a derme atrófica e menos elástica, relativamente acelular e avascular (FENSKE; LOBER, 1986; ACHILLES, 2004). O colágeno, a elastina e os glicosaminoglicanos sofrem alterações de estrutura e densidade e a espessura do tecido subcutâneo se reduz (FENSKE; LOBER, 1986). As manifestações clínicas desses fenômenos apresentam-se como sulcos profundos na pele, denominados rugas, que comprometem a estética e promovem a busca por procedimentos que venham a manter ou recuperar a aparência externa jovial (PERENACK, 2005).

Historicamente, os tratamentos utilizados para mascarar o envelhecimento facial eram focados exclusivamente na tração dos tecidos obtida por meio de procedimentos cirúrgicos, como a ritidoplastia (VARGAS; AMORIM; PITANGUY, 2009). Nos últimos 40 anos, uma série de substâncias injetáveis passou a ser desenvolvida com o intuito de preencher e suavizar as rugas na região buco facial. Como exemplo temos o silicone líquido, o colágeno bovino, a gordura autóloga, a hidroxiapatita, o ácido poli-L-láctico e, mais recentemente, o polimetilmetacrilato (PMMA) e o ácido hialurônico (AH) (YOON; HAN; KIM, 2003; EPPLEY; DADVAND, 2006). Tais produtos receberam o nome de materiais de preenchimento e se tornaram hoje um dos procedimentos mais comumente executados na medicina estética (NIAMTU, 2006; SÁNCHEZ et al., 2011).

O preenchimento facial é uma alternativa, não só para correção de alterações decorrentes do envelhecimento, mas também para produzir um aumento de volume artificial com fins cosméticos de estruturas da face, usualmente dos lábios (HÖNIG; BRINK; KORABIOWSKA, 2003). Os materiais de preenchimento tem ainda aplicação na área dos procedimentos médicos reconstrutivos, sendo utilizados para corrigir defeitos causados por trauma ou pela lipodistrofia facial secundária à terapia antiretroviral - TARV (SÁNCHEZ et al., 2011). Atualmente, a escolha desse tipo de tratamento tem crescido de forma expressiva, especialmente pela vantagem dessa técnica ser minimamente invasiva e de custo acessível (NIAMTU, 2006).

A literatura classifica os materiais de preenchimento atuais em não reabsorvíveis e reabsorvíveis, sendo os últimos aqueles que tem um efeito temporário de permanência nos tecidos (ZIMMERMANN; CLERICI, 2004).

Dos produtos não reabsorvíveis, o polimetilmetacrilato é o mais utilizado, com expressivo emprego especialmente nos casos de lipodistrofia facial em pacientes HIV positivos sob TARV (JONES, 2005; SKEIE et al., 2009). As apresentações comerciais variam de acordo com a concentração de PMMA empregada, que pode ser de 2, 10 e 30%, sendo a intermediária a mais usada pelos profissionais da área (ACHILLES, 2004).

O ácido hialurônico é o mais utilizado dentre os materiais de preenchimento reabsorvíveis. É usual o emprego desse produto na concentração de 20mg/mL, embora existam outras apresentações comercialmente disponíveis (5,5mg/mL e 25mg/mL). Tem efeito temporário, sendo enzimaticamente metabolizado ou fagocitado gradualmente em um período de 3 a 24 meses, dependendo do volume do agente implantado nos tecidos (ACHILLES, 2004). Um grande número de autores relata que o AH tem um tempo médio de permanência tissular de 9 meses (LUPTON; ALSTER, 2000; FERNÁNDEZ-ACEÑERO; ZAMORA; BORBUJO, 2003; ACHILLES, 2004).

À medida em que são produzidos e utilizados, há uma busca constante pelo material de preenchimento ideal (ZIMMERMANN; CLERICI, 2004). Teoricamente, além de não apresentarem potencial carcinogênico ou teratogênico, essas substâncias não deveriam ser migratórias, nem suscitar resposta inflamatória, sendo quimicamente inertes (HÖNIG; BRINK; KORABIOWSKA, 2003; ACHILLES, 2004). No entanto, nenhum dos produtos existentes até o momento demonstrou todas essas características e a literatura atual tem apontado uma série de casos nos quais ocorreram complicações buco faciais decorrentes da aplicação desses materiais (LOMBARDI et al., 2004; ZIMMERMANN; CLERICI, 2004; EDWARDS; FANTASIA; IOVINO, 2006; DA COSTA MIGUEL et al., 2009; JHAM et al., 2009).

Observa-se um crescimento no uso dos materiais de preenchimento facial devido ao custo relativamente baixo e à fácil execução dos procedimentos, impulsionados por uma busca incessante pela melhora da aparência física. Esse tema tem feito parte da rotina na prática clínica do cirurgião dentista, que passou a

se deparar com reações adversas nos tecidos bucais e peribucais provocadas pela injeção dessas substâncias.

Até pouco tempo, a literatura relacionada a esse assunto era baseada quase que exclusivamente em relatos de casos clínicos. Pesquisas na área da odontologia recentemente realizadas passaram a focar no entendimento das respostas causadas pelos materiais de preenchimento com finalidade estética (LOUREIRO BORGHETTI et al., 2011; VARGAS, 2011).

A presente tese foi desenvolvida para auxiliar na melhor compreensão do comportamento biológico frente a esses produtos estéticos. A análise de componentes envolvidos na resposta inflamatória buscou identificar, clínica e histologicamente, as respostas teciduais locais e à distância vinculadas ao uso de dois produtos mais utilizados na atualidade.

2 ARTIGO 1

O artigo a seguir intitula-se “**Dermal fillers: matter of interest in the dentist’s current practice**” e foi formatado e submetido de acordo com as normas da revista *Journal of Oral Pathology & Medicine* (Anexos A e B).

Dermal fillers: matter of interest in the dentist's current practice

Sabrina Pozatti Moure[1], Karlon Fróes de Vargas[1], Ruchielli Loureiro Borghetti[1], Karen Cherubini[1], Maria Antonia Zancanaro de Figueiredo[1]

[1] Division of Oral Medicine, School of Dentistry, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil

Running tittle: Dermal fillers: interest to the dentist

Key-words: dermal fillers, diagnosis, foreign body reaction

Corresponding author:
Dra. Maria Antonia Zancanaro de Figueiredo
Address: Serviço de Estomatologia do Hospital São Lucas da PUCRS
Av. Ipiranga, 6690 – 2º andar/sala 231, Porto Alegre, RS. Brasil. 90610-000
Phone/Fax: +55 51 3320.3254
Email: antonia.figueiredo@pucrs.br

Abstract

Currently, there has been a visible growth in demand for aesthetic procedures which maintain or restore the beauty, especially of the face. This need brought about the emergence of less invasive and more affordable techniques, when compared to traditional surgical procedures. There is a number of substances that have been developed and made available for use, although it is known that none of them is harmless to the tissue, causing possible adverse reactions. The areas of application are varied, being the perioral region one of the most targeted, by the need to reduce the nasolabial fold and increase in the volume of the lips. Adverse reactions in these locations are often detected in routine tests performed by the dental surgeon. They are usually observed as papular or nodular lesions in the oral mucosa and may even mimic other diseases. The objective of this paper is to review the existing literature linked to the theme, emphasizing the importance of knowing these types of changes which have been increasingly present in clinical dentistry.

Introduction

The search for the maintenance or achievement of facial beauty has always been present in human history. Until recently, treatment for this type of need focused on rhytidectomy through surgical techniques of tissue traction. Less invasive alternatives, with quick results and being economically more accessible, such as the injection of dermal fillers, have emerged in recent years aiming at the reduction of wrinkles and skin defect correction. Those techniques have been widely used by healthcare professionals, especially dermatologists and plastic surgeons (1, 2, 3).

However, filling materials have also been known by the chances of causing unwanted effects (4, 5, 6). Once they can be used in anatomical regions closer to the dental surgeon's field, this new reality is reflected in the routine of these professionals, who have increasingly reported orofacial tissue damage resulting from adverse effects of these products. Thus, it is necessary that the dental surgeon becomes able to detect and identify these lesions establishing differential diagnosis with other pathologies and treating the patient adequately.

Dermal fillers

The concept of beauty is often directly associated with youthfulness. A young face is one which has no wrinkles or lines, caused by structural and functional changes in organic tissues associated with the aging process. Over the years, the epidermis becomes thinner, the dermis becomes less elastic (7, 8) and the subcutaneous tissue is reduced because their constituents, such as collagen, elastin and glycosaminoglycans are gradually changed (7, 9, 10).

In the intention to restore this lost volume in the aging process of the face, a series of filling materials started to be developed and used in the last 40 years. Table 1 lists the currently most employed dermal fillers (2). Several other types of filling agents were developed in the past such as liquid silicone, paraffin, bovine collagen, autologous fat, hydroxyapatite, poly-L-lactic acid, but most fell into disuse due the numerous foreign body reactions and hypersensitivity (11). The dermal fillers are different with regard to chemical composition and length of permanence in tissues (6). Some of the most used fillers, such as polymethylmethacrylate (PMMA), are

classified as non-resorbable, since their constituents cannot be degraded by the body (12). Others are called resorbable, because they have a limited length of permanence in the tissues, such as hyaluronic acid (HA) (3, 13).

Table 1. Types of filling materials. Adapted from Carruthers et al.(2).

	Composition	**Brand name**	**Distributor**
Non-resorbable	Silicone	MDX-4-4011	No longer available
	PMMA microespheres and bovine collagen	Artecoll	Rofil Medical International
		Artefill	Artes Medical
	PMMA microespheres suspended in carboxygluconate/cellulose gel	Metacrill NewPlastic	Nutricel Laboratories Lebon Prod Quim Farm
Resorbable	Bovine collagen	Zyderm/Zyplast	Allergan
	Poly-L-lactic acid	Sculpra	Sanofi-Aventis Pharmaceutical
	Calcium hydroxyl appatite	Radiesse	Bioform Medical, Inc
	Hyaluronic acid (bacterial origin)	Restylane Puragen Perlane	Medicis Aesthetics Mentor Corp

Trademarks of PMMA most commonly used (Metacrill®, NewPlastic®) are composed of solid polymethylmethacrylate microspheres suspended in a colloid medium (carboxygluconate and carboxymethylcellulose, respectively). The microspheres are irregular in surface and have varying diameters ranging between 30 and 80µm (11, 12, 14). In the face, it is indicated and used for the filling of the nasolabial folds, lip and malar augmentation, facial contour definition, as well as the correction of lipodystrophy caused by antiretroviral therapies in HIV positive patients (6, 12, 15). PMMA is available for use at concentrations of 2, 10 and 30% and the indication of concentration varies proportionally according to the size of the defect to be treated (12). Microscopic examination of this material shows the presence of multiple translucent microspheres distributed in polymorphic cystic spaces (16).

The hyaluronic acid is found naturally in the connective tissue and several other human tissues (16). HA used as filling material can be obtained *in vitro* (through bacterial culture). This substance has been widely used for the correction of fine wrinkles and mild to moderate depressions (17). Several brands have been

developed (Restylane®, Puragen™, Perlane®) and are commercially available in different concentrations (5.5mg/mL, 20mg/mL and 25mg/mL), and the indication of each also varies in accordance to the extent of the defect to be corrected (3). When taken to the microscope, the material exhibits an amorphous and basophilic nature (16).

For years, the ideal dermal filler has been searched without success (6, 13). This should include specific criteria such as: not causing an inflammatory response; neither migrating nor being immunogenic; being chemically inert; not having potential to be carcinogenic or teratogenic and being biocompatible (4).

In the current literature, there are numerous reports of complications arising from the use of these products, such as local inflammatory processes of the foreign body reaction and migration of the substances (18-24).

Local inflammatory reaction

When an exogenous material is introduced in the body, there is a concatenated sequence of adjacent tissue inflammatory events (25).

Irritation caused from the filling material application generates local coagulation and activates the surrounding tissue. A series of chemical mediators released at this time causes hemodynamic changes which include changes in size and vascular flow, as well as increased permeability of blood vessels. Fibrinogen, platelet-activating factor (PAF) and cytokines are some of these mediators which, by causing vascular changes, facilitate the exudation of leukocytes (26). Polymorphonuclear neutrophils accumulate at the site of injury and are responsible for the effective first line of defense in the inflammatory process (3, 27).

The presence of neutrophils, in addition to hyperemia due to vascular changes and edema caused by plasma exudation in the early stages after injection of dermal fillers (3, 12), justify the clinical signs and symptoms that include pain, erythema, ecchymosis and increase in regional volume (3, 28-31). The duration and intensity of these effects depend on individual factors and product used, ranging from

a few days to a few weeks (13). Skin necrosis and infection can also occur sometimes causing irreversible damage to tissues (12).

Once the materials are not readily reabsorbed, and the irritative stimulus persists, the inflammatory process becomes chronic in the form of a mononuclear infiltrate of lymphocytes and plasma cells (Figure 1) (3, 25). To enable the infiltration of these cells into the site of injection, which takes place beginning to the end of the inflammatory process, it is necessary to generate a blood vascular network. Fibrinogen present in the initial coagulation turns into fibrin, which is a potent vasodilator and pro-angiogenic factor. It is known that platelets also play this role. Histamine released by mast cells present in the tissue stimulates neovascularization and vessel permeability increases, allowing extravasation of leukocytes. Even exudate leukocytes release cytokines which will provide a signal so that leukocytes and angioblasts proliferate (27, 32, 33).

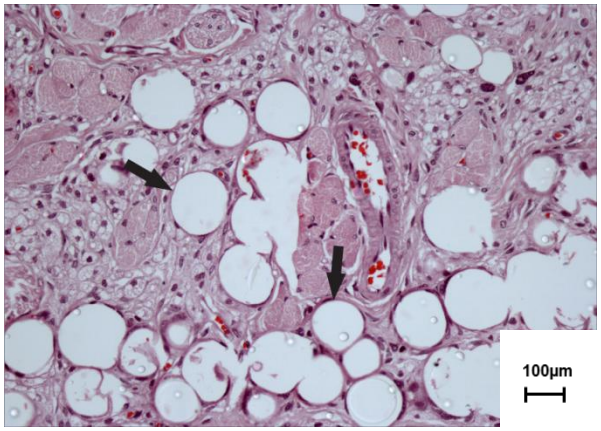

Figure 1 - Microscopic appearance of PMMA on connective tissue with presence of lymphoplasmacytic cells and macrophages in a section stained with hematoxylin and eosin (x400, original magnification). Note the similarity between the material´s microspheres (arrows) and adipocytes.

The progression of these inflammatory events requires extravasation and migration of macrophages to the site of injection of the material for phagocytosis (25). The attraction of phagocytes occurs mainly by the action of cytokines produced and secreted by cells already present in inflammation, such as neutrophils, lymphocytes, endothelial cells, fibroblasts, as well as the macrophages themselves. Examples are granulocyte macrophage colony-stimulating factors (GM-CSF), which are produced by T lymphocytes, endothelial cells, fibroblasts and macrophages (32). Interleukins

(IL) such as IL-2, which is produced by T lymphocytes (27) and transforming growth factor beta (TGFβ) secreted by neutrophils, endothelial cells, fibroblasts and macrophages themselves (34) can also illustrate this process. The recognition of exogenous material by the macrophage occurs via receptors that seize material surface molecules and induce the intake of these phagocytic cells enabled for later degranulation and digestion of the encapsulated material (27).

In biocompatible materials such as HA (3, 14, 28, 29, 35), a granulation tissue composed of newly formed blood vessels, macrophages and fibroblasts (25) represents the reparative phase of the chronic inflammatory response. On the other hand, when the material implanted in the tissue is immunogenic, the inflammatory process follows another route: the foreign body reaction, by the presence of foreign body giant cells (6, 25, 35, 36). These cells are formed from the fusion of macrophages, with the help of interleukins IL-3 and IL-4, when they cannot phagocytize the injected material (6, 25, 35, 36).

According to some authors, all filling materials can cause foreign body reaction in some patients (6). The etiopathogenesis for that is uncertain and no prediction can be made (14). Some possible causes include the injection of a large volume of material, the presence of impurities in the dermal filler, as well as cross-reactivity mediated by immune complement due to previous systemic infections. In addition, physical and chemical characteristics of the products are important factors regarding biocompatibility. Filling materials with irregular shape, porous surfaces and varied diameter particles, with those larger than 20μm tend to trigger lasting inflammation that can follow the path of the foreign body reaction (2, 25, 27, 37, 38).

The productive-reparative phase of the inflammatory process is characterized by the release of matrix metalloproteinases (MMPs) by macrophages and giant cells, to remodel the extracellular matrix (ECM) (25). In the case of resorbable materials in general, this last stage of inflammation will exist until the final degradation of the injected product. For non-resorbable materials, the reaction continues until one capsule is formed around the implant (13, 27). This fibrous capsule around the surface of the material is mainly caused by the production of TGFβ, predominantly expressed by macrophages, which leads to the formation of collagen fibers (39).

The late clinical signs that occur at the site of injection of filling materials appear in the form of painless papules or nodules, sometimes only noticeable by digital palpation (Figure 2) (3, 16). These lesions tend to be located in the oral submucosa (usually in the lips, gingivolabial sulcus and cheek mucosa) detected for weeks to years after injection of the material and are usually confused with pathologies of distinct etiology and behavior, such as cysts or tumors of the salivary glands (24).

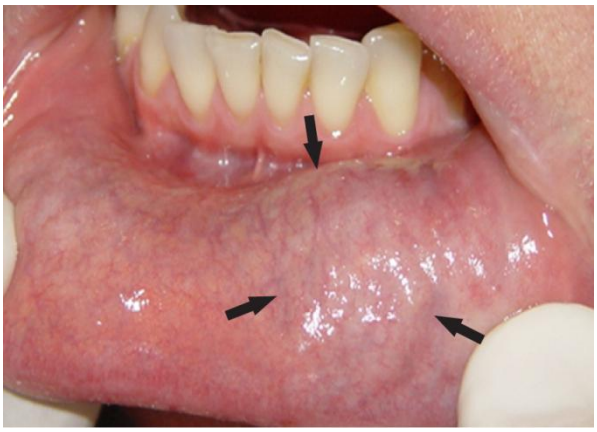

Figure 2 - Left lower lip submucosal nodule (arrow) noted on clinical examination in patient who had a cosmetic procedure with PMMA 10 years

Literature available on the adverse effects of dermal fillers is almost exclusively based on case reports (21, 40, 41). In 2004, a group of authors reported 11 cases of patients with nodules in the orofacial region, some of whom initially thought they were cases of salivary cysts or lesions of neoplastic origin. After biopsy, microscopic examination showed the presence of a foreign body reaction in all samples. The patients had undergone subcutaneous injections of various filling materials, including PMMA and HA (21). Several other authors also reported cases of submucosal nodules that were presumed to be mucocele or benign neoplastic lesions. However, after the histopathology of biopsied specimens, the diagnosis was foreign body reaction caused by PMMA and HA (24, 40-42).

One of the first experimental studies on this subject have been recently published. Loureiro Borghetti et al. (3) evaluated the clinical and histopathologic local responses 7, 60 and 90 days after the injection of 5.5mg/mL HA and 25mg/mL HA in rat tongues. The authors concluded that both concentrations of HA proved tolerable by the tissue, suggesting that this material has characteristics of biocompatibility.

Sánchez et al. (6) used skin biopsies to investigate the activation of macrophages and inflammatory response in patients with dermal fillers, including PMMA, and observed that there is a strong relationship between non-resorbable filling material and foreign body reaction.

Migration

The migration of the substance is another side effect reported due to the use of dermal fillers, although it is a very controversial issue in the literature (37, 43). Most researches mention the migration at a distance, without specifying its precise location (12).

A study observed the presence of renal and hepatic inflammatory infiltrate in rats subjected to injection of filling materials in the ears. Changes were interpreted as a result of the systematization of drugs, which could occasionally act as chemotactic substances, acting at a distance in organs of metabolism and excretion (44).

Other authors (38) analyzed microscopically structures such as lymph nodes, lung, spleen and liver of animals that received cheek, armpit and groin injections of various materials, most of which contained PMMA. The authors found no signs of migration, except in lymph nodes, and pointed out that there are three ways in which particles of materials can be transported: 1) hematogenous, if an inadvertent injection reaches a vein, 2) lymphatic, just as the hematogenous mechanism, it is necessary that a large lymphatic vessel be injured, and 3) by phagocytosis, when material fragments are phagocyted at the injection site and macrophages move through the lymphatic system.

Loureiro Borghetti et al. (3) injected different concentrations of HA in rat tongues. After 7, 60 and 90 days of monitoring, the authors have found, through histological analysis, the absence of any kidney alteration.

Conclusion

Day after day there has been a greater appeal for facial aesthetics and easy access to the most varied types of treatment. In a direct relationship, it is expected that dental surgeons have to increasingly deal with oral lesions arising from adverse reactions to filling materials.

Therefore it is important that healthcare professionals be aware of possible undesirable effects as well as how to handle patients undergoing cosmetic procedures using filling materials. It is suggested that the dentist becomes aware of papular or nodular lesions located in the oral mucosa, valuing all stages of anamnesis and physical examination, with special attention to the history of previous aesthetic treatments.

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3 ARTIGO 2

O artigo a seguir intitula-se “**Clinical and pathological characteristics of polymethylmethacrylate and hialuronic acid in the rat tongue**” e foi formatado e submetido de acordo com as normas da revista *International Journal of Oral & Maxillofacial Surgery* (Anexos C e D).

Clinical and pathological characteristics of polymethylmethacrylate and hialuronic acid in the rat tongue

Sabrina Pozatti Moure[1], Karlon Fróes de Vargas[1], Ruchielli Loureiro Borghetti[1], Fernanda Gonçalves Salum[1], Karen Cherubini[1], Maria Antonia Zancanaro de Figueiredo[1]

[1] *Division of Oral Medicine, School of Dentistry, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, Brazil*

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Corresponding author:
Dra. Maria Antonia Zancanaro de Figueiredo
Address: Serviço de Estomatologia do Hospital São Lucas da PUCRS
Av. Ipiranga, 6690 – 2º andar/sala 231, Porto Alegre, RS. Brasil. 90610-000
Phone/Fax: +55 51 3320.3254
Email: antonia.figueiredo@pucrs.br

Abstract

Adverse effects on the oral mucosa after the use of dermal fillers have been increasingly reported due to the increment of their use for facial aesthetics. The objective of this study was to evaluate both clinically and histologically the initial and late reactions, locally and at long distance, against 2 types of product: 10% polymethylmethacrylate and 20mg/mL hyaluronic acid. Each substance was randomly and separately injected in the rat tongues (polymethylmethacrylate, n=16; hyaluronic acid, n=18). They were compared with the control group (n=16) in 3 experimental times (7, 60 and 90 days) in the following analyses: clinical, intensity of local inflammatory response (hematoxylin and eosin), amount of newly formed blood vessels and macrophages (immunohistochemistry), density of collagen fibers (picrosirius) and systemic migration of the product in the liver and kidney (hematoxylin and eosin). The results showed inflammation triggered from the material injection, suggesting that both substances cause responses in local tissue, although there was biocompatibility when hyaluronic acid was used. This research highlights the importance of experimental studies on the subject, since adverse reactions have been observed routinely in the practice of dentists.

Introduction

Recently, dental surgeons have been dealing with nodular lesions preferentially distributed in the labial or buccal submucosa resembling inflammatory or neoplastic pathologies of minor salivary glands[9]. However, when biopsied and having their specimens evaluated microscopically, these lesions show an exogenous material associated with foreign body reaction. These products are dermal fillers used by dermatologists and plastic surgeons to restore the volume of subcutaneous tissue lost during the aging process or to enhance soft parts, such as the lips[14, 25]. Reports of numerous cases of foreign body reaction in the orofacial region by injecting filling materials draw attention to one important fact: it has become increasingly common to use these cosmetic procedures for seeking or maintaining a youthful appearance of the face. This occurs mainly due the fact that this technique is minimally invasive and more affordable when compared to traditional surgical traction[14].

The reaction caused by the presence of an exogenous substance inside the tissues occurs in the form of an inflammatory response that begins with the influx of neutrophils, causing pain and exudation. Later, lymphocytes, plasma cells and macrophages are detected at the site, with possible formation of giant cells. In this case, a foreign body reaction takes place. On the periphery of the inflammation area there are intense signs of fibroplasia and neovascularization[27].

In addition to the reactions that occur at the site of injection of these substances, there is also migration. This is a very controversial issue in literature, and most papers on migration at a distance do not specify its precise location. A study by Rosa and de Macedo[20] noted the presence of hepatic and renal inflammatory infiltrates in rats that received the application of different filling materials in the ear. The authors suggest that these substances could act at a distance in organs of metabolism and excretion.

The most widely used dermal fillers are polymethylmethacrylate (PMMA) and hyaluronic acid (HA). PMMA is perennial in the tissues due to the body's difficulty to degrade its constituents[12]. On the other hand, HA is resorbable, having a limited length of permanence, ranging from 6 to 9 months[13]. Both are indicated for filling wrinkles and correcting nasolabial folds, as well as soft tissue augmentation[25]. One of

PMMA's peculiarities is its use in cases of facial lipodystrophy in HIV patients treated with antiretrovirals[24].

Most studies have mentioned the reactions resulting from the use of facial filling materials based almost exclusively on case reports[5, 7, 13]. Recent research suggests the involvement of inflammatory cells such as lymphocytes, macrophages and giant cells in the immune response to the dermal filler[21]. Therefore, an experimental study was undertaken on the topic, evaluating and comparing clinical and histopathologic responses at early and late study times using different materials.

Materials and methods

This longitudinal randomized experimental study used 50 *Wistar* female rats (Rattus norvegicus) from the same breeder. All procedures were performed according to institutional standards for the care and use of experimental animals after the approval of local ethics committees.

The rats were kept in cages placed in ventilated shelves with the regular temperature of 22 ± 1°C and light-dark cycles of 12 hours. They were fed with Nuvilab-CR1 and filtered water *ad libitum*.

In the beginning of the experiment, each animal weighed about 200g and had an average of 2 months of age. They were divided into 3 groups according to the indicated treatment: PMMA (10% NewPlastic®; Lebon Chemicals and Pharmaceuticals Ltd., Rio de Janeiro, Brazil) (n=16); HA (20mg/mL Puragen™; Mentor Corporation, Santa Barbara, USA) (n=18) and control (NaCl 0.9%) (n=16). Each group was subdivided into 3 experimental times of 7, 60 and 90 days, according to the interval between treatment and euthanasia.

The animals were weighed prior to subsequent anesthesia using intraperitoneal injection of xylazine hydrochloride 20mg/mL (0.05mL/100g) with ketamine hydrochloride 50mg/mL (0.1mL/100g). Once anesthetized, the rats were placed in supine position on the operating table for the injection of the substance to be tested. The needle (26G ½, 13 x 4.5) was cautiously introduced in the tissues with its bevel facing up and the long axis as parallel as possible to the mucosa. The

submucosa was dissected at 7mm and standardized with the use of an endodontic cursor. Thus, 0.07mL of each filling material was introduced in the middle third of the ventral tongue, 2mm to the left of the midline and 7mm to the front of the frenum.

Clinical alterations

In each experimental time, the animals were sedated for clinical evaluation and the presence or absence of fundamental lesions such as plaques, papules and ulcerations was observed.

After clinical evaluation, euthanasia was proceeded by asphyxiation with isoflurane and subsequently the tongue, the right medial lobe of the liver and the right kidney were removed. These fragments were fixed in neutral buffered formalin at 10% and processed for hematoxylin and eosin (H-E) staining. Immunohistochemistry and picrosirius were also applied in tongue samples.

Training was conducted with an experienced pathologist aiming to standardize the criteria for analysis. Before reading the slides, the examiner was calibrated and blinded. The intra-examiner calibration was performed using the Intraclass Correlation through re-analysis of 30 slides or fields with an interval of 7 days, showing excellent correlation between the readings related to the intensity of inflammatory response ($p = 0.904$), number of newly formed blood vessels and macrophages ($p = 0.967$) and density of collagen fibers ($p = 1.000$).

For the microscopic analysis of the tongues, in the PMMA and HA groups, slides where exogenous material could be identified were considered viable and evaluation of the tissue near implant was made. In the control group, the area being evaluated corresponded to the anatomic region where the material was applied.

Intensity of inflammatory response

Through the use of a biological microscope (Zeiss® Axioskop 40, Zeiss, Oberkochen, Germany), at x100, x200 and x400 magnifications, the examiner qualitatively assessed the presence of inflammatory cells (neutrophils, eosinophils,

lymphocytes, plasma cells, macrophages) in the tongue sections stained by H-E. Giant cell analysis was done by individual descriptive evaluation of the slides. From the criteria already used by other studies[8, 14], the observation of certain characteristics defined the inflammation score, always bearing in mind the area where there was more intense tissue response:

0- Absent: absence of inflammation;
1- Mild: presence of sparse mononuclear cells;
2- Moderate: presence of lymphoplasmocitary infiltrates and/or sparse neutrophil and eosinophils;
3- Intense: presence of neutrophil and eosinophil infiltrates.

Number of newly formed blood vessels and macrophages

The immunohistochemical reactions in tongue sections were carried out using streptavidin-biotin complex with the following primary antibodies: anti-CD34 (NovoCastra, Newcastle, UK, 1:250 dilution) and anti-CD68 (NovoCastra, Newcastle, UK, 1:200 dilution). Positive controls were made through histological sections of the rat's intestine (CD34) and lung (CD68). In the negative control, there was omission of the primary antibody.

Using a Zeiss® microscope (Axioskop 40, Carl Zeiss, Jena, Germany) coupled to a camera (Cool Snap-Pro cf, Media Cybernetics, Bethesda, USA) connected to a Dell® computer (model Optiplex GX620, Round Rock, USA), for each rat, 10 microscopic fields were captured at a larger magnification (x400) for each marker. The manual count of structures was performed in each field of 166μm^2 considering the following criteria of positivity: wall thickness and size of blood vessel in stained slides with CD34 and cellular morphology of macrophage in slides stained with CD68.

Density of collagen fibers

It was possible to capture 3 to 5 fields of slides with tongues stained by picrosirius in a polarized light microscope (Zeiss® Axioskop 40 Zeiss, Oberkochen,

Germany) coupled to a camera (Cool Snap-Pro cf, Media Cybernetics, Bethesda, USA) connected to a Dell® computer (Optiplex GX620 model, Round Rock, USA). The chosen area should cover the largest amount of collagen fibers by using the x100 magnification. Images were transported to Image-Pro Plus®, version 4.5.1 (Media Cybernetics, Inc.; 2005) where the collagen percentage, represented by red birefringent, was calculated in the total slide area (673µm^2).

Migration

Migration was evaluated in the liver and kidney slides stained by H-E in a biological microscope (Zeiss® Axioskop 40 Zeiss, Oberkochen, Germany) at x100, x200 and x400 magnifications, based on the presence or absence of inflammatory response or traces of injected material.

All data was tabulated and analyzed through the SPSS 17 software (SPSS Inc.), with the Kruskal-Wallis non-parametric test, complemented by its Multiple Comparisons test at a significance level of 5%, since the distribution of data did not adjust to the Normal Distribution.

Results

Clinical alterations

In clinical analysis at 7 days, all animals showed lesions in the form of ulcers close to the PMMA injection site while no change was found in the HA group. At 60 days, 1 of 5 rats that received PMMA showed papules and 50% of those receiving HA developed white plaques in the region. When assessed 90 days after the material injection, both test groups showed white plaques: 2 animals from the PMMA and 1 from the HA group. The control group did not show any clinical alteration at the 3 experimental times.

Intensity of inflammatory response

At 7 days, inflammatory response at the site of injection of PMMA was intense (Fig. 1a), differing significantly from the moderate response at other times (Fig. 1b). Animals treated with HA presented a distinct behavior: in the first week, they showed a response that ranged from moderate to intense (Fig. 2a), dropping significantly until the 90-day period, when a mild to moderate inflammatory process was observed (Fig. 2b). No changes were observed in the control group (Table 1).

Table 1. Comparison of local inflammatory response intensity of each material throughout the different studied time spans.

Material	Score	**Time**		
		Day 7	**Day 60**	**Day 90**
PMMA	0- Absent	0	0	0
	1- Mild	0	0	0
	2- Moderate	2	5	5
	3- Intense	4	0	0
	Total (n)	6	5	5
	Mean Rank	11.83^{A}	6.50^{B}	6.50^{B}
HA	0- Absent	0	0	0
	1- Mild	0	1	3
	2- Moderate	3	5	3
	3- Intense	3	0	0
	Total (n)	6	6	6
	Mean Rank	13.50^{A}	8.75^{AB}	6.25^{B}
Control	0- Absent	6	6	4
	1- Mild	0	0	0
	2- Moderate	0	0	0
	3- Intense	0	0	0
	Total (n)	6	6	4
	Mean Rank	8.50^{A}	8.50^{A}	8.50^{A}

Kruskal-Wallis non-parametric test, $p<0.05$. Distinct letters in line differ significantly.

The test groups showed a significantly higher inflammatory response than the control group at 7, 60 and 90 days. PMMA tended to present more intense inflammatory scores than HA, though no statistically significant difference could be seen (Table 2).

Table 2. Comparison of local inflammatory response intensity in each studied time span between the different materials.

Time	Score	Material		
		PMMA	**HA**	**Control**
Day 7	0- Absent	0	0	6
	1- Mild	0	0	0
	2- Moderate	2	3	0
	3- Intense	4	3	0
	Total (n)	6	6	6
	Mean Rank	13.00^{A}	12.00^{A}	3.50^{B}
Day 60	0- Absent	0	0	6
	1- Mild	0	1	0
	2- Moderate	5	5	0
	3- Intense	0	0	0
	Total (n)	5	6	6
	Mean Rank	12.50^{A}	11.58^{A}	3.50^{B}
Day 90	0- Absent	0	0	4
	1- Mild	0	3	0
	2- Moderate	5	3	0
	3- Intense	0	0	0
	Total (n)	5	6	4
	Mean Rank	11.50^{A}	8.75^{A}	2.50^{B}

Kruskal-Wallis non-parametric test, $p<0.05$. Distinct letters in line differ significantly.

The evaluation of the presence of giant cells showed that this event was remarkably present in the PMMA group at all experimental times (Table 3).

Table 3. Giant cells (GC) in the implantation site of materials in each studied time span.

Material		Time		
		Day 7	**Day 60**	**Day 90**
PMMA	*Presence of GC (# rats)*	5	3	4
	Total (n)	6	5	5
HA	*Presence of GC (# rats)*	1	0	0
	Total (n)	6	6	6
Control	*Presence of GC (# rats)*	0	0	0
	Total (n)	6	6	4

Number of newly formed blood vessels and macrophages

The PMMA sites of injection showed a gradual increase, with statistically different newly formed blood vessels at all times (Fig. 1c, 1d and 1e). HA showed a predominance of these structures at 7 and 60 days (Fig. 2c and 2d), followed by a significant decrease in the last experimental time (Fig. 2e). The control group showed no difference in the number of newly formed vessels between the 3 study times (Table 4). When comparing the behaviour of substances injected at each time, it was observed that the test groups always presented more newly formed blood vessels than the control group. During the first 7 days, the rats injected with HA had a significantly higher number of that event than those which received PMMA. This relationship reached the same level after 60 days and was reversed in the last experimental period (Table 4).

With regard to the number of macrophages present in the tissue where the substances were applied, no significant difference between the times in the PMMA (Fig. 1f, 1g and 1h) and control groups was seen. HA, on the other hand, showed a greater number of these cells at 7 and 60 days (Fig. 2f and 2g) (Table 4). The test groups presented a statistically higher number of macrophages than the control group at all experimental times. The only significant difference between the filling materials occurred after 90 days, when PMMA had these cells in greater amounts (Table 4).

Density of collagen fibers

The percentage of collagen fibers near the area where PMMA was present was higher at 7 and 60 days (Fig. 1i and 1j). This value decreased significantly until 90 days of experiment (Fig. 1k). HA, however, reached a density peak of these fibers at the intermediate time (Fig. 2j). The control group showed no differences between the 3 study times (Table 4). When treatments were compared in the first 7 days, the rats that received PMMA had a higher amount of collagen fibers in the area than the other groups (with statistically significant difference only between the PMMA group and control). At 60 days, both test groups had a higher density of collagen fibers compared to the control group, therefore showing a statistical difference. In the last

experimental time, the amount of collagen fibers was higher in the HA group followed by PMMA and control groups, with statistically significant difference only between HA and control groups (Table 4).

Table 4. Median and Interquartile Interval distribution of materials in different time spans for each of the analysis.

Analysis	**Material**	**Time**		
		Day 7	**Day 60**	**Day 90**
		Median (P25-P75)	Median (P25-P75)	Median (P25-P75)
Number of newly formed blood vessels (#/166µm^2)	**PMMA**	1.00^{Cb} (0.00-2.00)	2.00^{Ba} (1.00-2.00)	2,00^{Aa} (1,00-3,00)
	HA	2.00^{Aa} (1.00-3.00)	2.00^{Aa} (1.00-3.00)	1.00^{Bb} (0.00-2.00)
	Control	0.00^{Ac} (0.00-0.00)	0.00^{Ab} (0.00-1.00)	0.00^{Ac} (0.00-0.00)
Number of macrophages (#/166µm^2)	**PMMA**	1.00^{Aa} (1.00-2.00)	1.00^{Aa} (0.00-3.00)	2.00^{Aa} (1.00-3.00)
	HA	1.00^{Aa} (0.00-2.50)	1.00^{Aa} (0.00-2.00)	0.00^{Bb} (0.00-2.00)
	Control	0.00^{Ab} (0.00-0.00)	0.00^{Ab} (0.00-0.00)	0.00^{Ac} (0.00-0.00)
Density of collagen fibers (%/673µm^2)	**PMMA**	3.99^{Aa} (3.17-4.88)	5.30^{Aa} (3.00-10.69)	1.41^{Bab} (1.09-1.69)
	HA	2.56^{Bab} (1.59-3.38)	5.00^{Aa} (3.34-8.40)	1.57^{Ba} (1.22-3.57)
	Control	0.43^{Ab} (0.21-0.91)	0.25^{Ab} (0.00-0.60)	0.76^{Ab} (0.34-0.80)

Kruskal-Wallis non-parametric test, $p<0.05$. Medians followed by distinct upper case letters in the line differ significantly. Medians followed by distinct lower case letters in the column differ significantly.

Migration

Neither alterations compatible with an inflammatory response nor the presence of traces of the injected substances could be seen in the liver and kidney samples.

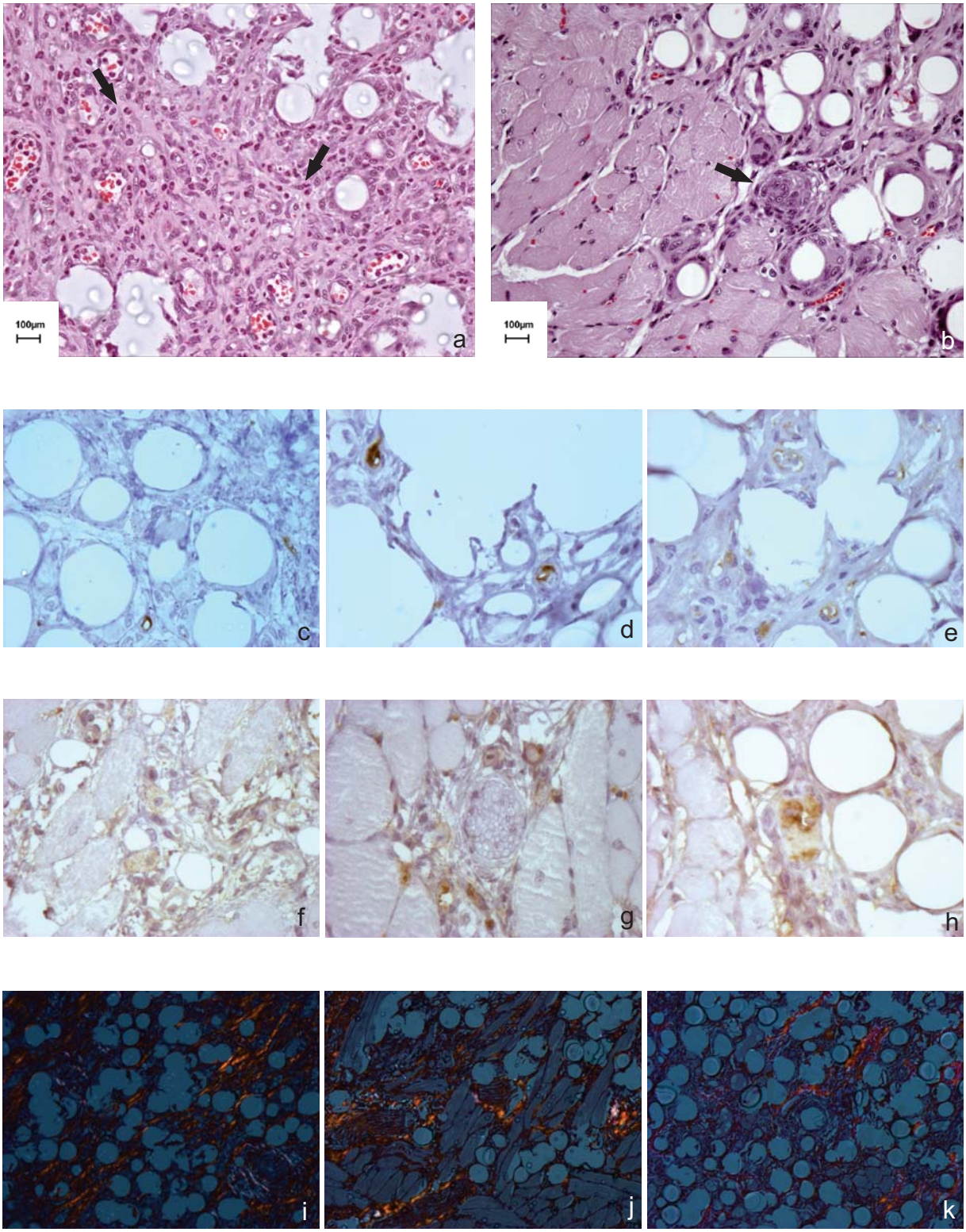

Fig. 1. PMMA local inflammatory response. Intense inflammation with infiltration of neutrophils (arrows) at 7 days (a) and moderate inflammation with lymphoplasmocitary infiltrates at 90 days (b) with the presence of giant cells (arrow) (H-E, x400). CD34 expression on new-formed blood vessels at 7 (c), 60 (d) and 90 days (e) detected by immunohistochemistry (x400). CD68 expression on macrophages at 7 (f), 60 (g) and 90 days (h) detected by immunohistochemistry (x400). Collagen fibers stained with picrosirius under polarized microscopy at 7 (i), 60 (j) and 90 days (k) (picrosirius, x200).

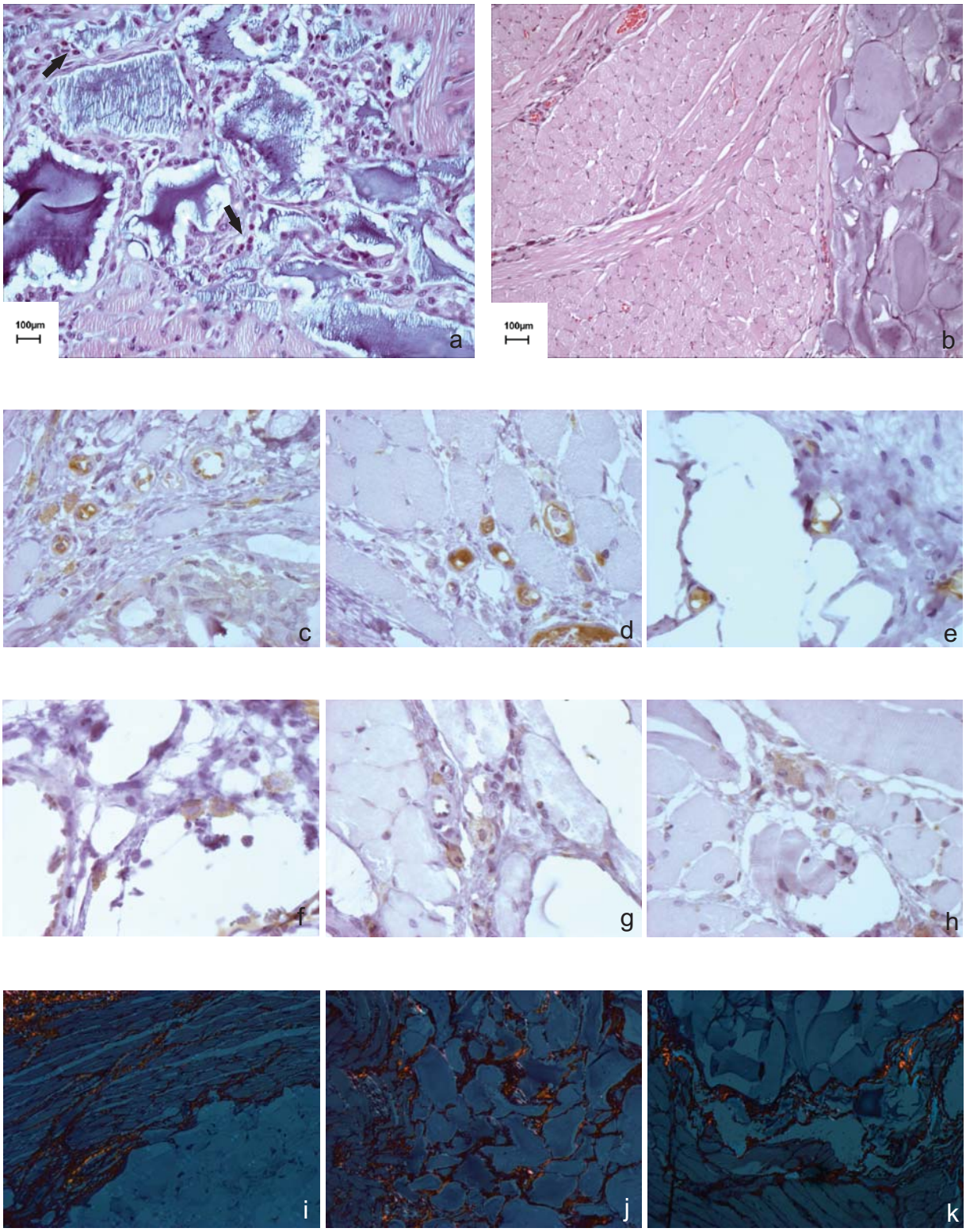

Fig. 2. HA local inflammatory response. Intense inflammation with infiltration of neutrophils (arrows) at 7 days (a) and mild inflammation with sparse lymphoplasmocitary cells at 90 days (b) (H-E, x400). CD34 expression on new-formed blood vessels at 7 (c), 60 (d) and 90 days (e) detected by immunohistochemistry (x400). CD68 expression on macrophages at 7 (f), 60 (g) and 90 days (h) detected by immunohistochemistry (x400). Collagen fibers stained with picrosirius under polarized microscopy at 7 (i), 60 (j) and 90 days (k) (picrosirius, x200).

Discussion

In this research, 2 of the most commonly used filling materials by dermatologists and plastic surgeons in the perioral region were used in order to obtain a better understanding of local and distant responses that those materials can cause to tissues. The ventral tongue of the rat was the structure chosen for the injection of substances with the intent to eliminate any interference with the results, since this region is free from trauma or chronic irritation[14]. During the experiment, the control group showed no changes in any of the analysis, which allowed it to be considered as a physiological standard for comparison.

Responses triggered by the injection of dermal fillers illustrated the inflammatory process in all its stages. Generally speaking, the presence of exogenous material initially generates a clot at the site, which serves as the foundation for future extravasation of inflammatory cells[15]. There are synthesis and accumulation of chemical mediators such as histamine, cytokines and prostaglandins, which modify the vascular bed and blood flow to neutrophil margination and diapedesis, the first exuded leukocytes[15]. In the analysis after 7 days, there was an intense inflammatory response that occurred at the expense of a polymorphonuclear neutrophil infiltration in both materials, though PMMA has shown a higher number of cases of intense inflammation than HA, possibly because it is less tolerated by tissues[14, 17, 25]. Previous research using the same materials obtained similar results[14, 25]. Although there are reports that HA is able to induce allergic processes in some patients[11], very few eosinophils have been seen in the tissues surrounding the studied materials and this presence was considered compatible with physiological conditions[19].

So that the extravasation of these inflammatory cells can take place, it is necessary to generate a network of blood vessels. In the beginning of coagulation there is fibrinogen which is converted to fibrin - a potent vasodilator and pro-angiogenic factor that promotes proliferation, migration and differentiation of endothelial cells. In addition, histamine released by mast cells present in the tissue, platelets and neutrophils recruited in the early stages of the inflammatory process stimulate neovascularization[15]. In the first week after the material injection, the animals from the test groups showed statistically larger numbers of newly formed

blood vessels when stained with CD34 and compared to the control. The same was observed over the 60 and 90 days of experiment, reinforcing the idea that the vascular supply is necessary to make the inflammatory process feasible from start to finish.

Due to the inability of the initial responses to degrade the injected filling material, inflammation became chronic at 60 and 90 days of experiment, represented by the presence of a lymphoplasmocitary infiltrate, featuring a moderate intensity response[1, 2]. Nevertheless, in the last monitoring period, half of the HA cases had a mild response, with sparse presence of lymphocytes and plasma cells. This behavior was previously described in other studies[11, 14] and, besides corroborating its resorbable nature, it suggests the compatibility of this product with the tissues.

Reinforcing that, dermal fillers showed that the intensity of the inflammatory response was related to the number of macrophages. When stained with CD68, the number of phagocytes in the test groups was statistically larger than in the control group throughout the entire experiment, remaining stable in the PMMA group in the 3 evaluated periods and decreasing at 90 days in the HA group. The explanation lies in the fact that in the PMMA group, the cells involved in chronic inflammatory process that remained in place recruited even more macrophages[15]. Exuded lymphocytes produce and release interleukins (IL), such as IL-2, which attract macrophages to the injured site[15]. Endothelial cells stimulate the attraction of phagocytes by releasing cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF)[16] and secrete transforming growth factor beta (TGFβ)[22]. Moreover, lymphocytes, neutrophils and macrophages themselves also assist in this process[16].

In biocompatible materials, the resolution of inflammatory response occurs through a granulation tissue composed of newly formed blood vessels, macrophages and fibroblasts[1]. If the material implanted in tissue is immunogenic, the inflammatory response follows another pathway represented by the presence of giant cells originated from the fusion of macrophages[1, 21]. Most rats injected with PMMA showed giant cells in the vicinity of the material. In the HA group, however, a small number of these cells was seen in just one animal and exclusively in the first week of the experiment, reinforcing the idea that this material has biocompatibility characteristics[11, 14, 19].

The pathogenesis and the natural course of the foreign body reaction after the injection of a filling agent remains unknown[9, 11]. Physicochemical properties of the materials, including particle size, chemical composition and structure and surface charge are the main factors that influence the inflammatory response pathway to be followed[10, 11, 21]. PMMA is a synthetic material composed of particles of irregular surface and varying diameters, ranging between 30 and 80μm, reasons why it usually causes more intense inflammatory responses, such as foreign body reaction[3, 11, 12]. The attempt to inject a large volume in a single session and the presence of impurities in the dermal filler may possibly contribute to the pathogenesis of this reaction[17].

In the rats treated with PMMA, density of collagen fibers was increased at 7 and 60 days, suffering a decrease at 90 days. A possible explanation for this finding is that inflammatory cells remaining close to the injection site produce and release matrix metalloproteinases and cytokines, inciting collagenase activity that leads to loosely arranged collagen fibers[18]. Furthermore, implants with particles that are irregular, porous and large in diameter, such as PMMA, tend to induce long-lasting inflammatory responses with low deposition of collagen fibers, in the form of a characteristically thin fibrous capsule around each microsphere[3, 12, 15, 27]. In the HA group, the peak density of collagen fibers was at 60 days, followed by a decrease at 90. This phenomenon was interpreted as reflecting the transition between the granulation tissue and the healing of the injured area associated with material degradation, when there is wound remodelling[23]. In agreement with this finding, Yoon et al.[26] did not observe the proliferation of collagen fibers 4 months after applying 20mg/mL HA.

In inflammation, the histopathological behavior translates into clinical manifestations. The ulcerated lesions in the tongue of animals that received PMMA at 7 days were interpreted as a reflection of the acute inflammatory response[14]. The edema coupled with the increase of volume caused by the injection of the material may have distended and traumatized tissues, leading to loss of epithelium. At 60 and 90 days, the white plaques and papules observed in some rats in the test groups are justified by keeping the increase in local volume, which may favor the injury and result in thickening of the keratin fibers or hyperplasia of collagen fibers[9, 14]. Several

authors mention these clinical signs in patients treated with dermal fillers, which may occur from weeks to years after injection[4, 6, 27].

Literature does not clearly indicate the migration ability of these filling agents. The absence of changes in the evaluated organs of metabolism and excretion agrees with most previous studies[12, 14], while Rosa and de Macedo[20] have found marked hepatic and renal inflammation in an experimental study in rats.

The results of this research showed that both filling materials caused inflammatory responses and that HA seems to be better tolerated by the tissue. PMMA had a distinct tissue response through the formation of a foreign body reaction. This finding raises questions about the inflammatory response in the long run, since the consequences can be quite damaging. These topics must be clarified through future research in the medical and dental care areas, since these materials have been widely used in facial and perioral tissues.

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Ethics: This study was approved by the Scientific and Ethic Committee (protocol #0005/10) and by the Ethic Committee for Animal Use (protocol #10/00151) of the Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brazil. (Anexos E e F)

4 DISCUSSÃO GERAL

O apelo pela melhora ou manutenção da aparência facial tem aumentado nos últimos anos, visando principalmente a retardar os sinais do envelhecimento (PERENACK, 2005). Os recursos da medicina estética para essa necessidade eram focados em técnicas cirúrgicas de tração dos tecidos, mas passaram a dividir espaço com procedimentos menos invasivos e mais acessíveis economicamente, como a injeção de materiais de preenchimento (YOON; HAN; KIM, 2003; EPPLEY; DADVAND, 2006; VARGAS; AMORIM; PITANGUY, 2009). Muitos desses produtos utilizados tem resultados satisfatórios e são seguros, apesar das literaturas médica e odontológica relatarem um número considerável de complicações buco faciais decorrentes da sua aplicação (LOMBARDI et al., 2004; ZIMMERMANN; CLERICI, 2004; EDWARDS; FANTASIA; IOVINO, 2006; DA COSTA MIGUEL et al., 2009; JHAM et al., 2009). Os efeitos adversos refletem um novo problema na prática do cirurgião dentista, impulsionando a realização de pesquisas que almejam a uma melhor compreensão sobre o tema bem como ao manejo adequado dos pacientes acometidos por essas complicações (LOUREIRO BORGHETTI et al., 2011; VARGAS, 2011).

A opção por se trabalhar com ratos ocorreu pela carência de pesquisas experimentais sobre o tema, já que a maioria das informações disponíveis na literatura é baseada em relatos de casos (EDWARDS; FANTASIA; IOVINO, 2006; DA COSTA MIGUEL et al., 2009). Estudos em animais costumam ser mais fidedignos pela possibilidade de padronizar variáveis importantes como o operador e as técnicas de aplicação dos materiais e de análise (GOMES et al., 2007).

O AH e o PMMA foram os materiais de preenchimento escolhidos por serem os mais utilizados por dermatologistas e cirurgiões plásticos, sendo um deles reabsorvível e o outro não (ZIMMERMANN; CLERICI, 2004). Elegeram-se as concentrações intermediárias desses produtos porque são as mais frequentemente indicadas para preenchimento de rugas suaves a moderadas dispostas na região peribucal (SÁNCHEZ et al., 2011; VARGAS et al., 2011). O ventre posterior de língua foi a região anatômica escolhida para injeção dos materiais pela menor vulnerabilidade ao trauma ou à irritação crônica, visando a eliminar quaisquer fatores

que pudessem interferir nos resultados (LOUREIRO BORGHETTI et al., 2011; VARGAS, 2011). Além disso, a aplicação do material em estruturas anatômicas da pele, onde estão presentes folículos pilosos, glândulas sebáceas e sudoríparas, poderia dificultar e confundir a análise histológica (FIGUEIREDO et al., 2001), principalmente quando da utilização do PMMA. Os tempos entre as injeções dos materiais e a eutanásia dos ratos foram pré-estabelecidos, para que se tivesse uma resposta inicial (7 dias), usualmente relatada na literatura como complicações agudas, e tardia (60 e 90 dias), momento em que o processo inflamatório inicia a degradação do material reabsorvível e que lesões crônicas começam a ser relatadas (GHISLANZONI et al., 2006; ZIELKE et al., 2008; LOUREIRO BORGHETTI et al., 2011).

A utilização de escores na avaliação histológica local corada pela técnica de rotina (HE) permitiu identificar e valorizar os tipos de células envolvidos nas respostas inflamatórias iniciais e tardias, seguindo critérios utilizados previamente em outros estudos (FIGUEIREDO et al., 2001; GOMES et al., 2007; LOUREIRO BORGHETTI et al., 2011). Na técnica de imunoistoquímica, o marcador CD34 foi usado para a identificação de vasos sanguíneos neoformados (IRION et al., 2008) na intenção de analisar de que forma a angiogênese do processo inflamatório se estruturou ao longo dos tempos experimentais. A escolha pelo marcador CD68 baseou-se em relatos da literatura que descrevem a marcação positiva desses anticorpos em macrófagos, células com importante função nas reações a corpo estranho por materiais de preenchimento (BONNEMA et al., 2003; DA COSTA MIGUEL et al., 2009; JHAM et al., 2009; SÁNCHEZ et al., 2011). Complementando a análise, a fase produtivo-reparativa da resposta inflamatória foi feita por meio do método histoquímico de coloração com picrosírius sob luz polarizada, uma vez que esta técnica é específica para detecção de fibras colágenas (ABRAHÃO et al., 2006).

No que se refere às alterações clínicas, a análise inicial mostrou um predomínio de lesões ulceradas na língua dos animais tratados com PMMA, como descrito em estudos anteriores (ZIMMERMANN e CLERICI, 2004; CHRISTENSEN et al., 2005; DADZIE et al., 2008). A exposição do tecido conjuntivo poderia deixar o ambiente mais suscetível aos agentes externos, exacerbando a reação inflamatória local nos animais desse grupo experimental. No entanto, ambos os materiais de

preenchimento utilizados promoveram uma resposta moderada aos 60 dias de avaliação, o que sugere que as reações inflamatórias foram desencadeadas pela presença do produto implantado. As alterações tardias observadas em alguns animais dos grupos teste condizem com vários relatos da literatura e apresentaram-se na forma de placas brancas e pápulas. Possivelmente se justificam pela hiperplasia de fibras colágenas e pela manutenção do aumento de volume local vinculado à presença do produto injetado (ZIMMERMANN; CLERICI, 2004; CHRISTENSEN et al., 2005; EDWARDS; FANTASIA; IOVINO, 2006; DADZIE et al., 2008; DA COSTA MIGUEL et al., 2009; LOUREIRO BORGHETTI et al., 2011).

Os resultados do presente estudo ilustraram as distintas fases do processo inflamatório secundário à injeção de cada um dos materiais de preenchimento. Na avaliação histológica da língua dos animais, os neutrófilos exsudados atingiram o local nos momentos iniciais, representando a primeira linha de defesa. Mais tarde, corroborando os achados descritos na literatura, linfócitos, plasmócitos, assim como macrófagos marcados com CD68, fizeram a defesa efetiva contra a substância exógena (CHRISTENSEN et al., 2005; ANDERSON et al., 2008; CAKMAK et al., 2011). Quando o material estudado era o PMMA, confirmando relatos de vários autores, um dado relevante encontrado foi a expressiva presença de células gigantes (CAKMAK et al., 2011; VARGAS, 2011); ao contrário dos casos que receberam injeção de AH em que essas células multinucleadas estiveram ausentes ou se apresentaram de forma esporádica (LEMPERLE; MORHENN; CHARRIER, 2003). Essa fase exsudativa ocorreu por conta da geração de uma rede de vasos sanguíneos presente do início ao fim do processo inflamatório, evidenciada pela marcação imunoistoquímica com CD34. Ao longo do experimento, a fase produtivo-reparativa tendeu à cicatrização, na medida em que o AH era degradado. Diversos autores afirmam haver mínima resposta fibrosa nos tecidos decorrente da implantação desse material (ZIMMERMANN; CLERICI, 2004; CHRISTENSEN et al., 2007), embora em 2011 Loureiro Borghetti et al. tenha sugerido a presença de fibroplasia circundando o produto. No que se refere ao PMMA, observou-se uma característica peculiar com redução significativa da densidade de fibras colágenas aos 90 dias de experimento. Esse resultado pode ser justificado pelo frouxo arranjo das fibras colágenas causado pela permanência de células inflamatórias que, em processos crônicos como esse, liberam mediadores químicos capazes de regular a

produção e a degradação da matriz extracelular (MEC), estimulando a atividade das colagenases (MOURE et al., 2011). Ademais, é descrita a formação de uma cápsula fibrosa caracteristicamente delgada ao redor das microesferas do PMMA (LEMPERLE et al., 2004; ZIMMERMANN; CLERICI, 2004; LUTTIKHUIZEN; HARMSEN; VAN LUYN, 2006; CARRUTHERS et al., 2009), o que levanta a hipótese de que características físico-químicas peculiares do produto possam ter determinado tal comportamento.

Interpretando os dados das análises histológicas locais, foi exequível reforçar a ideia de que a inflamação é um processo concatenado de múltiplos eventos, no qual uma resposta local, mesenquimal e complexa ocorre com o propósito de debelar o efeito do material implantado. Os resultados obtidos no presente estudo, a exemplo de outros autores, sugerem que o AH apresente características de biocompatibilidade (DOVER et al., 2005; GHISLANZONI et al., 2006; LOUREIRO BORGHETTI et al., 2011) e que o PMMA seja capaz de suscitar reação a corpo estranho, possivelmente por suas características físico-químicas (LEMPERLE; MORHENN; CHARRIER, 2003; LEMPERLE et al., 2004; CARRUTHERS et al., 2009).

A avaliação histológica do fígado e rim dos animais buscou observar a presença de migração sistêmica mencionada por diversos autores (LEMPERLE et al., 2004; ROSA; DE MACEDO, 2005; LOUREIRO BORGHETTI et al., 2011), porém relatada superficialmente na literatura. Um achado curioso foi o intenso processo inflamatório nos órgãos de metabolismo e excreção referido em experimento desenvolvido por Rosa em 2001. Contrapondo-se a esse autor e indo ao encontro da maioria dos relatos prévios (LEMPERLE et al., 2004; LOUREIRO BORGHETTI et al., 2011), não foram visualizadas quaisquer alterações nos tecidos hepático e renal avaliados na presente pesquisa. Ainda assim, acredita-se ser necessário o uso de metodologias que simulem a injeção inadvertida de materiais de preenchimento em vasos venosos (LEMPERLE et al., 2004) com o objetivo de investigar a capacidade de migração dos mesmos para outros órgãos, em especial o pulmão, devido ao risco de complicações tromboembólicas.

O PMMA mostrou, nesta pesquisa, ser capaz de suscitar reação a corpo estranho, confirmando achados de estudos anteriores (LOMBARDI et al., 2004;

ZIMMERMANN; CLERICI, 2004; EDWARDS; FANTASIA; IOVINO, 2006; DA COSTA MIGUEL et al., 2009; JHAM et al., 2009). Essa característica do produto gera maior preocupação em virtude do seu custo ser baixo e seu uso não ter qualquer restrição e controle de venda. Isso permite que profissionais despreparados e tecnicamente inaptos sejam tentados a utilizar este material de forma indiscriminada (VARGAS; AMORIM; PITANGUY, 2009, MERCER et al., 2010). Entretanto, alguns autores relatam que eventualmente todos os materiais de preenchimento, até mesmo o AH, são potencialmente capazes de desencadear reações a corpo estranho (LEMPERLE; MORHENN; CHARRIER, 2003; ZIMMERMANN; CLERICI, 2004; CHRISTENSEN, 2007). Afirmações como essa reforçam a necessidade de que se considere sempre a capacidade de resposta de cada paciente.

Estudos futuros, incluindo pesquisas na área de biomateriais, deverão contribuir para um melhor entendimento do processo inflamatório causado pelo uso de materiais de preenchimento. Analisar as características fisico-químicas dos produtos poderá auxiliar na compreensão dos motivos que levam determinado material a ser capaz de induzir alterações na resposta inflamatória a longo prazo, como a reação a corpo estranho, ou mesmo de migrar sistemicamente. A clara definição etiopatogênica acerca das complicações, por uso de materiais de preenchimentos, poderá contribuir para o aprimoramento da manufatura bem como auxiliar na escolha do produto a ser utilizado na área médica. Outrossim, norteará o manejo dos pacientes acometidos pelas enfermidades, inclusive no campo de atuação do cirurgião dentista, já que esses materiais tem sido largamente utilizados na região peribucal.

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ANEXOS

ANEXO A – Confirmação de submissão (Artigo 1)

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Para: Maria Antonia Z de Figueiredo
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(ii)Experimental subjects: Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version, 2002 www.wma.net/e/policy/b3.htm) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

(iii) Suppliers: Suppliers of materials should be named and their location (town, state/county, country) included.

Results: Present your results in logical sequence in the text, tables, and illustrations. Do not repeat in the text all the data in the tables, illustrations, or both: emphasize or summarize only important observations.

Discussion: Emphasize the new and important aspects of the study and conclusions that follow from them. Do not repeat in detail data given in the Results section. Include in the Discussion the implications of the findings and their limitations and relate the observations to other relevant studies.

Main Text of Review Articles comprise an introduction and a running text structured in a suitable way according to the subject treated. A final section with conclusions may be added.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited. 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. See also above under Ethical Guidelines.

Conflict of Interest Statement: All sources of institutional, private and corporate financial support for the work within the manuscript must be fully acknowledged, and any potential grant holders should be listed. Please see Conflicts of Interest for generally accepted definitions on conflict of interest? See also above under Ethical Guidelines.

5.4. References
References should be kept to the pertinent minimum and numbered consecutively in the order in which they appear in the text. Identify references in text, tables, and 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 identification of that figure or table in the text. Use the style of the examples below, which are based on the formats used in Index Medicus. Try to avoid using abstracts as references. Include manuscripts accepted, but not published; designate the abbreviated title of the journal followed by (in press). Information from manuscripts not yet accepted, should be cited in the text as personal communication. The references must be verified by the author(s) against the original documents. Titles should be abbreviated in accordance with the style used in Index Medicus and the Vancouver System.

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting. EndNote reference styles can be searched for here: www.endnote.com/support/enstyles.asp . Reference Manager reference styles can be searched for here: www.refman.com/support/rmstyles.asp

Examples of the Journal's reference style:

(1) Standard journal article
(List all authors when 6 or less; when 7 or more, list only the first 3 and add et al.)
BUCHNER A, SCIUBBA JJ. Peripheral epithelial odontogenic tumors: a review. Oral Surg Oral Med Oral Pathol 1987; 63: 688-97.
HEINIC GS, GREENSPAN D, MACPHAIL LA, et al. Oral Histoplasma capsulatum infection in association with HIV infection: a case report. J Oral Pathol Med 1992; 21: 85-9.

(2) Corporate author
European Collaborative Study. Risk factors for mother-to-child transmission of HIV-1. Lancet 1992; 339: 1007-12.

(3) No author given
Anonymous. `The importance of being early' [leader]. Br Dent J 1991; 170: 167.

(4) Journal supplement
MØLLER-PETERSEN J. Evaluation of diagnostic tests. Design and phases. Scand J Clin Lab Invest 1992; 52: suppl. (208): 35-50.
CROSS SS, SCHOLFIELD JH, KENNEDY A, COTTON DWK. Measuring the fractal dimension of tumour borders. J Pathol 1992; 168: 117A (abstr).

(5) Journal paginated by issue
HILLAM C. Dentistry in Europe in the 1790's. Dent Historian 1992; 22: (May): 31-4.

(6) Book
PINDBORG JJ. Atlas of diseases of the oral mucosa. Copenhagen: Munksgaard, 1992: 50-66.

(7) Chapter in a book
VAN DER WAAL I. Salivary gland neoplasms. In: PRABHU SR, WILSON DF, DAFTARY DK, JOHNSON NW, eds. Oral diseases in the tropics. Oxford: Oxford Medical, 1992; 478-86.

(8) Published proceedings paper
DRINNAN AJ. Review of the literature: educational aspects of oral medicine. In: MILLARD HD, MASON DK, eds. World workshop on oral medicine. Chicago: Year Book Medical, 1989; 5-11.

(9) Agency publication
MUIR C, WATERHOUSE J, MACK T, POWELL J, WHELAN S. Cancer incidence in five continents: Vol. 5. Lyon: International Agency for Research on Cancer, 1987; IARC Scientific Publications No. 88.

(10) Dissertation or thesis
CHUNGPANICH S. The diagnostic and prognostic potential of nucleolar organizer regions in oral epithelial dysplasia. MMedSci Thesis, University of Sheffield, 1989.

5.5. Tables, Figures and Figure Legends

Tables: should be numbered consecutively with Arabic numerals. Type each table on a separate sheet, with titles making them self-explanatory. Due regard should be given to the proportions of the printed page.

Figures: All figures should clarify the text and their number be kept to a minimum. Text on figures should be in CAPITALS. Line drawings should be professionally drawn; half-tones should exhibit high contrast.

All figures and artwork must be provided in electronic format. Figure legends should be a separate section of the manuscript, and should begin with a brief title for the whole figure and continue with a short description of each panel and the symbols used: they should not contain any details of methods.

Submit your figures as EPS, TIFF or PDF files. Use 300 dpi resolution for photographic images and 600 dpi resolution for line art.Full details of the submission of artwork are available at http://authorservices.wiley.com/bauthor/illustration.asp.

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A completed Copyright Transfer Agreement (CTA), found at www.wiley.com/go/ctaaglobal must be received by Production Editor before any manuscript can be published. Authors must send the completed original CTA by regular mail upon receiving notice of manuscript acceptance, i.e. do not send the CTA at submission.

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Proofs will be sent via e-mail as an Acrobat PDF (portable document format) file. The e-mail server must be able to accept attachments up to 4 MB in size. Acrobat Reader will be required in order to read this file.

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ANEXO C – Confirmação de submissão (Artigo 2)

From: **International Journal of Oral & Maxillofacial Surgery** <IJOMS@elsevier.com>
Date: 2011/11/15
Subject: Submission Confirmation for CLINICAL AND PATHOLOGICAL CHARACTERISTICS OF POLYMETHYLMETHACRYLATE AND HIALURONIC ACID IN THE RAT TONGUE
To: sabrinamoure@gmail.com

Dear Mrs. Moure,

We acknowledge, with thanks, the receipt of your manuscript submitted to International Journal of Oral & Maxillofacial Surgery.

You may check on the progress of your paper by logging on to the Elsevier Editorial System as an author. The URL is http://ees.elsevier.com/ijoms/.
Your username is Your username is: sabrinamoure.

If you need to retrieve password details,
please go to: http://ees.elsevier.com/ijoms/automail_query.asp

Your manuscript will be given a reference number once an Editor has been assigned. Your paper will then be forwarded to the expert reviewers of the Editorial Board for review. Once the results of the reviewing process are available we will advise you.

Thank you for showing your interest in publishing in the International Journal of Oral and Maxillofacial Surgery.

Kind regards,

Jacqui Merrison
IJOMS Editorial Office

ANEXO D – Normas da revista escolhida para submissão (Artigo 2)

International Journal of Oral & Maxillofacial Surgery

Guide for Authors
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.

Online Submission
Submission and peer-review of all papers is now conducted entirely online, increasing efficiency for editors, authors, and reviewers, and enhancing publication speed. Authors requiring further information on online submission are strongly encouraged to view the system, including a tutorial, at http://ees.elsevier.com/ijoms A comprehensive Author Support service is available to answer additional enquiries at authorsupport@elsevier.com. Once a paper has been submitted, all subsequent correspondence between the Editorial Office (ijoms@elsevier.com) and the corresponding author will be by e-mail.

Editorial Policy
A paper is accepted for publication on the understanding that it has not been submitted simultaneously to another journal, has been read and approved by all authors, and that the work has not been published before. The Editors reserve the right to make editorial and literary corrections. Any opinions expressed or policies advocated do not necessarily reflect the opinions and policies of the Editors.

Declarations
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:
Funding: None
Competing interests: None declared
Ethical approval: Not required

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.

The corresponding author is responsible for ensuring that all authors are aware of their obligations.

Before a paper is accepted all the authors of the paper must sign the Confirmation of Authorship form. This form confirms that all the named authors agree to publication if the paper is accepted and that each has had significant input into the paper. Please download the form and send it to the Editorial Office. (pdf version or word version) It is advisable that to prevent delay this form is submitted early in the editorial process.

Acknowledgements
All contributors who do not meet the criteria for authorship as defined above should be listed in an acknowledgements section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support. Authors should disclose whether they had any writing assistance and identify the entity that paid for this assistance.

Conflict of interest
At the end of the main text, all authors must disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. If an author has no conflict of interest to declare, this should be stated.

Role of the funding source
All sources of funding should be declared as an acknowledgement at the end of the text. Authors should declare the role of study sponsors, if any, in the study design, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. If the study sponsors had no such involvement, the authors should so state.

Ethics
Work on human beings that is submitted to the International Journal of Oral and Maxillofacial Surgery should comply with the principles laid down in the Declaration of Helsinki (Recommendations guiding physicians in biomedical research involving human subjects. Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975, the 35th World Medical Assembly, Venice, Italy, October 1983, and the 41st World Medical Assembly, Hong Kong, September 1989). The manuscript should contain a statement that the work has been approved by the appropriate ethical committees related to the institution(s) in which it was performed and that subjects gave informed consent to the work. Studies involving experiments with animals must state that their care was in accordance with institution guidelines. Patients' and volunteers' names, initials, and hospital numbers should not be used.

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• research papers: no more than 6000 words and 40 references
• review papers - no limit on length or number of references
• technical notes (surgical techniques, new instruments, technical innovations) - no more than 2000 words, 10 references and 4 figures
• case reports - no more than 2000 words, 10 references and 4 figures book reviews
• letters to the editor - please see detailed guidelines provided at the end of the main guide for authors
• IAOMS announcements
• general announcements.

All authors must have contributed to the paper, not necessarily the patient treatment. Technical notes and case reports are limited to a maximum of 4 authors, in exceptional circumstances, 5.

Criteria for Publication
Papers that will be considered for publication should be: • focused
• based on a sound hypothesis and an adequate investigation method analysing a statistically relevant series, leading to relevant results that back the conclusion
• well written in simple, scientific English grammar and style
• presented with a clear message and containing new information that is relevant for the readership of the journal
• Note the comment above relating to case reports.

Following peer-review, authors are required to resubmit their revised paper within **3 months;** in exceptional circumstances, this timeline may be extended at the editor's discretion.

Presentation of Manuscripts
General points
Papers should be submitted in journal style. Failure to do so will result in the paper being immediately returned to the author and may lead to significant delays in publication. Spelling may follow British or American usage, but not a mixture of the two. Papers should be double-spaced with a margin of at least 3 cm all round.

Format
Papers should be set out as follows, with each section beginning on a separate page: • title page
• abstract
• text
• acknowledgements
• references
• tables
• captions to illustrations.
Please note that the qualifications of the authors will not be included in the published paper and should not be listed anywhere on the manuscript.

Title page
The title page should give the following information: • title of the article
• full name of each author
• name and address of the department or institution to which the work should be attributed
• name, address, telephone and fax numbers, and e-mail address of the author
responsible for correspondence and to whom requests for offprints should be sent
• sources of support in the form of grants
• key words.
If the title is longer than 40 characters (including spaces), a short title should be supplied for use in the running heads.

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

Text
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

Materials and Methods
• 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
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Discussion
• Discuss - do not recapitulate results• Point out exceptions and lack of correlations. Do not try to cover up or 'fudge' data• Show how results agree/contrast with previous work• Discuss the implications of your findings• State your conclusions very clearly

Headings: Headings enhance readability but should be appropriate to the nature of the paper. They should be kept to a minimum and may be removed by the Editors. Normally only two categories of headings should be used: major ones should be typed in capital letters; minor ones should be typed in lower case (with an initial capital letter) at the left hand margin.

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Abbreviations, symbols, and nomenclature: Only standardized terms, which have been generally accepted, should be used. Unfamiliar abbreviations must be defined when first used. For further details concerning abbreviations, see Baron DN, ed. Units, symbols, and abbreviations. A guide for biological and medical editors and authors, London, Royal Society of Medicine, 1988 (available from The Royal Society of Medicine Services, 1 Wimpole Street, London W1M 8AE, UK).
The minus sign should be -.
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Titles of journals should be abbreviated according to Index Medicus (see www.nlm.nih.gov.uk) . When citing papers from monographs and books, give the author, title of chapter, editor of book, title of book, publisher, place and year of publication, first and last page numbers. Internet pages and online resources may be included within the text and should state as a minimum the author(s), title and full URL. The date of access should be supplied and all URLs should be checked again at proof stage.

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All illustrations (e.g. graphs, drawings or photographs) are considered to be figures, and should be numbered in sequence with Arabic numerals. Each figure should have a caption, typed double-spaced on a separate page and numbered correspondingly. **The minimum resolution for electronically generated figures is 300 dpi.**

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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 and 6 references. 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 E – Aprovação da Comissão Científica e de Ética da Faculdade de Odontologia da PUCRS

Comissão Científica e de Ética
Faculdade da Odontologia da PUCRS

Porto Alegre 11 de março de 2010

O Projeto de: Tese

Protocolado sob nº:	0005/10
Intitulado:	Resposta tecidual em ratos submetidos à injeção submucosa de dois materiais de preenchimento com finalidade estética: análises clínica e histológica
Pesquisador Responsável:	Profa. Maria Antonia Zancanaro de Figueiredo
Pesquisadores Associados	Sabrina Pozatti Moure; Karlon Fróes de Vargas; Ruchielli Loureiro Borghetti
Nível:	Doutorado

Foi ***aprovado*** pela Comissão Científica e de Ética da Faculdade de Odontologia da PUCRS em *11 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 F – Aprovação da Comissão de Ética Para o Uso de Animais

PUCRS CEUA PUC Comitê de Ética para o Uso de Animais
Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMITÊ DE ÉTICA PARA O USO DE ANIMAIS

Ofício 139/10 – CEUA

Porto Alegre, 19 de agosto de 2010.

Senhora Pesquisadora:

O Comitê de Ética para o Uso de Animais apreciou e aprovou a emenda de realização de imunohistoquímica, intitulado: **"Resposta tecidual em ratos submetidos à injeção submucosa de dois materiais de preenchimento com finalidade estética: análises clínica e histológica"** ao protocolo de pesquisa, registro CEUA 10/00151, intitulado: **"Estudo experimental em ratos submetidos à injeção submucosa de ácido hialurônico em distintas concentrações: avaliação clínica e histológica"**, aprovado em 18 de março de 2010.

Sua investigação está autorizada a partir da presente data.

Atenciosamente,

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

Ilma. Sra.
Profa. Dra. Maria Antonia de Figueiredo
Faculdade de Odontologia
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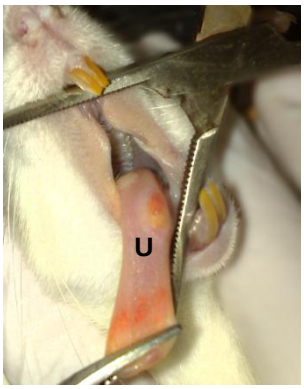
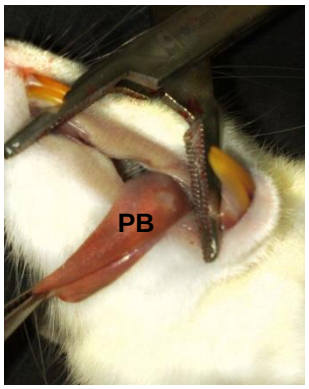
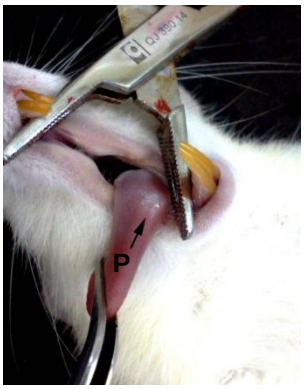
Apêndices

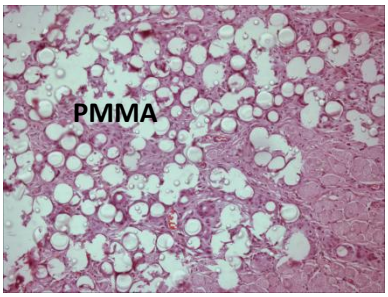
APÊNDICES

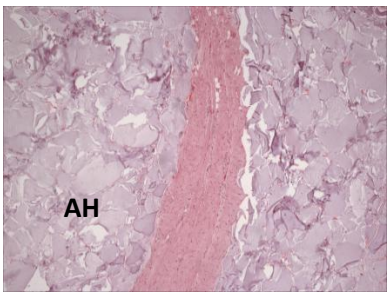




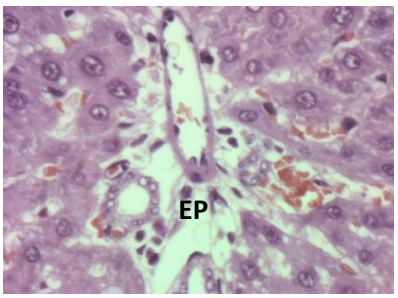


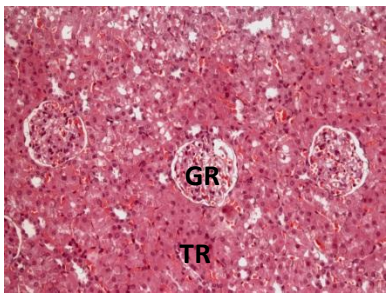
Alterações clínicas presentes nas regiões próximas às implantações dos materiais. **U**: lesão ulcerada; **PB**: placa branca; **P**: pápula.





Aspecto histológico dos materiais de preenchimento: polimetilmetacrilato (HE; 200x) e ácido hialurônico (HE; 100x). **PMMA**: polimetilmetacrilato; **AH**: ácido hialurônico.





Aspecto histológico sem alterações do fígado e do rim, respectivamente, 60 dias após a injeção de PMMA. Fígado: espaço porta (**EP**) (HE; 400x). Rim: glomérulos (**GR**) e túbulos renais (**TR**) (HE; 200x).

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PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO EM ESTOMATOLOGIA CLÍNICA

FICHA DE AVALIAÇÃO CLÍNICA

IDENTIFICAÇÃO

Rato nº: ____ **Peso inicial:** _____Kg
Peso final: _____Kg

Lâmina nº: _____

Material:
□ PMMA 10%
□ AH 20mg/mL
□ Controle

Tempo:
□ 7 dias
□ 60 dias
□ 90 dias

AVALIAÇÃO CLÍNICA LOCAL

□ Úlcera
□ Placa branca
□ Pápula
□ Outras formas: _____

Sinais secundários:
□ sim □ não
Especifique (sangramento, supuração, abscesso):
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Fotos: _____

Data da avaliação: __/__/____.

Ficha de avaliação clínica utilizada durante o experimento.

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FICHA DE AVALIAÇÃO HISTOLÓGICA

IDENTIFICAÇÃO

Rato nº: ____ **Peso inicial:** _____Kg
Peso final: _____Kg

Lâmina nº: _____

Material:
□ PMMA 10%
□ AH 20mg/mL
□ Controle

Tempo:
□ 7 dias
□ 60 dias
□ 90 dias

AVALIAÇÃO HISTOLÓGICA LOCAL

HE

Intensidade da resposta inflamatória

- Escores:

□ 0 - Ausente □ 1 - Leve □ 2 - Moderada □ 3 - Intensa

IMUNOISTOQUÍMICA

Vasos sanguíneos neoformados (CD34)

- Nº vasos neoformados/campo (166µm^2):

Campo 1: ____ Campo 6: ____
Campo 2: ____ Campo 7: ____
Campo 3: ____ Campo 8: ____
Campo 4: ____ Campo 9: ____
Campo 5: ____ Campo 10: ___

Macrófagos (CD68)

- Nº macrófagos/campo (166µm^2):

Campo 1: ____ Campo 6: ____
Campo 2: ____ Campo 7: ____
Campo 3: ____ Campo 8: ____
Campo 4: ____ Campo 9: ____
Campo 5: ____ Campo 10: ___

PICROSÍRIUS

Densidade de fibras colágenas

- % fibras colágenas/campo (673µm^2)

Campo 1: ____ Campo 2: ____ Campo 3: ____ Campo 4: _____ Campo 5: ____

MIGRAÇÃO

HE

Fígado
□ presença de resposta inflamatória
□ presença de resquícios do material

Rim
□ presença de resposta inflamatória
□ presença de resquícios do material

Fotos: _____

Data da avaliação: __/__/____.

Ficha de avaliação histológica utilizada durante o experimento.