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AVALIAÇÃO CLÍNICA E HISTOLÓGICA DE RATOS SUBMETIDOS À INJEÇÃO EM VENTRE LINGUAL DE ÁCIDO HIALURÔNICO EM DISTINTAS CONCENTRAÇÕES

PORTO ALEGRE

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Orientadora: Profa. Maria Antonia Zancanaro de Figueiredo

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“A mente que se abre a uma nova idéia jamais voltará ao seu tamanho original”.

Albert Einstein

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RESUMO

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O ácido hialurônico (AH) é o material de preenchimento estético reabsorvível mais empregado na atualidade. Seu uso indiscriminado tem frequentemente evidenciado reações indesejáveis nos usuários destes produtos. A literatura científica disponível não esclarece, de forma conclusiva, a etiologia dos efeitos adversos que podem ocorrer a partir da sua injeção. A presente pesquisa teve por objetivo avaliar, por meio da análise clínica e histológica, a resposta tissular local e sistêmica de duas concentrações de AH em língua de ratos. Cinquenta e quatro ratos fêmeos (*Rattus norvegicus*, Wistar) foram distribuídos em 3 grupos de tratamento (AH 5,5mg/mL, AH 25mg/mL e NaCl 0,9 % - controle) e 3 tempos experimentais (7 dias, 60 dias e 90 dias). Antes da eutanásia, procedeu-se a avaliação clínica, através de parâmetros de presença e ausência de sinais. Posteriormente, removeu-se língua e rim direito para análise microscópica. Nos cortes de língua corados por hematoxilina e eosina (HE), mensurou-se parâmetros de ausência ou presença de reação inflamatória, sendo esta classificada como leve, moderada ou severa. Nos cortes corados com picrosírius, estabeleceu-se o percentual de fibras colágenas presentes. Nas amostras obtidas do rim (HE), foram observadas a presença ou ausência de resposta inflamatória em toda a extensão da lâmina. As alterações clínicas presentes não foram estatisticamente significativas entre os grupos (Kruskal-Wallis; $p>0,05$). Histologicamente, os grupos AH apresentaram maior grau de inflamação que os controles em todos os tempos do experimento (Kruskal-Wallis, $p< 0,01$). Quanto à fibroplasia, o grupo AH 25mg/mL tendeu a formar mais colágeno se comparado ao grupo controle (ANOVA Mixed Models; $p=0.016$). Em 100% das amostras de rim analisadas, não se observou resposta inflamatória. A partir da metodologia utilizada neste estudo, os resultados obtidos permitem concluir que (1) O AH nas concentrações de 5,5 e 25mg/mL provocou alterações clínicas variáveis e ocasionais em todos os períodos; (2) O AH nas

concentrações utilizadas, em até 7 dias, promoveu um grau moderado de inflamação tecidual; (3) A reação inflamatória circunjacente às partículas do AH diminuiu de intensidade entre 60 e 90 dias, reduzindo a possibilidade de reações adversas pelo uso do material; (4) O AH 25mg/mL suscitou maior formação de colágeno se comparado ao grupo controle; (5) Não houve resposta inflamatória e/ou migração sistêmica renal a partir da injeção do AH a 5,5mg/mL e 25mg/mL em até 90 dias de observação.

Palavras-chave: ácido hialurônico; biocompatibilidade, histologia, efeitos adversos

SUMMARY

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Hyaluronic Acid (HA) is currently the most applied resorbable filling material. Its indiscriminate use has showed the adverse reactions. Scientific literature does not conclusively clarify the aetiology of the adverse effects, which can occur after material injection. The current research has aimed at evaluating, through clinical and histological analysis, local and systemic tissue response to the injection of 2 different hyaluronic acid concentrations in rat's tongue. Fifty-four female Wistar rats (*Rattus norvegicus*) were randomically allocated into 3 groups according to the treatment (5.5mg/mL HA, 25mg/mL HA and 0.9 % NaCl - control) and 3 experimental times (7 days, 60 days and 90 days). Immediately before euthanasia, clinical analysis was performed guided by the parameters of presence and absence of inflammatory signs or any other alteration of the normal pattern. After euthanasia, the tongue and the right kidney of each animal were removed for microscopic analysis. Presence or absence of inflammatory reaction was measured in the tongue sections stained by hematoxylin and eosin (HE) and reactions were classified as mild, moderate or severe. The proportion of collagen fibers present was established in the sections stained with picrosirius. Presence or absence of inflammatory response in the total extension of each slide was observed in the kidney samples (HE). The clinical changes present were not statistically significant among groups (Kruskal-Wallis; $p>0.05$). The groups that received HA injection presented a higher degree of inflammation if compared to the control groups in all monitoring periods (Kruskal-Wallis, $p<0.01$). Regarding fibroplasias, the 25mg/mL HA group tended to form more collagen if compared to the control group (ANOVA Mixed Models; $p=0.016$). In 100% of the analyzed kidney samples, there was no inflammatory reaction. According to the methodology used in this study, the obtained results allows the conclusion

that (1) HA at 5.5 and 25mg/mL presented variable and occasional clinic changes in all evaluation periods; (2) HA in the chosen concentrations, within 7 days, promoted a moderate degree of tissue inflammation; (3) After 60 and 90 days, inflammatory reaction surrounding HA particles became milder, reducing risks of adverse reactions to the material; (4) HA at 25mg/mL provoked larger concentrations of collagen if compared to the control group; (5) There was no inflammatory response and/or renal systemic migration due to the injection of HA at 5.5mg/mL and 25mg/mL in up to 90 days of monitoring.

Key words: hyaluronic acid; biocompatibility; histology; adverse effects

SUMÁRIO

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1 INTRODUÇÃO

O processo de envelhecimento causa invariavelmente alterações estruturais e funcionais nos tecidos. Este fenômeno provoca a perda da gordura subcutânea, tornando a derme mais fina e menos elástica. Estas modificações produzem sulcos profundos na pele, denominados rugas, as quais comprometem a estética e promovem a busca pela manutenção da juventude (PERENACK, 2005; MATARASSO, CARRUTHERS, JEWELL, 2006; ATIYEH, DIBO, 2009; VARGAS, AMORIM, PITANGUY, 2009).

A cirurgia plástica dominou por muito tempo o campo do rejuvenescimento facial. Entretanto, devido à grande procura dos pacientes por procedimentos menos invasivos, de recuperação rápida e com retorno de uma aparência satisfatória em curto prazo, surgiram as técnicas de preenchimento facial, com o objetivo de suavizar rugas ou vincos na região perioral e periocular, aumentar artificialmente lábios e região malar, bem como corrigir defeitos faciais (PARADA et al., 2005; FISCHER, METZLER, SCHALLER, 2007; SMITH, 2008).

A substância de implante considerada ideal deve apresentar determinadas características que vão além da sua biocompatibilidade. O material não deve ser carcinogênico nem causar reação inflamatória ou alérgica, não deve ser modificado pelos líquidos e tecidos orgânicos, deve resistir a tensões mecânicas, ser de fácil aplicação e remoção e quimicamente inerte. Entretanto, até o momento, nenhum produto disponível no mercado apresenta todos esses requisitos sugeridos (ACHILLES, 2004; DASTOOR, MISCH, WANG, 2007).

Os materiais de preenchimento com finalidade estética são classificados em temporários e permanentes, variando de acordo com o seu tempo de permanência nos tecidos.

Destacam-se na literatura como as substâncias mais utilizadas pela bioplastia (plástica sem cortes), o ácido hialurônico, o ácido poli-L-láctico, o colágeno bovino e o polimetilmetacrilato (ACHILLES, 2004; DASTOOR, MISCH, WANG, 2007; VARGAS et al., 2011).

O ácido hialurônico (AH) é um dos mais recentes materiais temporários desenvolvidos para o preenchimento de partes moles. Antes da sua disponibilização, o colágeno bovino era a substância costumeiramente utilizada nesses procedimentos. As indicações frequentes de uso do AH são para as regiões glabelar, periorbital, perioral e malar. As contra-indicações estão relacionadas aos portadores de doença autoimune, presença de material de preenchimento permanente no local da aplicação e história de alergia prévia ao ácido hialurônico (VEDAMURTHY, 2004; ROMAGNOLI, BELMONTESI, 2008).

O AH é um polissacarídeo de alto peso molecular, sendo um importante componente da matriz extracelular. É considerado um implante biossintético temporário (ROMAGNOLI, BELMONTESI, 2008; HEDÉN et al., 2009) e sua sobrevida na estrutura da derme varia de 3 a 12 meses (GOLD, 2007; ALLEMANN, BAUMANN, 2008). As propriedades hidrofílicas do AH atraem água para a matriz extracelular aumentando desta forma a elasticidade da pele (MATARASSO, CARRUTHERS, JEWELL, 2006). Para conseguir aumentar a durabilidade das várias preparações do AH, a estabilização usualmente é obtida por meio de ligações intermoleculares com outras substâncias, como por exemplo, o 1,4-butanediol-diglicidil-éter (DE MAIO, RZANY, 2007; MOERS-CARPI et al., 2007; ALLEMANN, BAUMANN, 2008).

O produto a base de AH pode ter origem aviária ou não-animal, sendo que cada tipo apresenta suas características específicas. As fontes mais comuns são a crista de galo (aviária) e a cultura de bactérias (não-animal) (GOLD, 2007). Há várias preparações que são indicadas

de acordo com os distintos níveis de profundidade da injeção do material. As diferenças se baseiam na concentração do AH, no grau de ligações intermoleculares e, consequentemente, na taxa de degradação do material (GOLD, 2007; KOGAN et al., 2007; ALLEMANN, BAUMANN, 2008; CARRUTHERS et al., 2010).

A escolha da concentração do AH em preenchimentos faciais leva em consideração a profundidade das rugas e linhas de expressão bem como a quantidade de volume desejado. As anestesias tópica, infiltrativa ou por bloqueio são meios facultativos para controle da dor durante a injeção do material. (MATARASSO, CARRUTHERS, JEWELL, 2006; ROMAGNOLI, BELMONTESI, 2008).

A diversidade de produtos disponibilizados, seu uso indiscriminado e o crescimento dessa modalidade plástica entre os diferentes profissionais da área da saúde aumentaram de forma significativa a procura pelos serviços corretivos estéticos, evidenciando as reações adversas, que podem prejudicar a estética facial e, até mesmo, comprometer a saúde geral do paciente (LEMPERLE, ROMANO, BUSSO, 2003; DE CASTRO et al., 2007; SOUSA et al., 2008; VARGAS et al., 2011).

Os efeitos observados e descritos na literatura a partir da injeção de AH costumam ser variados. Estas complicações vão desde uma simples reação inflamatória até a necrose tecidual ou comprometimento hepático/renal (ROSA, 2001; ROSA, DE MACEDO, 2005; DE CASTRO et al., 2007). No mesmo local onde foi injetado o material exógeno pode ocorrer reação inflamatória sob a forma de um granuloma (SANCHIS-BIELSA et al., 2009; VARGAS, AMORIM, PITANGUY, 2009), nódulo, edema e eritema (VEDAMURTHY, 2004; LUPO, 2006; ZIELKE et al., 2008). Casos de hipersensibilidade (GHISLANZONI et al., 2006; PATEL, BRUCK, KATZ, 2006), formação de abscesso (TOY, FRANK, 2003;

ROMAGNOLI, BELMONTESI, 2008; ROUSSO, PITMAN, 2010) e embolização arterial (SCHANZ et al., 2002) também têm sido relacionados a aplicação de AH.

A migração do material de preenchimento introduzido nos tecidos ainda é um tema bastante controverso na literatura. A maioria dos trabalhos relata a migração do mesmo à distância, contudo sem especificar a sua precisa localização (ROSA, 2001).

Os profissionais da odontologia devem estar aptos a detectar os efeitos adversos dos materiais de preenchimento facial, uma vez que estes afetam a estética de uma região de reconhecido interesse e atuação dos cirurgiões-dentistas. Alterações vinculadas a essas substâncias podem repercutir diretamente na mucosa bucal, dificultando, algumas vezes sua identificação (MALY et al., 2004; GONÇALES et al., 2009).

Os materiais de preenchimento têm potencial de uso para complementar alguns procedimentos na odontologia estética, influenciando no sorriso ou contorno de estruturas faciais dos pacientes. Entretanto, não existem disponíveis na literatura científica, ensaios clínicos adequadamente delineados e em quantidade suficiente que permitam determinar, de forma segura, os efeitos a longo prazo destas substâncias. Relatos de casos clínicos não são suficientes para entender de maneira efetiva os riscos e benefícios da aplicação desses materiais (MALY et al., 2004; GHISLANZONI et al., 2006; DE CASTRO et al., 2007; ALIJOTAS-REIG, GARCIA-GIMENEZ, 2008; GONÇALES et al., 2009; ROUSSO, PITMAN, 2010). Portanto, justifica-se a realização de estudos padronizados, que busquem identificar e comparar as respostas teciduais entre os produtos mais utilizados na bioplastia facial.

A presente dissertação compreende 2 artigos científicos. O primeiro deles faz uma revisão da literatura sobre o tema em questão e tem por objetivo fundamentar, com base científica, o experimento realizado. O segundo artigo descreve a investigação, em modelo

animal, das alterações clínicas e histológicas na língua e rim de ratos a partir da utilização de ácido hialurônico em distintas concentrações e tempos variados de acompanhamento.


PUCRS

2 ARTIGO 1

O artigo de revisão a seguir intitula-se **“HYALURONIC ACID FACIAL FILLER — IMPLICATIONS IN DENTISTRY”** e foi formatado e submetido de acordo com as normas do periódico *Gerodontology* (Anexos A e B), o qual possui Qualis B2.

HYALURONIC ACID FACIAL FILLER — IMPLICATIONS IN DENTISTRY

Running title: Hyaluronic acid filler in dentistry

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Keywords: hyaluronic acid; facial filler; diagnosis; foreign body granuloma

2.1 ABSTRACT

Objective: The aim of this study is to provide a review on the effects of hyaluronic acid as a facial filling substance used for esthetic improvement. **Background:** The high demand for restoration of volume and filling of facial depression has given rise to the rapid emergence of new materials in the market. Facial fillers represent a breakthrough in non-invasive rejuvenating approaches for the skin and subcutaneous tissue. Hyaluronic acid is often used in the treatment of wrinkles and in lip augmentation. **Methods:** The article reviews the literature regarding the implications of hyaluronic acid, focused on the work of dentistry professionals, considering the description, indications and adverse side effects of the substance. **Conclusion:** The variety and the indiscriminate use of substances currently available, together with the growth of this class of cosmetic procedure in the different fields of action in the health industry have led to an increase in the demand for corrective cosmetic procedures, unfolding adverse reactions that may impair facial esthetics and even put the patient's general health condition at risk.

2.2 INTRODUCTION

The aging process causes structural and functional changes in organic tissues, and is potentiated of environmental factors. These phenomena thin the skin structure out, in a process where it loses elasticity. As a result, the loss of subcutaneous fat and skin collagen generates facial depression and folds, which compromise esthetic appearance and encourage the development of procedures to keep or recover younger looks (1-3).

Cosmetic surgery has for long been the most commonly adopted approach in facial rejuvenation (4,5). Traditionally, the treatment against face aging was focused on the surgical

traction of tissues (6). In a scenario of an increasing demand for less invasive procedures, which could afford fast recovery and the recovery of satisfactory looks in the short run, facial filling techniques have been developed (7).

For decades a variety of substances have been used to smooth out wrinkles or folds in the perioral and periocular regions in the skin tissue, to artificially augment lips and the malar region, and to correct facial defects. Ideally, these materials should be safe, efficient, with lasting esthetic outcomes and low incidence of adverse effects (3,8).

Hyaluronic acid has recently been advocated for use as facial filler. This review aims to relate the use of this material with oral diagnoses and procedures.

2.3 History

The approach of augmentation of soft tissues in order to improve facial esthetics started in 1800, when Neuber reported the use of fat collected from the arm to fill facial depressions. Subsequently, paraffin was also used, though the substance was prohibited in 1930 due to reports of severe cases of granuloma. Unpredictable tissue reactions in the long run have created the need to study pure substances to be used as facial fillers. In 1962, liquid silicone was launched as a cosmetic corrective agent, but years later it was banned because of the high potential to cause adverse effects. Starting in 1980 and until recently, bovine collagen was the biomaterial of choice in face filling procedures (9-10). Nevertheless, its use in skin is associated to a 3 to 5 percent risk of late hypersensitivity reaction, and requires a double skin test before treatment is started (11).

The substances used for cosmetic purposes have gained great popularity especially due to the effects of botulinum toxin, commonly known by its commercial name, Botox®.

From the 1980's on, several products have been launched in the market, such as hydroxyapatite, poly-L-lactic acid, hyaluronic acid and polymethylacrylate (9,10).

The variety and indiscriminate use of products currently available and the growing adoption of this technique amongst professionals acting in different areas of the health industry have considerably increased the demand for cosmetic corrective services. This scenario unveiled the occurrence of a set of adverse reactions that may impair facial esthetics and even put the patient's general health condition at risk (12-14).

Apart from biocompatibility, other characteristics define the ideal cosmetic filler, such as: non-carcinogenicity, stability to organic fluids and tissues, absence of inflammatory or allergic reaction, resistance to mechanical effort, low cost, easy application and removal. In spite of the technological advancements and the existence of several biomaterials in use, no product currently available in the market meets all these requirements (9,13,15).

The literature ranks cosmetic fillers into 2 classes, temporary (or resorbable), and permanent (non-resorbable), depending on the time span they remain in tissues (16). The advantage of resorbable products lies in the fact that the result may be reverted after some period has elapsed, while permanent fillers require surgical removal in the event of migration or tissue rejection of the material (10).

2.4 Hyaluronic acid

Hyaluronic acid (HA) is a polysaccharide of the family of glycosaminoglycans isolated for the first time in 1934 from the bovine eye vitreous humor by Karl Meyer, the North American scientist, and John Palmer, his assistant. They discovered a substance containing 2 saccharides, 1 of which was uronic acid, known as "the sugar acid".

Subsequently the researchers suggested that the substance should be called hyaluronic acid, which the combination of 2 terms, hyaloid (from *hualos*, the Greek for glass), and uronic acid. In 1986 the term hyaluronan was presented as a more updated term, though the most commonly employed name to date is still that proposed by Meyer and Palmer (17-19).

In the last 2 decades HA has been considerably used in eye surgery, wound healing and treatment approaches to conditions of joints. With the advent of greater biotechnological achievements, several HA particle sizes have been developed. Nowadays, HA utilization for esthetic purposes has increased expressively (5,20), and the substance has also been employed often in the treatment of facial wrinkles and folds, as well as in lip augmentation procedures (21).

2.5 Chemical structure of hyaluronic acid

The first description of the chemical structure of the HA molecule was presented by Weissman and Meyer in 1954, when it was demonstrated that HA is formed by 2 uronic acid units and 1 amine sugar unit, forming a polymer of disaccharides, themselves composed of monosachharide D-glucuronic acid and D-N-acetylglucosamine, linked together via alternating β-1,4 and β-1,3 glycosidic bonds (16,17). It is estimated that a 60-kg human body contains 12 g of hyaluronic acid (2).

Hyaluronan is an essential component of the extracellular matrix of every tissue. The highest hyaluronan concentration was reported in the umbilical cord (4 mg/kg), synovial fluid (3-4 mg/L), although the skin also presents a large content of the polysaccharide (18). It was discovered that the role played by hyaluronan in the skin structure consisted essentially in increasing volume and lubricating tissues, since the hydrophilic properties it exhibits attract

water to the extracellular matrix and increase skin elasticity. Hence the expectation that HA could definitely act as an anti-wrinkle agent (22).

2.6 Filling material

Currently HA is the most commonly employed resorbable biomaterial for esthetic improvement purposes. The most used agent in stabilization is 1,4-butanediol-diglycidyl-ether. Product safety is based on the washing-off process of these cross-linking residues, which affords to obtain a pure, atoxic and biocompatible filling material (18,23). Hyaluronic acid injections do not have to be preceded by a skin test, and the literature indicates a minimal hypersensitivity risk (24).

An HA product may be developed from avian or even non-animal raw materials, which present specific and individual characteristics (25). The most common sources of these raw materials include rooster comb (avian) and bacterial growth (non-animal), especially *Streptococcus equi*, a non-pathogenic bacterium to humans (18). Several preparations of the product are prescribed according to the depth of injection and to the esthetic outcome expected. The differences are based on the concentration of HA, in the intensity of cross-linking and, as a result, on the rate of compound degradation (26).

Due to the fact that it is resorbable, HA is metabolized by enzymes or gradually phagocyted. These processes occur within 3 to 24 months after applications, depending on how much HA is injected in tissues (3). Other authors have reported a gradual absorption of the substance between 6 months and 1 year after applications, and that HA permanence in tissues is 9 months on average (27-31).

When choosing the concentration of HA used in facial filling procedures, 2 main aspects are considered: the depth of wrinkles and expression lines, as well as the level of augmentation desired. As a rule, 3 concentrations of the product are used. Low concentration HA is used to fill the so-called “smoker lines” that form around the upper lip, as well as crow’s feet. Intermediate concentration HA is used in lip augmentation procedures, while high concentration HA products are injected in nasolabial folds (32). Topical, infiltrative or block anesthesia are the approaches of choice to control pain during the injection of the filling material, but tooth block may stop the natural lip movements and compromise the final result, due to the distortion that occurs in the site the substance is injected. In turn, infiltration anesthesia may lead to the loss of a landmark in terms of the volume of material to be injected, which ultimately increases or reduces augmentation, in comparison to the desired result (33).

2.7 Dentistry and facial filling materials

Dentistry professionals have to become aware of the effects of facial filling materials, since these may affect the esthetic result in the region they are injected - an area of great interest of dental surgeons. Some of the possible problems caused by these substances may have a repercussion in the oral mucosa, and lead to confusion or misinterpretation.

On the other hand, filling substances are increasingly present in esthetic complementation and oral rehabilitation approaches (9,30). With the advent of dental implants, a large number of patients began to replace their total prostheses for fixed protocol prostheses. However, this class of prostheses does not allow the same esthetic result to the patient’s face, since the lack of resin flank in these fixed prostheses often increases the

nasogenian fold and consequently worsens an aged look. For this reason, the use of fillers in the lower lip and in the nasogenian fold is required for a great number of patients who replace their total prostheses for implant-supported dentures. This procedure aims at attenuating the aging look caused by the loss of lip support.

2.8 Effects of hyaluronic acid filling

Although it has been classified as a non-immunogenic substance (34), it is known that HA may trigger an unfavorable tissue response due to the remnants of bacterial proteins in the composition of the product, to the incorrect application or even to the presence of a biofilm on the tissue (14,35,36). The filling substances may cause a wide array of complications, from a simple inflammatory reaction to tissue necrosis (12,37-39), which may become visible immediately or a longer time lapse after the application.

The immediate and/or transient complications are the most common adverse effects of HA fillings and as a rule are events related to inflammatory processes or to technical problems. These effects are observed soon after injection, with zero to 14 days (40) and include erythema, ecchymosis and swelling in the region the product was applied (13,41-44). Hematomas may form due to injury to a blood vessel, while necrosis may appear when the injection perforates an artery. These changes have been reported in the glabella and in the nasolabial fold (45-47). Hypersensitivity (48) and vasculitis (49) have also been observed in some clinical case reports.

When transient effects occur, biopsies are not usually prescribed (39). However, the procedure is necessary when some clinical signs become apparent, like the migration of the injected material and granuloma caused by a foreign body (39, 50). The procedure is

warranted because the changes mentioned may be manifested as papules or nodes (51-54), and frequently may be mistaken for pathologies with distinct etiology and behavior, like cysts and/or salivary gland neoplasias (54).

Also, an inflammatory reaction like a granuloma may be observed in the site the exogenous material is injected (3,42,55). The process starts with the arrival of neutrophils and lymphocytes, which is accompanied by pain and exudation. Right from the start, the material injected is invaded by inflammatory cells. The foreign body there is too large to allow phagocytosis by one macrophage only. Therefore, these cells gather together to form giant cells and aim at isolating the exogenous substance. Surrounding the zone of granulomatous inflammation more intense signs of fibroplasia are observed, in a process that occurs in order to limit the tissue response to the presence of the filling material and thus reduce local inflammation (28). In histological examinations, HA is observed as a blue mass with a bizarre configuration and variable sizes surrounded by neutrophils, eosinophils and multinucleated giant cells (15,56-59).

In 2003, Fernández-Aceñero, Zamora and Borbujo (29) described the case of a patient who presented several nodes in the upper lip caused by an irregular increase in tissue volume that had been evolving for 2 months. The patient reported having had lip augmentation injections with HA. Based on the assumed diagnosis of foreign body, an incisional biopsy was conducted. A well-outlined mass was detected in the subcutaneous adipose tissue plane, and was diagnosed as granuloma. The presence of exogenous material in the biopsied area was confirmed by histopathology.

The mechanism through which filling substances trigger a foreign body reaction, the reasons behind the variation in intensity, and the unpredictable character of their mode of action are yet to be elucidated (54,60). The filling material often migrates to the oral mucosa,

forming a stiff nodule within tissues (29,61,62). This stresses the need for a complete physical examination that should include visual inspection and tissue palpation. A patient's previous experience with a filling substance is not always reported spontaneously. Several times it is necessary to insist in collecting more thorough information during anamnesis, with a view to obtaining as many details as possible concerning past filling material applications.

2.9 CONCLUSION

The unplanned use of filling materials has revealed a series of adverse reactions that put the esthetic result and the patient's general health at risk. Even though many professionals of the health industry consider bioplasty procedures safe, with no hazards to the patient, adverse effects are observed in some cases. These are significant complications that include deformity and tissue destruction by an inflammatory response.

The easy access to filling substances has led to a widespread adoption of incisionless cosmetic interventions in facial rejuvenation approaches. In this scenario, several professionals of different areas in the health industry tap into the popularity and low cost of these products, performing the filling technique in their patients and exposing them to unnecessary and serious hazards. These patients are often informed of the advantages but not of the likely adverse effects they may experience after an intervention of this kind.

When performed by experienced professionals and under a correct selection of the case and of the biomaterial to be used it is possible to minimize the effects of age on the skin. Nevertheless, in some circumstances some sort of sequelae may occur. The excessive and indiscriminate utilization of these materials may pave the way to the surfacing of disappointing issues related to safety and efficacy. Dental surgeons have to familiarize

themselves with these procedures, since the possible adverse effects of filling materials may emulate other pathologies in the orofacial region, making it difficult to diagnose and conduct the appropriate clinical management of the patient (63,64).

2.10 REFERENCES

1. Brandt FS, Cazzaniga A. Hyaluronic acid fillers: Restylane and Perlane. Facial Plast Surg Clin North Am 2007;15(1):63-76.

2. Matarasso SL, Carruthers JD, Jewell ML. Consensus recommendations for soft-tissue augmentation with nonanimal stabilized hyaluronic acid (Restylane). Plast Reconstr Surg 2006;117(suppl 3):3S-34S.

3. Vargas AF, de Amorim NG, Pitanguy I. Complicações tardias dos preechimentos permanentes. Rev Bras Cir Plást 2009; 24(1):71-81.

4. De Maio M. The minimal approach: an innovation in facial cosmetic procedures. Aesthetic Plast Surg 2004;28(5):295-300.

5. Brandt FS, Cazzaniga A. Hyaluronic acid gel fillers in the management of facial aging. Clin Interv Aging 2008;3(1):153-9.

6. Grover R. Optimizing treatment outcome with Restylane SubQ: the role of patient selection and counseling. Aesthetic Surg J 2006; 26(suppl 1):S18-S21.

7. Smith KC. Reversible vs. nonreversible fillers in facial aesthetics: concerns and considerations. Dermatol Online J 2008;14(8):3.

8. Parada MB, Michalany NS, Hassun KM, et al. A histologic study of adverse effects of different cosmetic skin fillers. Skinmed 2005; 4(6):345-9.

9. Dastoor SF, Misch CE, Wang HL. Dermal fillers for facial soft tissue augmentation. J Oral Implantol 2007;33(4):191-204.

10. John HE, Price RD. Perspectives in the selection of hyaluronic acid fillers for facial wrinkles and aging skin. Patient Prefer Adherence 2009;3:225-30.

11. Lowe NJ, Maxwell CA, Lowe P, et al. Hyaluronic acid skin fillers: adverse reactions and skin testing. J Am Acad Dermatol 2001; 45(6):930-3.

12. De Castro ACB, Collares MVM, Portinho CP, et al. Necrose facial extensa após infiltração com polimetilmetacrilato. Rev Bras Otorrinolaringol 2007;73(6):850.

13. Lemperle G, Romano JJ, Busso M. Soft tissue augmentation with artecoll: 10-year history, indications, techniques, and complications. Dermatol Surg 2003; 29(6):573-87.

14. Niamtu J III. The use of restylane in cosmetic facial surgery. J Oral Maxillofac Surg 2006;64(2):317-25.

15. Lemperle G, Morhenn V, Charrier U. Human histology and persistence of various injectable filler substances for soft tissue augmentation. Aesth Plast Surg 2003;27(5):354-66.

16. Edwards PC, Fantasia JE. Review of long-term adverse effects associated with the use of chemically-modified animal and nonanimal source hyaluronic acid dermal fillers. Clin Interv Aging 2007;2(4):509-19.

17. McDonald J, Hascall VC. Hyaluronan minireview series. J Biol Chem 2002; 277(7):4575-9.

18. Romagnoli M, Belmontesi M. Hyaluronic acid-based fillers: theory and practice. Clin Dermatol 2008;26(2):123-59.

19. Matarasso SL. Understanding and using hyaluronic acid. Aesthetic Surg J 2004; 24(4):361-4.

20. Yoon ES, Han SK, Kim WK. Advantages of the presence of living dermal fibroblasts within restylane for soft tissue augmentation. Ann Plast Surg 2003; 51(6):587-92.

21. Carruthers J, Carruthers A. A prospective, randomized, parallel group study analyzing the effect of BTX-A (Botox) and nonanimal sourced hyaluronic acid (NASHA, Restylane) in combination compared with NASHA (Restylane) alone in severe glabellar rhytides in adult female subjects: treatment of severe glabellar rhytides with a hyaluronic acid derivative compared with the derivative and BTX-A. Dermatol Surg 2003;29(8):802-9.

22. Allemann IB, Baumann L. Hyaluronic acid gel (Juvéderm) preparations in the treatment of facial wrinkles and folds. Clin Interv Aging 2008;3(4):629-34.

23. Yeom J, Bhang SH, Kim BS, et al. Effect of cross-linking reagents for hyaluronic acid hydrogel dermal fillers on tissue augmentation and regeneration. Bioconjug Chem 2010;21(2):240-7.

24. Eppley BL, Dadvand B. Injectable soft-tissue fillers: clinical overview. Plast Reconstr Surg 2006;118(4):98e-106e.

25. Manna F, Dentini M, Desideri P, et al. Comparative chemical evaluation of two commercially available derivatives of hyaluronic acid (Hylaform from rooster combs and Restylane from streptococcus) used for soft tissue augmentation. J Eur Acad Dermatol Venereol 1999; 13(3):183-92.

26. De Maio M, Rzany B. Substâncias de preenchimento em medicina estética. São Paulo: Livraria Santos Editora; 2007.

27. Lupton JR, Alster TS. Cutaneous hypersensitivity reaction to injectable hyaluronic acid gel. Dermatol Surg 2000;26(2):135-7.

28. Achilles R. Imunologia e Biocompatibilidade. In: ROCA, ed. Tratado de Medicina Estética. São Paulo: Roca; 2004:1215-23.

29. Fernández-Aceñero MJ, Zamora E, Borbujo J. Granulomatous foreign body reaction against hyaluronic acid: report of a case after lip augmentation. Dermatol Surg 2003;29(12):1225-6.

30. Rosa SC, de Macedo JLS. Reações adversas a substâncias de preenchimento subcutâneo. Rev Soc Bras Cir Plást 2005; 20(4):248-52.

31. Resende VCL, Haddad A, Regazzini DV, et al. Avaliação tardia de 1366 casos de preenchimento dérmico com implante de ácido hialurônico em face. Rev Bras Cir Plást 2008;23(suppl 3):34.

32. Saylan Z.Facial fillers and their complications. Aesthetic Surg J 2003;23(3):221-4.

33. Klein AW. In search of the perfect lip: 2005. Dermatol Surg 2005; 31(11 Pt 2):1599-603.

34. Hamilton RG, Strobos J, Adkinson NF Jr. Immunogenicity studies of cosmetically administered nonanimal-stabilized hyaluronic acid particles. Dermatol Surg 2007;33 (suppl 2):S176-S185.

35. Gelfer A, Carruthers A, Carruthers J, et al. The natural history of polymethylmethacrylate microspheres granulomas. Dermatol Surg 2007; 33(5):614-20.

36. Rohrich RJ, Monheit G, Nguyen AT, et al. Soft-tissue filler complications: the important role of biofilms. Plast Reconstr Surg 2010;125(4):1250-6.

37. Fernández-Cossío S, Castaño-Oreja MT. Biocompatibility of two novel dermal fillers: histological evaluation of implants of a hyaluronic acid filler and a polyacrylamide filler. Plast Reconstr Surg 2006;117(6):1789-96.

38. Lemperle G, Morhenn VB, Pestonjamasp V, et al. Migration studies and histology of injectable microspheres of different sizes in mice. Plast Reconstr Surg 2004;113(5):1380-90.

39. Zimmermann US, Clerici TJ. The histological aspects of fillers complications. Semin Cutan Med Surg 2004;23(4):241-50.

40. Monheit GD, Rohrich RJ. The nature of long-term fillers and the risk of complications. Dermatol Surg 2009;35 (suppl 2):1598-604.

41. Narins RS, Brandt F, Leyden J, et al. A randomized, double-blind, multicenter comparison of the efficacy and tolerability of Restylane versus Zyplast for the correction of nasolabial folds. Dermatol Surg 2003 ; 29(6):588-95.

42. Zielke H, Wolber L, Wiest L, et al. Risk profiles of different injectable fillers: results from the Injectable Filler Safety Study (IFS Study). Dermatol Surg 2008;34(3):326-35.

43. Lupo MP. Hyaluronic acid fillers in facial rejuvenation. Semin Cutan Med Surg 2006;25(3):122-6.

44. Cox SE. Clinical experience with filler complications. Dermatol Surg 2009; 35 (suppl 2):1661-6.

45. Glaich AS, Cohen JL, Goldberg LH. Injection necrosis of the glabella: protocol for prevention and treatment after use of dermal fillers. Dermatol Surg 2006;32(2):276-81.

46. Bellman B. Complication following suspected intra-arterial injection of Restylane. Aesthetic Surg J 2006;26(3):304-5.

47. Grunebaum LD, Allemann IB, Dayan S, et al. The risk of alar necrosis associated with dermal filler injection. Dermatol Surg 2009;35:1635-40.

48. Patel VJ, Bruck MC, Katz BE. Hypersensitivity reaction to hyaluronic acid with negative skin testing. Plast Reconstr Surg 2006;117(6):92e-4e.

49. Alijotas-Reig J. Recurrent urticarial vasculitis related to nonanimal hyaluronic acid skin filler injection. Dermatol Surg 2009;35 (suppl 1):395-7.

50. Bjarnsholt T, Tolker-Nielsen T, Givskov M, et al. Detection of bacteria by fluorescence in situ hybridization in culture-negative soft tissue filler lesions. Dermatol Surg 2009;35 (suppl 2):1620-4.

51. Dadzie OE, Mahalingam M, Parada M, et al. Adverse cutaneous reactions to soft tissue fillers - a review of the histological features. J Cutan Pathol 2008; 35(6):536-48.

52. Anwar MU, Sharpe DT. Skin nodules after semipermanent cosmetic dermal filler. Aesthetic Plast Surg 2007; 31(4):401-2.

53. Toy BR, Frank PJ. Outbreak of Mycobacterium abscessus infection after soft tissue augmentation. Dermatol Surg 2003;29(9):971-3.

54. Jham BC, Nikitakis NG, Scheper MA, et al. Granulomatous foreign-body reaction involving oral and perioral tissues after injection of biomaterials: a series of 7 cases and review of the literature. J Oral Maxillofac Surg 2009;67(2):280-5.

55. Hönig JF, Brink U, Korabiowska M. Severe granulomatous allergic tissue reaction after hyaluronic acid injection in the treatment of facial lines and its surgical correction. J Craniofac Surg 2003;14(2):197-200.

56. Okada S, Okuyama R, Tagami H, et al. Eosinophilic granulomatous reaction after intradermal injection of hyaluronic acid. Acta Derm Venereol 2008;88(1):69-70.

57. Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. Semin Immunol 2008; 20(2):86-100.

58. Bardazzi F, Ruffato A, Antonucci A, et al. Cutaneous granulomatous reaction to injectable hyaluronic acid gel: another case. J Dermatolog Treat 2007;18(1):59-62.

59. Ghislanzoni M, Bianchi F, Barbareschi M, et al. Cutaneous granulomatous reaction to injectable hyaluronic acid gel. Br J Dermatol 2006;154(4):755-8.

60. Edwards PC, Fantasia JE, Iovino R. Foreign body reaction to hyaluronic acid (Restylane): an adverse outcome of lip augmentation. J Oral Maxillofac Surg 2006; 64(8):1296-9.

61. Lombardi T, Samson J, Plantier F, et al. Orofacial granulomas after injection of cosmetic fillers. Histopathologic and clinical study of 11 cases. J Oral Pathol Med 2004; 33(2):115-20.

62. Sanchis-Bielsa JM, Bagan JV, Poveda R, et al. Foreign body granulomatous reactions to cosmetic fillers: a clinical study of 15 cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 108(2):237-41.

63. Maly A, Regev E, Meir K, et al. Tissue reaction to liquid silicone simulating low-grade liposarcoma following lip augmentation. J Oral Pathol Med 2004; 33(5):314.

64. Gonçales ES, Almeida AS, Soares S, et al. Silicone implant for chin augmentation mimicking a low-grade liposarcoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 107(4):e21-e23.

3 ARTIGO 2

O artigo a seguir intitula-se **“TISSUE RESPONSE IN RAT TONGUE SUBMITTED TO HYALURONIC ACID INJECTION IN DIFFERENT CONCENTRATIONS”** e foi aceito pelo periódico *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology* (Anexos C e D), o qual apresenta Qualis A2.

CLINICAL AND HISTOLOGICAL EVALUATION OF EFFECTS OF HYALURONIC ACID IN RAT TONGUE

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3.1 ABSTRACT

*Objective***:** This study aimed to investigate local and systemic effects of two concentrations of hyaluronic acid (HA) in rats.

Study design: Fifty-four female Wistar rats were allocated into 3 treatment groups (25mg/mL HA; 5.5mg/mL HA; 0.9% NaCl) and 3 subgroups of experimental time: 7, 60 and 90 days. The rats were evaluated clinically and then euthanized. The tongue and right kidney were removed and stained by HE and picrosirius (only tongue).

Results: The clinical changes did not differ significantly between groups (Kruskal-Wallis; $p>0.05$). Histologically there was significantly greater inflammatory response in the groups with HA compared to control (Kruskal-Wallis; $p<0.001$). All kidney samples were normal. The 25mg/mL HA had a higher collagen formation (ANOVA Mixed Models; $p=0.016$).

Conclusion: HA causes moderate inflammatory reaction until 7 days and mild until 60 and 90 days, compatible with tissue repair, and a slightly increased collagen formation around the material was detected.

3.2 INTRODUCTION

Wrinkles reduction and the correction of cutaneous defects by using injectable materials have been largely applied by dermatologists and plastic surgeons. The growth of injectable materials manufacturers is due to the increase in demand during the past years and also to the range of new materials available in the market.

The ideal filling material should be safe and effective, not immunogenic, easy to apply and remove, non-resorbable, biocompatible, low-cost and it should not cause inflammatory or allergic reactions[1-4]. However, up to this moment, no product available in the market has all the requirements mentioned above.

HA is a polysaccharide present in the extracellular matrix of living beings which is able to attract water to tissues[5]. That factor makes HA a very convenient material in clinical practice for increasing skin volume. In humans, the amount of HA in the skin decreases with age, which represents an important role in the aging process, resulting in the reduction of dermal tissue elasticity and hydration[6-9] .

Sources commonly used for commercializing HA-based products are rooster's comb and bacteria (S*treptococcus*)[10,11]. The most common adverse effects of this resorbable substance involve edema, erythema and ecchymosis[12,13]. Hypersensitivity reaction, angioedema and foreign body reaction have also been reported[14-16].

In the present study, the objective was to evaluate early and late effects, local or systemic, with the injection of two concentrations of hyaluronic acid in rats' ventral tongue in different monitoring periods.

3.3 MATERIALS AND METHODS

This research was initiated following approval from the Scientific and Ethic Committee (protocol 0007/10) and then from the Ethic Committee for Animal Use (protocol 10/00151) of the Pontifical Catholic University of Rio Grande do Sul (PUCRS), Brazil, and the procedures were carried out in accordance with institutional guidelines for animal care and use.

3.3.1 Materials

The material used for this experiment was 5.5mg/mL hyaluronic acid (Prevelle®, *Mentor Corporation, CA, EUA*) and 25 mg/mL (Teosyal®, *Lab.Teoxane, Geneva, Switzerland*). Two extreme concentrations were chosen in order to compare their response on

tissues, due to the fact that there is a high applicability on the facial region, area of interest to dental surgeons and subject to adverse effects. The materials were injected at the beginning of the experiment and the monitoring happened in the respective study periods (7, 60 and 90 days).

3.3.2 Animal model

The sample was composed of 54 female Wistar rats (*Rattus novergicus*) aged 2 months and weighing approximately 200g; they were obtained from the animal facility of the Federal University of Pelotas (UFPEL, Brazil). Animals were individually numbered on the tail and housed in plastic cages placed in ventilated racks (Alesco, Monte Mor, SP, Brazil) at a temperature of 22°C with a 12-h light/dark cycle. Animals were fed a standard diet of rat chow (Nuvilab, Colombo, PR, Brazil) and given water *ad libitum*. The animals were randomly allocated into 3 groups, according to the treatment received: group 1 (n=18): 5.5mg/mL HA; group 2 (n=18): 25mg/mL HA; group 3 (n=18): control- 0.9%NaCl, and into 3 experimental periods: A- 7 days; B- 60 days and C- 90 days.

3.3.3 Anesthesia

Initially the female rat number 1 was weighed in a digital scale (Urano model UDI 2500/0,5) so that the dosage of anesthetic could be calculated. This procedure was performed with an intraperitoneal injection of a mixture of 20mg/mL Xylazine Hydrochloride (0.05mL/100g) (ROMPUM®, Bayer S.A. - Saúde Animal, SP, Brasil), sedative, analgesic and muscle relaxant, with 50mg/mL Ketamine Hydrochloride (0.1mL/100g) - (DOPALEN®, Agribrands do Brasil Indústria e comércio Ltda., SP, Brasil), an anesthetic for veterinary use.

Animals from the respective groups (1, 2 and 3), randomly chosen, were successively anesthetized.

3.3.4 Material application

As sedation could be observed, the animal was placed on a surgical table, in supine position and having their paws tied using elastic strips. The tongue of rat 1 was pulled out with tweezers exposing the ventral tongue region. Using a disposable insulin syringe 26G ½ (13 x 4.5), 0.07 mL of the filling material (5.5mg/mL HA) was injected in the middle third of the ventral tongue, 7mm ahead of the frenum, on the left side, 2mm from the midline. The needle was inclined as parallel as possible to the mucosa, with the bevel facing up, 7mm deep, and this measure being standardized by an endodontic silicone stop.

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

3.3.5 Clinical evaluation

After 7 days, preceding euthanasia of the animals from group 1 A, there was sedation and subsequent clinical evaluation of each rat's tongue. The same procedure was performed in the other monitoring periods. In the clinical analysis, possible tissue alterations such as swelling, nodules, ulceration, necrosis and/or suppuration could be observed.

3.3.6 Euthanasia

Seven days after material injection, animals from group 1A, randomically chosen, were individually submitted to euthanasia with isoflurane. Following the same procedure,

according to the respective monitoring periods, the animals from the other groups (60 and 90 days) systematically went through the same euthanasia procedure as the one performed for the group mentioned above.

3.3.7 Sample processing

After being euthanized, all animals were necropsied, having their tongue and right kidney removed for microscopic analysis. Sample fixation was carried out with the use of 10% neutral buffered formalin for a minimum of 24 hours. Samples of the tongue and kidney were sectioned longitudinally into 2 fragments. The inclusion was done so that the edge of the tongue sample had its long axis parallel to the paraffin block section plan. For each specimen, there were two histological sections of 6µm each in order to obtain 2 slides, later stained with hematoxylin and eosin (HE) and picrosirius, the latter being a specific staining for collagen fiber analysis[17].

3.3.8 Histological evaluation

In groups 1 and 2, fragments in which exogenous material was present were considered viable for histological analysis in tongue. In slide analysis, the examiner was previously calibrated and blinded with the use of masks in all evaluated slides. The histological section analysis occurred in the Oral Medicine Unit (São Lucas Hospital in PUCRS, Brazil) through light microscopy (Olympus® model BX50) in approximate magnifications of x40, x100, x200 and x400. In order to establish a standard criteria judgment, there was a training session with an experienced pathologist. The intra-examiner calibration was performed with the reanalysis of 40 slides in a 7-day interval between observations (Kappa= 0.889 ± 0.061; $p<0.001$).

3.3.8.1 Inflammatory reaction

For groups 1 and 2, the histological evaluation was performed with an analysis of the microscopic fields adjacent to the filling material, always attempting to identify the region with a higher intensity of inflammatory response. Thus, the absence or presence of lymphocytes, plasma cells, macrophages, giant cells, neutrophils, eosinophils, fibroplasia, edema and hyperemia was analyzed with x200 magnification. For group 3, field selection and analysis were carried out according to the anatomical references where the filling material was injected.

In the histopathological analysis, the criteria illustrated in figure 1, were considered[18,19].

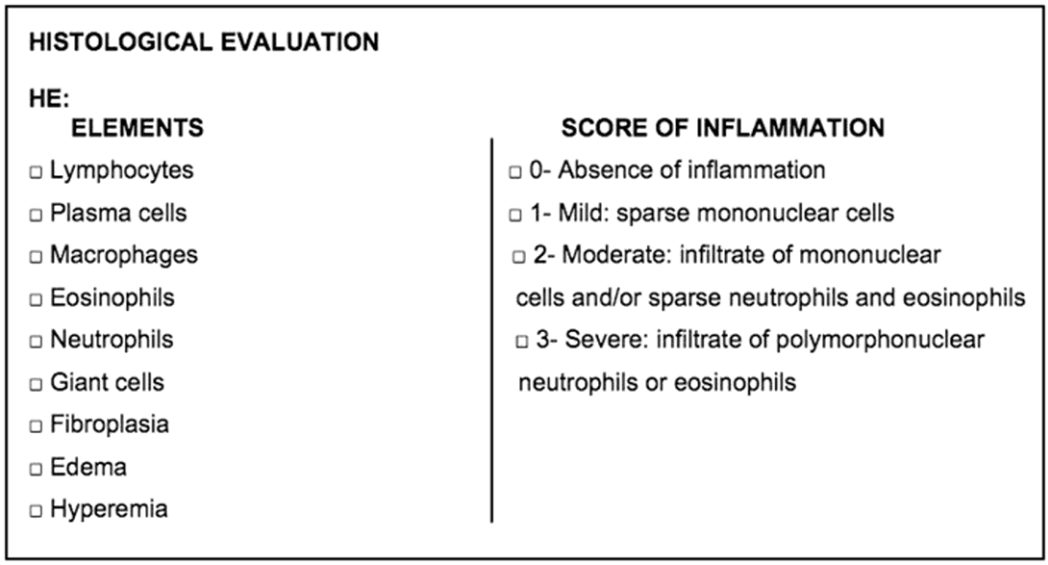

Fig. 1. Representation of the histological score sheet.

3.3.8.2 Connective tissue reaction

In tongue slides stained by picrosirius, 3 to 5 areas (μm^2), which contained most connective tissue, were selected using x100 magnification. Those photomicrographs were

exported to the *Image Pro Plus*® version 4.5.1 software (Media Cybernetics, Inc.; 2005), in which images obtained with polarized light are recognized and converted into shades of red (collagen representative area). Thus, the proportion of collagen fibers was determined by the calculation of the areas occupied by them in comparison to the total area of each field.

3.3.8.3 Migration

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

3.3.9 Statistical analysis

In order to perform the statistical analysis, the following softwares were used: SPSS 17 (*SPSS Inc*.) and SYSTAT 13 (*Systat Software Inc*.). Kruskal-Wallis tests and Conover-Inman Post Hoc tests were used for all pairwise comparisons, considering the differences with significance levels set at 5% ($p<0.05$).

To analyze the collagen content from picrosirius slides (which has a numeric variable), Mixed Models with Fisher Post Hoc analysis were used with significance levels set at 5%. The fixed effects of the statistical model were group, time and time and group interaction whereas the random effect was the animal whose histological slide had multiple reading fields.

3.4 RESULTS

During this research, 3 rats died, therefore 51 rats were included in this study, according to the following distribution: group 1A (n=6); 2A (n=5); 3A (n=6); group 1B (n=6), 2B (n=6); 3B (n=6); group 1C (n=6); 2C (n=5); 3C (n=5).

3.4.1 Clinical evaluation

The clinical evaluation parameters have not shown statistically significant differences in this study. No animal presented erythema, necrosis and/or suppuration. Out of 34 animals, 7 of them, which received HA injections, presented a volume increase in the ventral tongue in groups 2B (n=1) and 1C (n=1). The presence of ulcerations (Fig.2) was observed exclusively in the first week in groups 5.5 mg/mL HA (n=1) and 25mg/mL HA (n=2), while white plaques (Fig. 2) were noticed in the 90-day period, in every test group (n=2).

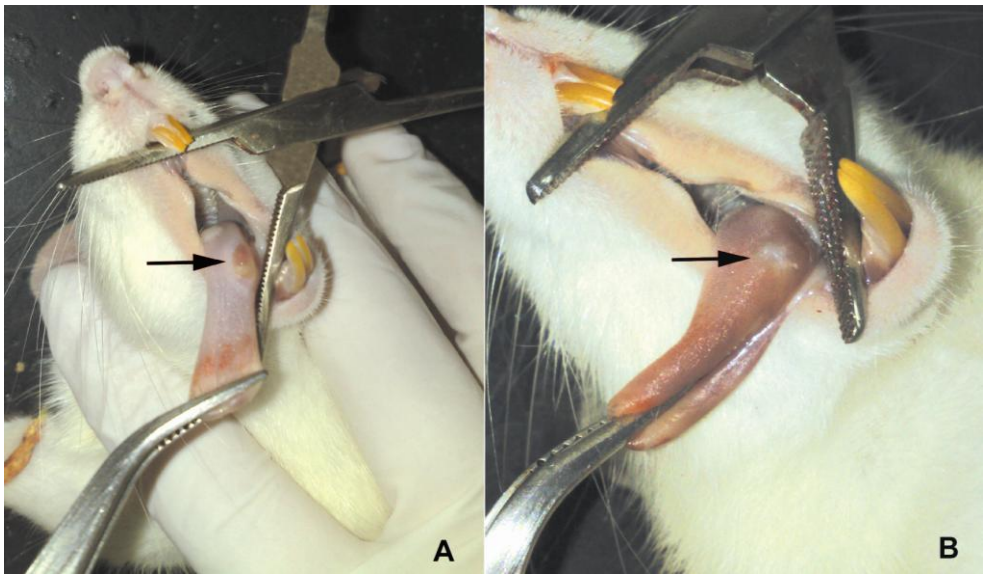

Fig.2. Clinical findings. **A**, ulceration HA (7 days). **B**, white plaque HA (90 days).

3.4.2 Histological evaluation

It was observed that HA histological pattern (Fig. 3) is basophilic, of variable shape and size and distributed within the tissue.

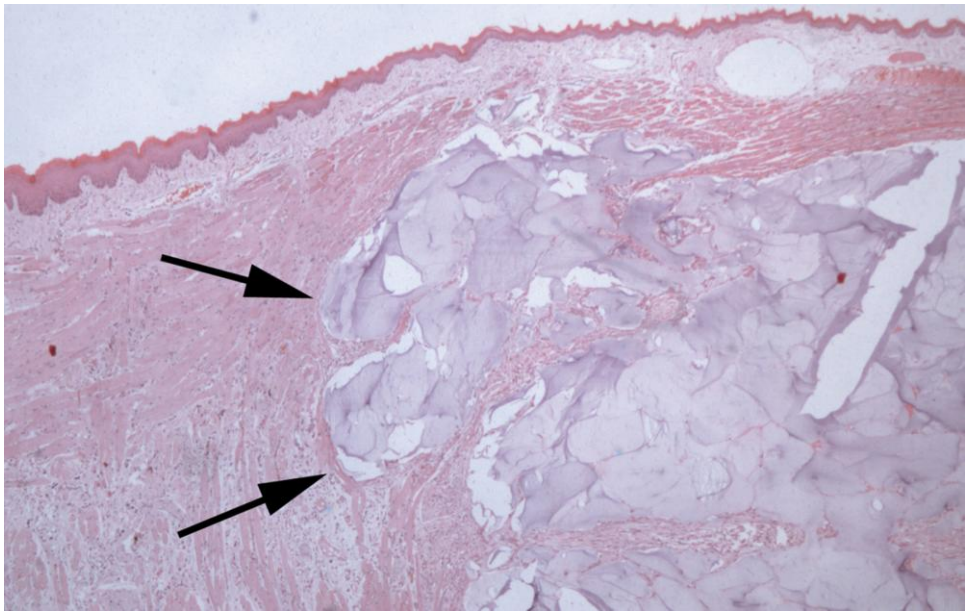

Fig. 3. Photomicrograph of HA (arrow) within tissue. (HE, magnification: x40).

3.4.2.1 Inflammatory reaction

One week after material application, there was predominance of severe/moderate inflammatory reaction, ranging from moderate/mild to absence of reaction from 60 to 90 days (Fig. 4). For the same study period, inflammatory reaction in test groups was more intense ($p<0.05$), decreasing in 60 and 90 days (Fig. 4).

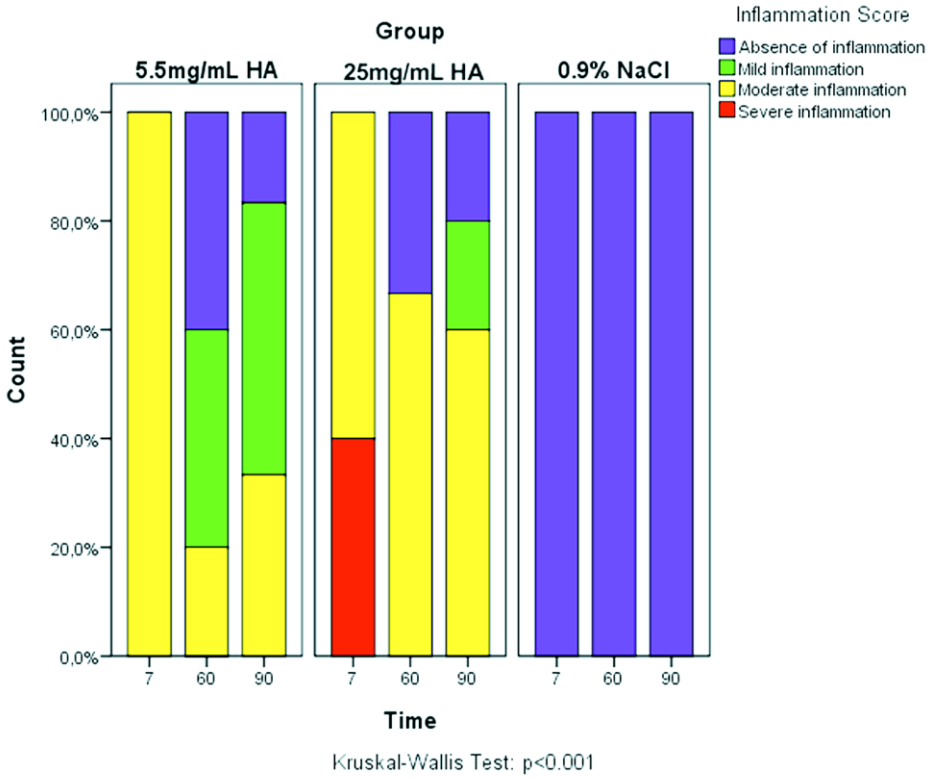

Fig.4. Score distribution representation for the respective groups and monitoring periods.

3.4.2.2 Variables

Mononuclear cells were not present in the control group in any of the monitoring periods. Lymphocytes remained significantly present in all periods for both HA concentrations ($p<0.05$). However, in these test groups, there was no significant variation with time. The same pattern related for lymphocytes was observed for plasma cells and macrophages.

Neutrophils and eosinophils had strong presence in the 7-day period ($p<0.01$), decreasing to control group levels in 60 and 90 days, showing that the highest inflammatory score observed in the first week occurred at the expense of an acute reaction of these 2 types of cell.

Although there was no significant statistic difference ($p>0.05$), giant cells were detected in 1 case in subgroup A and in another in subgroup B. Abscess (Fig. 5), edema and hyperemia were observed in 2 cases in the first 7 days of HA 25mg/mL group.

The presence of fibroplasia was detected in all monitoring periods of group tests, except for the control group where it was not detected in any monitoring period ($p<0.01$).

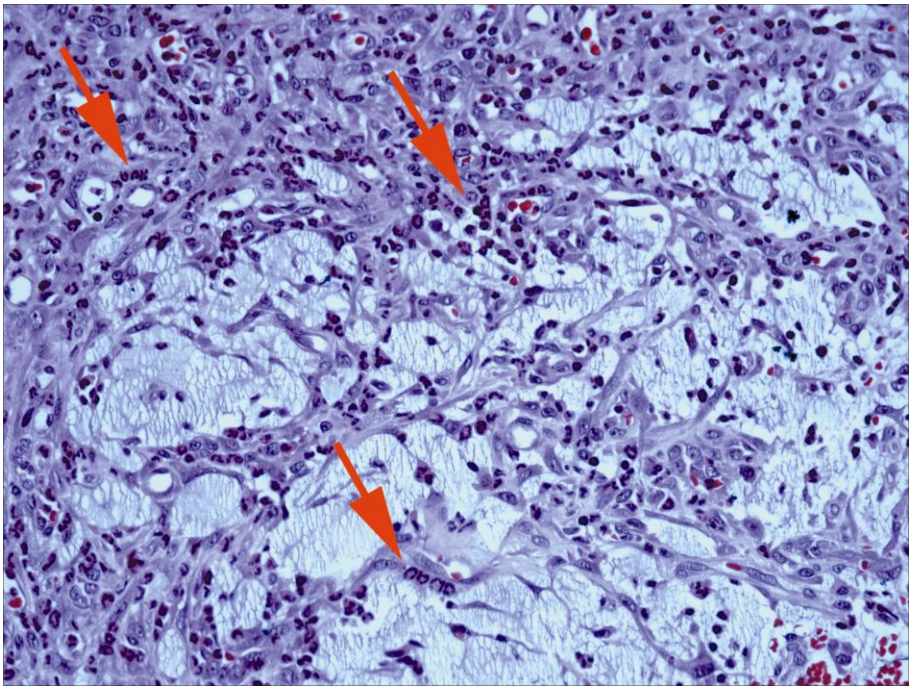

Fig 5- Photomicrograph showing occurrence of abscess (arrows). (HE, magnification: x400).

3.4.2.3 Connective tissue reaction

The mixed model for verifying the percentage of collagen fibers surrounding the material detected differences among groups ($p=0.016$), did not detect differences with time ($p=0.409$), neither interaction between group and time ($p=0.475$). The average percentage of fibroplasia for each group is described in table I. Figure 6 represents collagen formation around HA.

Table I. Distribution of average percentage of fibroplasia in study groups regardless of evaluation period.

Group	*Mean*	*95% Confidence Interval*	
		Lower	*Upper*
5.5mg/mL HA	0.161	0.059	0.263
25mg/mL HA	0.281	0.176	0.387
0.9% NaCl	0.057	-0.052	0.167

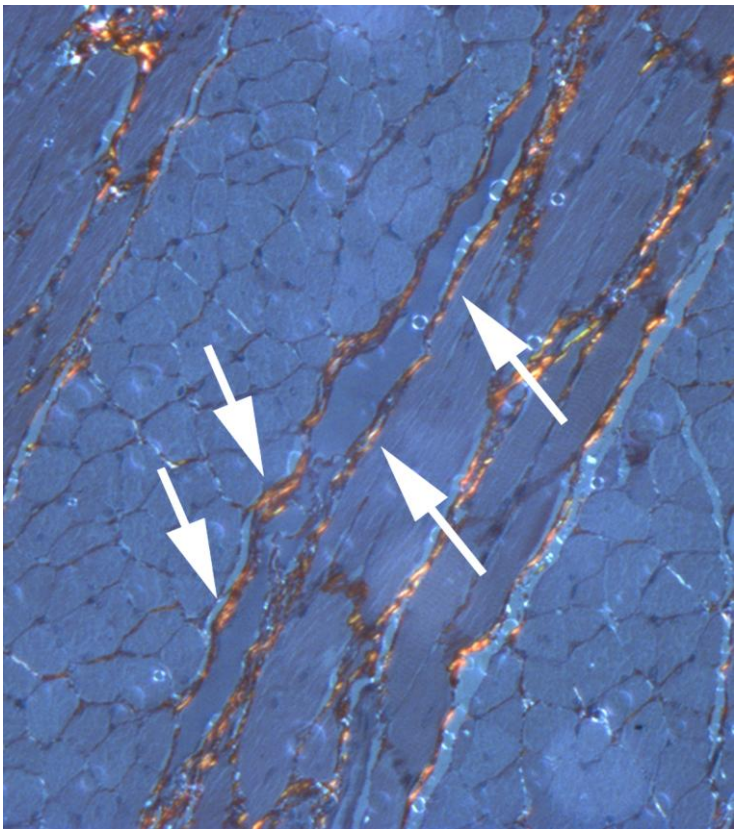

Fig. 6. Photomicrograph showing the formation of collagen fibers (arrows) adjacent to 25mg/mL HA (60 days) (Picrosirius, magnification: x100).

Group 1 (5.5mg/mL HA) tended to produce more collagen if compared to the control group, however p-value did not reach the significance level (p=0.172). When compared to

group 2 (25mg/mL HA), there was less collagen, but p-value was not statistically significant either. Fisher's test (Table II) detected difference between groups 2 and 3 (0.9% NaCl).

Table II. Fisher's Least-Significant-Difference Test of effect group.

Group	*Group*	*Difference*	*p Value*	*95% CI of the difference*	
				Lower	*Upper*
5.5mg/mL HA	0.9%NaCl	0.104	0.172	-0.046	0.253
25mg/mL HA	0.9%NaCl	0.224	0.004	0.072	0.376
25mg/mL HA	5.5mg/mL HA	0.120	0.108	-0.027	0.267

CI= Confidence Interval

3.4.2.4 Migration

The kidney samples, in their entirety, neither showed any evidence of inflammatory response, nor traces of filling material.

3.5 DISCUSSION

The range of filling materials available in the market, indiscriminate use and the growth of this plastic modality among health care professionals have significantly increased the demand of aesthetic corrective services, bringing to light the adverse reactions which can damage facial aesthetics and even harm the patient's general health [20,21]. Those secondary effects derived from filling materials are frequently identified by dental surgeons because of their practice field.

In this research, 2 different filling materials were used, based on non-animal origin hyaluronic acid which has been recently available in the market. In lower concentrations, the product is used to reduce superficial and expression wrinkles, while in higher concentrations, it is indicated for deeper wrinkles and tissue volume increase[22,23]. Concerning histological aspects HA is presented as a basophilic material, irregular in shape and size[24-26].

The region of choice for injecting materials was the ventral tongue which is anatomically more protected and, consequently, less vulnerable to local trauma factors. The latter could generate a secondary inflammatory response since rats are rodents and would be placed in groups in the same cage, which could put the results obtained in this research at risk.

Inflammation signs clinically detected in the place of material injection were: volume increase (1 in the 25mg/mL HA group at 60 days and the other in 5.5 HA mg/mL group at 90 days), white plaques (2 occurrences in the 90-day period, one in each HA group) and ulcerations (1 case in 5.5mg/mL HA group and 2 in 25mg/mL HA group, both in a 1-week period). The volume increase observed in the physical examination was detected through digital inspection in the injected area, displaying a firm zone through palpation. This could be related to some functional trauma. White plaques may have been developed due to an increase in local volume, and even because of the presence of edema in the first week, favoring injury in the area, culminating with the thickening of the keratin layer. It is possible to suppose that the ulcerations noticed may be related to the material application technique. Studies on HA in humans[27-30], have mentioned that frequent complaints involve edema and erythema on the first 7 days. Those authors have also verified the presence of nodules and local material migration, the latter being explained by the large volume of injected material. Other clinical studies have found the presence of ulceration, nodules and abscess. The occurrence of abscesses has been empirically justified by authors as a possible consequence of intravascular injection of the material[31,32]. HA secondary effects such as dermis erosion, cystic nodules, hypersensitivity reaction and granulomas have already been reported in literature[14,26,33-37]. The substance concentrations (5.5 and 25mg/mL) have shown similar behavior during the experiment: acute inflammation after a 7-day period and chronic inflammation after 60-day and 90-day periods. The inflammatory response pattern varied during late monitoring periods.

These filling materials were inert at times, thus unable to provoke circumjacent inflammatory response, showing compatibility with tissues. Confirming those findings, Fernández-Cossío et al. (2006)[2], in a histological evaluation about filling materials biocompatibility (polyacralamyde and 20mg/mL hyaluronic acid) in rats, have reported the presence of minimal inflammatory infiltrate surrounding HA during 8 months of experiment. Lemperle, Morhen and Charrier (2003)[1] have observed that 1 month following 20mg/mL HA application, there was minimal cell response involving skin tissue. Macrophages and giant cells were diffusely distributed, not showing immune response, which demonstrates material biocompatibility in tissues.

In only 2 cases, the histological evaluation of rat tongue samples showed abscess formation 7 days after application of 25mg/mL HA. Those slides received a score corresponding to severe inflammation also due to infiltrate of neutrophils and eosinophils. Clinical cases of abscess after filling with HA have already been reported[38,39]. Nonetheless those cases are considered rare and can be associated to impurity resulting from the product manufacturing process[23,39]. Despite the fact that other authors[31,40] relate this fact to the material injection technique, in the current research due attention was paid to method standardization and sterility control by the procedure with the use of disposable and sterilized material.

Yoon et al (2003)[41] compared the response in mice of 20mg/mL HA (Restylane®) exclusive luse – control group, with human dermal fibroblasts culture associated to Restylane® - test group. It was noticed, in histological analysis, that there was presence of human collagen surrounding the test group while 20mg/mL HA group was incapable of forming collagen matrix during 16 weeks after the experiment. In our research, the histological evaluation showed that surrounding the HA implant there was collagen fiber

formation, which was larger in groups where the material concentration was 25mg/mL. This can explain why the volume in dermis remains in the injected area, even with the substance resorption. Christensen (2007)[42], in a review on adverse reactions to filling materials, observed that hydrophilic gels, such as HA, are in constant exchange of water molecules with their vicinity and, consequently, there is a minimal fibrous response. However, fibroplasia can increase, if associated to a granuloma, where exogenous material remains encapsulated, with the presence of foreign body giant cells, macrophages, eosinophils and neutrophils[43].

Systemic migration of filling materials is still a highly controversial topic in scientific literature. A study with mice[20] observed the presence of hepatic and renal inflammatory infiltrates in animals submitted to the injection of different filling materials in the ears. Four types of substance were used, among them 20mg/mL hyaluronic acid. Such alterations were interpreted as a result of drug systematization, which could occasionally behave as chemotactic substances, acting in long distance affecting some metabolism or excretion organ. In our study, the totality of rat kidney samples showed absence of inflammatory response and there was no trace of HA derivatives.

Both concentrations of hyaluronic acid proved to be tolerable by tissues, suggesting that these materials present biocompatibility characteristics. New investigations are needed in order to clarify the etiology of possible reactions caused by this substance, and also to illustrate its biostability in a longer experiment period, as well as its efficiency and safety, once this product has been applied regularly in oral region, recognized area of expertise of dental professionals.

3.6 REFERENCES

1. Lemperle G, Morhenn V, Charrier U. Human histology and persistence of various injectable filler substances for soft tissue augmentation. Aesthetic Plast Surg 2003;27(5):354-66.
2. Fernández-Cossío S, Castaño-Oreja MT. Biocompatibility of two novel dermal fillers: histological evaluation of implants of a hyaluronic acid filler and a polyacrylamide filler. Plast Reconstr Surg 2006;117(6):1789-96.
3. Dastoor SF, Misch CE, Wang HL. Dermal fillers for facial soft tissue augmentation. J Oral Implantol 2007;33(4):191-204.
4. Vargas KF, Borghetti RL, Moure SP, Salum FG, Cherubini K, Figueiredo MAZ. Use of polymethylmethacrylate as permanent filling agent in the jaw, mouth and face regions - Implications on dental practice. Gerodontology 2010. In Press.
5. Requena L, Requena C, Christensen L, Zimmermann US, Kutzner H, Cerroni L. Adverse reactions to injectable soft tissue fillers. J Am Acad Dermatol 2011;64(1): 1-34.
6. Allemann IB, Baumann L. Hyaluronic acid gel (Juvéderm) preparations in the treatment of facial wrinkles and folds. Clin Interv in Aging 2008; 3(4):629–34.
7. Murray CA, Zloty D, Warshawski L. The evolution of soft tissue fillers in clinical practice. Dermatol Clin 2005; 23(2): 343-63.
8. John HE, Price RD. Perspectives in the selection of hyaluronic acid fillers for facial wrinkles and aging skin. Patient Prefer Adherence 2009; 3:225-30.
9. Vargas AF, de Amorim NG, Pitanguy I. Complicações tardias dos preechimentos permanentes. Rev Bras Cir Plást 2009; 24(1):71-81.

10. Manna F, Dentini M, Desideri P, De PO, Mortilla E, Maras B. Comparative chemical evaluation of two commercially available derivatives of hyaluronic acid (hylaform from rooster combs and restylane from streptococcus) used for soft tissue augmentation. J Eur Acad Dermatol Venereol 1999;13(3):183-92.
11. Kogan G, Soltés L, Stern R, Gemeiner P. Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications. Biotechnol Lett 2007;29(1):17-25.
12. Lemperle G, Rullan PP, Gauthier-Hazan N. Avoiding and treating dermal filler complications. Plast Reconstr Surg 2006;118(Suppl 3): 92S-107S.
13. Nestor MS, Ablon GR, Stillman MA. The use of a contact cooling device to reduce pain and ecchymosis associated with dermal filler injections. J Clin Aesthet Dermatol 2010;3(3):29-34.
14. Patel VJ, Bruck MC, Katz BE. Hypersensitivity reaction to hyaluronic acid with negative skin testing. Plast Reconstr Surg 2006;117(6):92e-4e.
15. Cohen JL. Understanding, avoiding, and managing dermal filler complications. Dermatol Surg 2008;34 (Suppl 1):S92-9.
16. Zielke H, Wolber L, Wiest L, Rzany B. Risk profiles of different injectable fillers: results from the Injectable Filler Safety Study (IFS Study). Dermatol Surg 2008;34(3):326-35.
17. Vier-Pelisser FV, Figueiredo MA, Cherubini K, Braga Filho A, Figueiredo JA. The effect of head-fractioned teletherapy on pulp tissue. Int Endod J 2007;40(11):859-65.
18. Figueiredo JA, Pesce HF, Gioso MA, Figueiredo MA. The histological effects of four endodontic sealers implanted in the oral mucosa: submucous injection versus implant in polyethylene tubes. Int Endod J 2001;34(5):377-85.

19. Gomes MS, Barletta FB, Della Bona A, Vanni JR, Pereira Cda C, Figueiredo JA. Microbial leakage and apical inflammatory response in dog's teeth after root canal filling with different sealers, post space preparation and exposure to the oral environment. J Appl Oral Sci 2007; 15(5): 429-36.

20. Rosa SC, de Macedo JLS. Reações adversas a substâncias de preenchimento subcutâneo. Rev Soc Bras Cir Plást 2005; 20(4):248-52.

21. De Castro ACB, Collares MVM, Portinho CP, Dias PC, Pinto RA. Necrose facial extensa após infiltração com polimetilmetacrilato. Rev Bras Otorrinolaringol 2007;73(6):850.

22. Burgess CM. Principles of soft tissue augmentation for the aging face. Clin Interv Aging 2006;1(4):349-55.

23. Romagnoli M, Belmontesi M. Hyaluronic acid-based fillers: theory and practice. Clin Dermatol 2008;26(2):123-59.

24. Zimmermann US, Clerici TJ. The histological aspects of fillers complications. Semin Cutan Med Surg 2004;23(4):241-50.

25. Parada MB, Michalany NS, Hassun KM, Bagatin E, Talarico S. A Histologic Study of Adverse Effects of Different Cosmetic Skin Fillers. SKINmed 2005;4(6):345-9.

26. Ghislanzoni M, Bianchi F, Barbareschi M, Alessi E. Cutaneous granulomatous reaction to injectable hyaluronic acid gel. Br J Dermatol 2006;154(4):755-8.

27. Narins RS, Brandt F, Leyden J, Lorenc ZP, Rubin M, Smith S. A randomized, double-blind, multicenter comparison of the efficacy and tolerability of Restylane versus Zyplast for the correction of nasolabial folds. Dermatol Surg 2003; 29(6):588-95.

28. DeLorenzi C, Weinberg M, Solish N, Swift A. Multicenter study of the efficacy and safety of subcutaneous non-animal-stabilized hyaluronic acid in aesthetic facial contouring: interim report. Dermatol Surg 2006;32(2):205-11.
29. DeLorenzi C, Weinberg M, Solish N, Swift A. The long-term efficacy and safety of a subcutaneously injected large-particle stabilized hyaluronic acid-based gel of nonanimal origin in esthetic facial contouring. Dermatol Surg 2009;35(Suppl 1):313-21.
30. Levy PM, De Boulle K, Raspaldo H. Comparison of injection comfort of a new category of cohesive hyaluronic acid filler with preincorporated lidocaine and a hyaluronic acid filler alone. Dermatol Surg 2009; 35(Suppl 1):332-6.
31. Resende VCL, Haddad A, Regazzini DV, Ferreira LM. Avaliação tardia de 1366 casos de preenchimento dérmico com implante de ácido hialurônico em face. Rev Bras Cir Plást 2008;23(Suppl 3):34.
32. Bachmann F, Erdmann R, Hartmann V, Wiest L, Rzany B. The spectrum of adverse reactions after treatment with injectable fillers in the glabellar region: results from the Injectable Filler Safety Study. Dermatol Surg 2009; 35 (Suppl 2):1629-34.
33. Lupton JR, Alster TS. Cutaneous hypersensitivity reaction to injectable hyaluronic acid gel. Dermatol Surg 2000; 26(2):135-7.
34. Friedman PM, Mafong EA, Kauvar AN, Geronemus RG. Safety data of injectable nonanimal stabilized hyaluronic acid gel for soft tissue augmentation. Dermatol Surg 2002; 28(6):491-4.
35. Edwards PC, Fantasia JE, Iovino R. Foreign body reaction to hyaluronic acid (Restylane): an adverse outcome of lip augmentation. J Oral Maxillofac Surg 2006; 64(8):1296-9.

36. Cox SE. Clinical experience with filler complications. Dermatol Surg 2009; 35(Suppl 2):1661-6.

37. Grunebaum LD, Allemann IB, Dayan S, Mandy S, Baumann L. The risk of alar necrosis associated with dermal filler injection. Dermatol Surg 2009;35 (Suppl 2):1635-40.

38. Toy BR, Frank PJ. Outbreak of Mycobacterium abscessus infection after soft tissue augmentation. Dermatol Surg 2003;29(9):971-3.

39. Rousso JJ, Pitman MJ. Enterococcus faecalis complicating dermal filler injection: a case of virulent facial abscesses. Dermatol Surg 2010; 36(10):1638-41.

40. Lowe NJ, Maxwell CA, Lowe P, Duick MG, Shah K. Hyaluronic acid skin fillers: adverse reactions and skin testing. J Am Acad Dermatol 2001; 45(6):930-3.

41. Yoon ES, Han SK, Kim WK. Advantages of the presence of living dermal fibroblasts within restylane for soft tissue augmentation. Ann Plast Surg 2003; 51(6):587-92.

42. Christensen L. Normal and pathologic tissue reactions to soft tissue gel fillers. Dermatol Surg 2007; 33(Suppl 2):S168-75.

43. Dadzie OE, Mahalingam M, Parada M, El HT, Philips T, Bhawan J. Adverse cutaneous reactions to soft tissue fillers-a review of the histological features. J Cutan Pathol 2008; 35(6):536-48.

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4 DISCUSSÃO GERAL

O envelhecimento cutâneo é considerado um desafio para a medicina, especialmente quando cirurgiões plásticos e dermatologistas são instigados a solucionar problemas de preenchimento de rugas e sulcos mais profundos (ROSA, DE MACEDO, 2007). A busca constante do homem por alternativas que auxiliem na recuperação ou manutenção da eterna juventude tem sido, ao longo da história, alvo de grande interesse para diversos estudos.

O ácido hialurônico (AH) é um dos materiais reabsorvíveis mais utilizados na atualidade, sendo injetado com mais frequência na derme, e, eventualmente, em um plano tecidual mais profundo. Embora seja classificado como não imunogênico (GHISLANZONI et al., 2006) esta substância pode causar reações adversas, as quais são muitas vezes encontradas por cirurgiões-dentistas, devido a sua área de atuação. Um aspecto importante que impulsionou este estudo experimental foi o diagnóstico diferencial das lesões granulomatosas causadas por estes produtos, com patologias originadas do tecido adiposo (lipossarcoma) e das glândulas salivares (lesões císticas e neoplásicas malignas e benignas). Sabe-se que a aplicação destes materiais nos lábios e sulcos nasogenianos, é feita em área anatômica próxima das glândulas salivares acessórias. Assim, quando ocorre uma reação adversa de corpo estranho, pode originar uma lesão que clinicamente confunde-se com uma patologia desta natureza. Soma-se a isto, a dificuldade de se obter informações importantes durante a anamnese, pois muitas vezes o paciente omite este tipo de procedimento, ou, quando o relata, desconhece o nome do produto que foi utilizado no seu tratamento.

A grande demanda por procedimentos corretivos estéticos, como a bioplastia, proporcionou um rápido avanço no desenvolvimento de materiais de preenchimento, comprometendo, em alguns casos, a qualidade no que concerne a eficácia e a segurança dos

produtos utilizados. O número de pesquisas realizadas na atualidade, não acompanha a crescente disponibilização de novos produtos no mercado, necessitando-se, assim, de constantes investigações na busca de esclarecimentos que permitam aos profissionais e seus pacientes, utilizarem com segurança este tipo de terapia.

Através deste experimento, procurou-se avaliar as respostas clínicas e histológicas do AH, relacionado em 2008 por Romagnoli e Belmontesi, como o material de implante cutâneo que mais contempla características ideais, como segurança e efetividade, tempo aceitável de permanência nos tecidos, fácil aplicação e ser quimicamente inerte. Foram aplicadas concentrações extremas deste material (5,5mg/mL e 25mg/mL), buscando verificar e comparar possíveis variações no grau de resposta inflamatória tecidual a partir de distintos empregos do mesmo produto.

Rosa, em 2001, utilizou animais de laboratório em experimento através de implante subcutâneo de AH em orelha de camundongos. Em nosso estudo o ventre de língua foi o local eleito para injeção da substância, pelo fato dessa região estar anatomicamente mais protegida de traumas, visto que são animais roedores que ficariam alojados na mesma gaiola. Outra área mais externa, como o filtro, seria uma opção com maior facilidade de acesso, contudo mais vulnerável a traumatismo (como mordidas) e, por consequência, a desenvolver processo inflamatório. Uma vez que se pretendia verificar a presença ou ausência de resposta inflamatória, sem que houvesse qualquer interferência, optou-se pelo ventre lingual, evitando-se, assim, o comprometimento da fidelidade dos resultados. Além disso, a área do filtro dificultaria a avaliação histopatológica pela exuberante presença de anexos cutâneos (como por exemplo, os folículos pilosos).

Na presente pesquisa, as alterações clínicas ocorreram exclusivamente nos grupos que receberam AH, sendo observadas em todos os tempos experimentais. Aos 7 dias, notou-se a

presença de ulcerações, provavelmente justificadas pelo ato de injetar o material. Na avaliação clínica dos 60 e 90 dias (AH 5,5mg/mL), visualizou-se um aumento de volume local e, podendo o mesma estar associado a algum tipo de traumatismo. Foram encontradas também placas esbranquiçadas nos animais pertencentes aos grupos 1 e 2 aos 90 dias, as quais podem ter se desenvolvido em consequência de um aumento de volume regional, até mesmo o edema, presente na primeira semana, favorecendo, por consequência, a injúria local e, assim, um possível espessamento da camada de ceratina.

Clinicamente, as complicações relativas ao AH incluem eritema, equimose, nódulo, aumento de volume, ulceração e formação de abscesso, os quais podem se desenvolver em alguns dias ou até 1 ano após a injeção (ALIJOTAS-REIG, GARCIA-GIMENEZ, 2008; DADZIE et al., 2008; BACHMANN et al., 2009; SAGE CHAFFINS, KOUBA, 2009). Em estudo clínico prospectivo com 72 pacientes, Lowe e Grover (2006) mencionaram que as alterações observadas no local da injeção de AH 20mg/mL, em 64 semanas de acompanhamento, foram hematoma (n=3) e inchaço (n=4) num período imediato (3 dias) e migração e aumento de volume (n=1) em 8 semanas de acompanhamento. Em relato de uma série de casos de preenchimento com AH 20mg/mL, Cox em 2009, referiu a presença de erosão na derme após 3 horas da aplicação, evoluindo para necrose tecidual. Esta complicação é considerada rara e pode estar associada à injeção intravascular da substância. Pelo que foi observado em nosso estudo, a técnica para aplicar o material exerceu marcada influência sobre as reações indesejáveis, sendo que estas, podem ocorrer, também, devido a variação individual, bem como pela contaminação do material no seu processo de fabricação. Outro efeito adverso descrito por Cox (2009) foi um aumento de volume periorbital após 2 anos do procedimento, que, segundo o autor, ocorreu devido a aplicação superficial ou deslocamento da substância. Quadros de reação granulomatosa associados a abscessos, são reportados por

alguns autores (HÖNIG, BRINK, KORABIOWSKA, 2003; SAGE, CHAFFINS, KOUBA, 2009). Discute-se que os materiais degradáveis são posteriormente metabolizados, podendo levar a respostas de natureza inflamatória, bem como originar uma reação de corpo estranho ou resposta imunológica.

Reações de granuloma de corpo estranho estão comumente associadas ao uso dos materiais de preenchimento permanente, como o polimetilmetacrilato (PMMA), pois esta substância facilita o encapsulamento pelos macrófagos, já que se manifesta, ao exame histológico, como microesferas. Estas são percebidas clinicamente sob a forma de nódulos ou pápulas e podem mimetizar outras enfermidades, especialmente as patologias de glândulas salivares (DA COSTA et al., 2009). Este fato reforça a importância do cirurgião-dentista executar, invariavelmente, todos os passos da anamnese e exame físico, incluindo a palpação da área, favorecendo desta forma a o estabelecimento do diagnóstico conclusivo.

O AH apresenta-se microscopicamente como uma substância amorfa e basofílica de tamanho variável, distribuído na intimidade tecidual. Neste experimento, observou-se que aos 7 dias, todo o grupo teste 1 (AH 5,5mg/mL) apresentou resposta inflamatória moderada, ou seja, infiltrado de células mononucleares com esparsos polimorfonucleares neutrófilos e eosinófilos. Já o grupo teste 2 (AH 25mg/mL) variou de uma resposta moderada a severa, com presença de infiltrado de polimorfonucleares neutrófilos e eosinófilos. Rosa (2001) encontrou, neste mesmo período, o AH 20mg/mL circundado basicamente por neutrófilos, eosinófilos e escassas células mononucleares. Fernández-Cossío e Castaño-Oreja (2006) observaram um padrão de resposta inflamatória com infiltrado de leucócitos polimorfonucleares, após 1 semana do implante de AH 20mg/mL em ratos. No presente estudo aos 60 dias, no grupo com a menor concentração de AH, houve um predomínio de inflamação leve a ausente e no grupo teste 2, detectou-se um grau moderado de inflamação.

Contrapondo-se a estes resultados, Rosa (2001) constatou ainda uma intensa eosinofilia, interpretada como reação de hipersensibilidade. Lemperle, Morhenn e Charrier (2003), em biópsias humanas, destacaram pequena reação de corpo estranho adjacente ao AH, formada por macrófagos e células gigantes multinucleadas.

A fibroplasia presente foi determinada e quantificada através da técnica do picrosírius, coloração específica para fibras colágenas. O colágeno é uma proteína essencialmente importante que compõe a estrutura da derme, sendo responsável por fornecer suporte e resistência à pele (BAUMANN, KAUFMAN, SAGHARI, 2006). Através da análise utilizando a coloração picrosírius, luz polarizada e um *software* de análise de imagem, pode-se identificar e relacionar a proporção de colágeno presente, de acordo com o tipo de material utilizado. A análise das amostras demonstrou que o percentual de fibras colágenas neoformadas em áreas adjacentes aos materiais de preenchimento e no local de aplicação dos mesmos, aumentou gradativamente acompanhando o respectivo aumento da concentração do AH. Este é um efeito favorável, pois o mesmo não ocorreu por encapsulamento da substância, mas por neoformação de colágeno. O que se espera deste biomaterial é que promova a formação de um tecido novo, funcional e não patológico, uma vez que o mesmo é reabsorvido.

Fernández-Cossío e Castaño-Oreja (2006), observaram em ratos, a formação de uma cápsula uniforme, circunjacente ao AH 20mg/mL, constituída por uma fina camada de fibroblastos entre as fibras colágenas, num período de 1 mês. Aos 8 meses, essa cápsula fibrosa permanecia da mesma espessura, com mínimo infiltrado inflamatório ao redor do implante. Também relataram que uma eosinofilia foi observada neste último tempo, atribuída a condensação do produto. É descrito na literatura que, ao exame histológico, o AH apresenta uma constante resposta inflamatória, variando de um infiltrado crônico leve com presença ou

ausência de reação a corpo estranho, a um padrão de resposta inflamatória moderado a denso, formado por linfócitos, plasmócitos, macrófagos e fibrose (MICHEELS, 2001; ZIMMERMANN, CLERICI, 2004).

Quanto a migração do material, esta é definida por Lemperle et al. (2004) de acordo com 3 mecanismos: vias hematogênicas e linfáticas, se a injeção do material promover injúria a algum vaso de maior calibre, podendo atingir os capilares pulmonares e mais especificamente na via linfática, os linfonodos regionais. Finalmente, a via por fagocitose, através da ação dos macrófagos, que podem migrar posteriormente para outros sítios anatômicos. O processo de migração de materiais de preenchimento permanece um tema bastante controvertido. A maioria dos trabalhos relata a migração à distância, sem especificar a precisa localização da mesma. Rosa (2001) observou a presença de infiltrado inflamatório hepático e renal nos animais submetidos à injeção de materiais de preenchimento em suas orelhas. Utilizou neste experimento 4 tipos de substâncias, dentre elas o ácido hialurônico a 20mg/mL. Tais alterações foram interpretadas como decorrência da sistematização das drogas, que eventualmente poderiam atuar como uma substância quimiotática, agindo à distância em algum órgão de metabolismo e excreção. Nos períodos de experimento considerados em nossa pesquisa não se constatou presença de reação inflamatória em100% das amostras renais avaliadas. Este fato pode ser justificado pelo AH ser uma substância difícil de ser fagocitada pelos macrófagos, uma vez que, ao exame histológico, mostra-se distribuído em blocos e não em partículas menores, como o PMMA.

Diversos autores descrevem a biocompatibilidade e a efetividade do AH no preenchimento de sulcos, rugas e aumento de volume tecidual (BAUMANN et al., 2007; BEASLEY, WEISS, WEISS, 2010). Embora esta substância apresente uma baixa incidência de insucessos no tratamento, o paciente sempre deverá ser informado sobre eles, pois, ainda

que sua permanência nos tecidos seja temporária, uma eventual complicação pode desencadear um efeito permanente sobre a pele. Materiais que eventualmente possam desencadear efeitos indesejáveis, não podem ser considerados isentos de risco, portanto, o paciente deve estar ciente das potenciais complicações que podem advir do seu uso.

Constantes variações nos derivados do ácido hialurônico vêm sendo introduzidas no mercado. A variabilidade no tamanho das partículas e na viscosidade do gel podem não mudar apenas a longevidade e indicação do produto, mas também criar novos efeitos, como por exemplo, a instabilidade da substância nos tecidos. Nesse sentido, novos estudos longitudinais, padronizados, com maior tempo de acompanhamento, se fazem necessários buscando uma melhor compreensão dos efeitos provocados por esse material e aprofundar os conhecimentos relacionados à sua biocompatibilidade.

REFERÊNCIAS

ALIJOTAS-REIG, J. Recurrent urticarial vasculitis related to nonanimal hyaluronic acid skin filler injection. **Dermatologic Surgery**, New York, v. 35, p. 395-397, 2009. Suplemento 1.

ALIJOTAS-REIG, J.; GARCIA-GIMENEZ V. Delayed immune-mediated adverse effects related to hyaluronic acid and acrylic hydrogel dermal fillers: clinical findings, longterm follow-up and review of the literature. **Journal of the European Academy of Dermatology and Venereology**, Amsterdam, v. 22, n. 2, p. 150-161, 2008.

ALLEMANN, I. B.; BAUMANN, L. Hyaluronic acid gel (Juvéderm) preparations in the treatment of facial wrinkles and folds. **Clinical Interventions in Aging**, Auckland, v. 3, n. 4, p. 629-634, 2008.

ACHILLES, R. Imunologia e biocompatibilidade. In: ROCA, (Ed.). **Tratado de medicina estética**. São Paulo: Roca, 2004. p. 1215-1223.

ANDERSON, J. M.; RODRIGUEZ, A.; CHANG, D. T. Foreign body reaction to biomaterials. **Seminars in Immunology**, London, v. 20, n. 2, p. 86-100, 2008.

ANWAR, M. U.; SHARPE, D. T. Skin nodules after semipermanent cosmetic dermal filler. **Aesthetic Plastic Surgery**, New York, v. 31, n. 4, p. 401-402, 2007.

ATIYEH, B. S.; DIBO, S. A. Nonsurgical nonablative treatment of aging skin: radiofrequency technologies between aggressive marketing and evidence-based efficacy. **Aesthetic Plastic Surgery**, New York, v. 33, n. 3, p. 283-294, 2009.

BACHMANN, F. et al. The spectrum of adverse reactions after treatment with injectable fillers in the glabellar region: results from the Injectable Filler Safety Study. **Dermatologic Surgery**, New York, v. 35, p. 1629-1634, 2009. Suplemento 2.

BARDAZZI, F. et al. Cutaneous granulomatous reaction to injectable hyaluronic acid gel: another case. **Journal of Dermatological Treatment**, London, v. 18, n. 1, p. 59-62, 2007.

BAUMANN, L. et al. Comparison of smooth-gel hyaluronic acid dermal filler with cross-linked bovine collagen: a multicenter, double-masked, randomized, within-subject study. **Dermatologic Surgery**, New York, v. 33, p. S128-S135, 2007. Suplemento 2.

BAUMANN, L.; KAUFMAN, J.; SAGHARI, S. Collagen Fillers. **Dermatologic Therapy** Copenhagen, v. 19, n. 3, p. 134-140, 2006.

BEASLEY, K. L.; WEISS, M. A.; WEISS, R. A. Soft tissue augmentation using a two-way connector to supplement hyaluronic acid filler with 1% lidocaine hydrochloric acid with epinephrine 1:100,000: our experience and observations. **Dermatologic Surgery**, New York, v. 36, n. 4, p. 524-526, 2010.

BELLMAN, B. Complication following suspected intra-arterial injection of Restylane. **Aesthetic Surgery Journal**, St. Louis, MO, v. 26, n. 3, p. 304-305, 2006.

BJARNSHOLT, T. et al. Detection of bacteria by fluorescence in situ hybridization in culture-negative soft tissue filler lesions. **Dermatologic Surgery**, New York, v. 35, p. 1620-1624, 2009. Suplemento 2.

BRANDT, F. S.; CAZZANIGA, A. Hyaluronic acid fillers: restylane and Perlane. **Facial Plastic Surgery Clinics of North America**, Philadelphia, v. 15, n. 1, p. 63-76, 2007.

BRANDT, F. S.; CAZZANIGA, A. Hyaluronic acid gel fillers in the management of facial aging. **Clinical Interventions in Aging**, Auckland, v. 3, n. 1, p. 153-159, 2008.

BURGESS, C. M. Principles of soft tissue augmentation for the aging face. **Clinical Interventions in Aging**, Auckland, v. 1, n. 4, p. 349-355, 2006.

CARRUTHERS, J. et al. Volumizing with a 20-mg/ml smooth, cohesive, viscous hyaluronic acid filler and its role in facial rejuvenation therapy. **Dermatologic Surgery**, New York, v. 36, p. 1886-1892, 2010. Suplemento 3.

CARRUTHERS, J.; CARRUTHERS, A. A prospective, randomized, parallel group study analyzing the effect of BTX-A (Botox) and nonanimal sourced hyaluronic acid (NASHA, Restylane) in combination compared with NASHA (Restylane) alone in severe glabellar rhytides in adult female subjects: treatment of severe glabellar rhytides with a hyaluronic acid derivative compared with the derivative and BTX-A. **Dermatologic Surgery**, New York, v. 29, n. 8, p. 802-809, 2003.

CHRISTENSEN, L. Normal and pathologic tissue reactions to soft tissue gel fillers. **Dermatologic Surgery**, New York, v. 33, p. S168-S175, 2007. Suplemento 2.

COHEN, J. L. Understanding, avoiding, and managing dermal filler complications. **Dermatologic Surgery**, New York, v. 34, p. S92-S99, 2008. Suplemento 1.

COX, S. E. Clinical experience with filler complications. **Dermatologic Surgery**, New York, v. 35, p. 1661-1666, 2009. Suplemento 2.

DA COSTA, MC et al. Oral foreign body granuloma: unusual presentation of a rare adverse reaction to permanent injectable cosmetic filler. **Journal of Oral and Maxillofacial Surgery**, Philadelphia, v. 38, n. 4, p. 385-387, 2009.

DADZIE, O.E. et al. Adverse cutaneous reactions to soft tissue fillers-a review of the histological features. **Journal of Cutaneous Pathology**, Copenhagen, v. 35, n. 6, p. 536-548, 2008.

DASTOOR, S. F.; MISCH, C. E.; WANG, H. L. Dermal fillers for facial soft tissue augmentation. **Journal of Oral Implantology**, Lawrence, v. 33, n. 4, p. 191-204, 2007.

DE CASTRO, A. C. B. et al. Necrose facial extensa após infiltração com polimetilmetacrilato. **Revista Brasileira de Otorrinolaringologia**, Porto Alegre, v. 73, n. 6, p. 850, 2007.

DELORENZI, C. et al. Multicenter study of the efficacy and safety of subcutaneous non-animal-stabilized hyaluronic acid in aesthetic facial contouring: interim report. **Dermatologic Surgery**, New York, v. 32, n. 2, p. 205-211, 2006.

DELORENZI, C. et al. The long-term efficacy and safety of a subcutaneously injected large-particle stabilized hyaluronic acid-based gel of nonanimal origin in esthetic facial contouring. **Dermatologic Surgery**, New York, v. 35, p. 313-321, 2009. Suplemento 1.

DE MAIO, M. The minimal approach: an innovation in facial cosmetic procedures. **Aesthetic Plastic Surgery**, New York, v. 28, n. 5, p. 295-300, 2004.

DE MAIO, M.; RZANY, B. **Substâncias de preenchimento em medicina estética**. São Paulo: Livraria Santos, 2007.

EDWARDS, P. C.; FANTASIA, J. E. Review of long-term adverse effects associated with the use of chemically-modified animal and nonanimal source hyaluronic acid dermal fillers. **Clinical Interventions in Aging**, Auckland, v. 2, n. 4, p. 509-519, 2007.

EDWARDS, P. C.; FANTASIA, J. E.; IOVINO, R. Foreign body reaction to hyaluronic acid (Restylane): an adverse outcome of lip augmentation. **Journal of Oral and Maxillofacial Surgery**, Philadelphia, v. 64, n. 8, p. 1296-1299, 2006.

EPPLEY, B. L.; DADVAND, B. Injectable soft-tissue fillers: clinical overview. **Plastic and Reconstructive Surgery**, Baltimore, v. 118, n. 4, p. 98e-106e, 2006.

FERNÁNDEZ-ACEÑERO, M. J.; ZAMORA, E.; BORBUJO, J. Granulomatous foreign body reaction against hyaluronic acid: report of a case after lip augmentation. **Dermatologic Surgery**, New York, v. 29, n. 12, p. 1225-1226, 2003.

FERNÁNDEZ-COSSÍO, S.; CASTAÑO-OREJA, M. T. Biocompatibility of two novel dermal fillers: histological evaluation of implants of a hyaluronic acid filler and a polyacrylamide filler. **Plastic and Reconstructive Surgery**, Baltimore, v. 117, n. 6, p. 1789-1796, 2006.

FIGUEIREDO, J. A. et al. The histological effects of four endodontic sealers implanted in the oral mucosa: submucous injection versus implant in polyethylene tubes. **International Endodontic Journal**, Oxford, v. 34, n. 5, p. 377-385, 2001.

FISCHER, J.; METZLER, G.; SCHALLER, M. Cosmetic Permanent Fillers for Soft Tissue Augmentation: a new contraindication for interferon therapies. **Archives of Dermatology**, Chicago, n. 143, p. 507-510, 2007.

FRIEDMAN, P. M. et al. Safety data of injectable nonanimal stabilized hyaluronic acid gel for soft tissue augmentation. **Dermatologic Surgery**, New York, v. 28, n. 6, p. 491-494, 2002.

GELFER, A. et al. The natural history of polymethylmethacrylate microspheres granulomas. **Dermatologic Surgery**, New York, v. 33, n. 5, p. 614-620, 2007.

GHISLANZONI, M. et al. Cutaneous granulomatous reaction to injectable hyaluronic acid gel. **British Journal Dermatology**, Oxford, v. 154, n. 4, p. 755-758, 2006.

GLAICH, A. S.; COHEN, J. L.; GOLDBERG, L. H. Injection necrosis of the glabella: protocol for prevention and treatment after use of dermal fillers. **Dermatologic Surgery**, New York, v. 32, n. 2, p. 276-281, 2006.

GOLD, M. H. Use of hyaluronic acid fillers for the treatment of the aging face. **Clinical Interventions in Aging**, Auckland, v. 2, n. 3, p. 369-376, 2007.

GOMES, M. S. et al. Microbial leakage and apical inflammatory response in dog's teeth after root canal filling with different sealers, post space preparation and exposure to the oral environment. **Journal of Applied Oral Science**, Bauru, v. 15, n. 5, p. 429-436, 2007.

GONÇALES, A. A. S. et al. Silicone implant for chin augmentation mimicking a low-grade liposarcoma. **Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics**, St. Louis, MO, v. 107, n. 4, p. e21-e23, 2009.

GROVER, R. Optimizing treatment outcome with Restylane SubQ: the role of patient selection and counseling. **Aesthetic Surgery Journal**, New York, v. 26, p. S18-S21, 2006. Suplemento 1.

GRUNEBAUM, L. D. et al. The risk of alar necrosis associated with dermal filler injection. **Dermatologic Surgery**, New York, v. 35, p. 1635-1640, 2009. Suplemento 2.

HAMILTON, R. G.; STROBOS, J.; ADKINSON JUNIOR, N. F. Immunogenicity studies of cosmetically administered nonanimal-stabilized hyaluronic acid particles. **Dermatologic Surgery**, New York, v. 33, p. S176-S185, 2007. Suplemento 2.

HEDÉN, P. et al. Body shaping and volume restoration: the role of hyaluronic acid. **Aesthetic Plastic Surgery**, New York, v. 33, n. 3, p. 274-282, 2009.

HÖNIG, J. F.; BRINK, U.; KORABIOWSKA, M. Severe granulomatous allergic tissue reaction after hyaluronic acid injection in the treatment of facial lines and its surgical correction. **Journal of Craniofacial Surgery**, Boston, v. 14, n. 2, p. 197-200, 2003.

JHAM, B. C. et al. Granulomatous foreign-body reaction involving oral and perioral tissues after injection of biomaterials: a series of 7 cases and review of the literature. **Journal of Oral and Maxillofacial Surgery**, Philadelphia, v. 67, n. 2, p. 280-285, 2009.

JOHN, H. E.; PRICE, R. D. Perspectives in the selection of hyaluronic acid fillers for facial wrinkles and aging skin. **Patient Preference an Adherence**, Auckland, v. 3, p. 225-230, 2009.

KLEIN, A. W. In search of the perfect lip: 2005. **Dermatologic Surgery**, New York, v. 21, n. 11, pt. 2, p. 1599-1603, 2005.

KOGAN, G. et al. Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications. **Biotechnology Letters**, Kew, v. 29, n. 1, p. 17-25, 2007.

LEMPERLE G, MORHENN V, CHARRIER U. Human histology and persistence of various injectable filler substances for soft tissue augmentation. **Aesthetic Plastic Surgery**, New York, v. 27, n. 5, p. 354-366, 2003.

LEMPERLE, G.; RULLAN, P. P.; GAUTHIER-HAZAN, N. Avoiding and treating dermal filler complications. **Plastic and Reconstructive Surgery**, Baltimore, v. 118, p. 92S-107S, 2006. Suplemento 3.

LEMPERLE, G. et al. Migration studies and histology of injectable microspheres of different sizes in mice. **Plastic and Reconstructive Surgery**, Baltimore, v. 113, n. 5, p. 1380-1390, 2004.

LEMPERLE, G.; ROMANO, J. J.; BUSSO, M. Soft tissue augmentation with Artecoll: 10-year history, indications, techniques, and complications. **Dermatologic Surgery**, New York, v. 29, n. 6, p. 573-587, 2003.

LEVY, P. M.; DE BOULLE, K; RASPALDO, H. Comparison of injection comfort of a new category of cohesive hyaluronic acid filler with preincorporated lidocaine and a hyaluronic acid filler alone. **Dermatologic Surgery**, New York, v. 35, p. 332-336, 2009. Suplemento 1.

LOMBARDI, T. et al. Orofacial granulomas after injection of cosmetic fillers. Histopathologic and clinical study of 11 cases. **Journal of Oral Pathology and Medicine**, Copenhagen, v. 33, n. 2, p. 115-120, 2004.

LOWE, N. J. et al. Hyaluronic acid skin fillers: adverse reactions and skin testing. **Journal of the American Academy of Dermatology**, St. Louis, Mo, v. 45, n. 6, p. 930-933, 2001.

LOWE, N. J.; GROVER, R. Injectable hyaluronic acid implant for malar and mental enhancement. **Dermatologic Surgery**, New York, v. 32, n. 7, p. 881-885, 2006.

LUPO, M. P. Hyaluronic acid fillers in facial rejuvenation. **Seminars in Cutaneous Medicine and Surgery**, Philadelphia, v. 25, n. 3, p. 122-126, 2006.

LUPTON, J. R.; ALSTER, T. S. Cutaneous hypersensitivity reaction to injectable hyaluronic acid gel. **Dermatologic Surgery**, New York, v. 26, n. 2, p. 135-137, 2000.

MALY, A. et al. Tissue reaction to liquid silicone simulating low-grade liposarcoma following lip augmentation. **Journal of Oral Pathology and Medicine**, Copenhagen, v. 33, n. 5, p. 314, 2004.

MANNA, F. et al. Comparative chemical evaluation of two commercially available derivatives of hyaluronic acid (Hylaform from rooster combs and Restylane from streptococcus) used for soft tissue augmentation. **Journal of the European Academy Dermatology and Venereology**, Amsterdam, v. 13, n. 3, p. 183-192, 1999.

MATARASSO, S. L. Understanding and using hyaluronic acid. **Aesthetic Surgery Journal**, New York, v. 24, n. 4, p. 361-364, 2004.

MATARASSO, S. L.; CARRUTHERS, J. D.; JEWELL, M. L. Consensus recommendations for soft-tissue augmentation with nonanimal stabilized hyaluronic acid (Restylane). **Plastic and Reconstructive Surgery**, Baltimore, v. 117, p. 3S-34S, 2006. Suplemento 3.

MCDONALD, J.; HASCALL, V. C. Hyaluronan minireview series. **Journal of Biological Chemistry**, Baltimore, v. 227, n. 7, p. 4575-4579, 2002.

MICHEELS, P. Human anti-hyaluronic acid antibodies: is it possible? **Dermatologic Surgery**, New York, v. 27, n. 2, p. 185-191, 2001.

MOERS-CARPI, M. et al. A Multicenter, Randomized Trial Comparing Calcium Hydroxylapatite to Two Hyaluronic Acids for Treatment of Nasolabial Folds. **Dermatologic Surgery**, v. 33, p. S144-S151, 2007. Suplemento 2.

MONHEIT, G. D.; ROHRICH, R. J. The nature of long-term fillers and the risk of complications. **Dermatologic Surgery**, New York, v. 35, p. 1598-1604, 2009. Suplemento 2.

MURRAY, C. A.; ZLOTY, D.; WARSHAWSKI, L. The evolution of soft tissue fillers in clinical practice. **Dermatologic clinics**, Philadelphia, v. 23, n. 2, p. 343-363, 2005.

NARINS, R. S. et al. A randomized, double-blind, multicenter comparison of the efficacy and tolerability of Restylane versus Zyplast for the correction of nasolabial folds. **Dermatologic Surgery**, New York, v. 29, n. 6, p. 588-595, 2003.

NESTOR, M. S.; ABLON, G. R.; STILLMAN, M. A. The use of a contact cooling device to reduce pain and ecchymosis associated with dermal filler injections. **Journal of Clinical Aesthetic Dermatology**, Edgemont, v. 3, n. 3, p. 29-34, 2010.

NIAMTU, J III. The use of restylane in cosmetic facial surgery. **Journal of Oral and Maxillofacial Surgery**, Philadelphia, v. 64, n. 2, p. 317-325, 2006.

OKADA, S. et al. Eosinophilic granulomatous reaction after intradermal injection of hyaluronic acid. **Acta Dermato-Venereologica**, Stockholm, v. 88, n. 1, p. 69-70, 2008.

PARADA, M. B. et al. A histologic study of adverse effects of different cosmetic skin fillers. **SKINmed**, Darien, v. 4, n. 6, p. 345-349, 2005.

PATEL, V. J.; BRUCK, M. C.; KATZ, B. E. Hypersensitivity reaction to hyaluronic acid with negative skin testing. **Plastic and Reconstructive Surgery**, Baltimore, v. 117, n. 6, p. 92e-94e, 2006.

PERENACK, J. Treatment Options to Optimize Display of Anterior Dental Esthetics in the Patient With the Aged Lip. **Journal of Oral and Maxillofacial Surgery**, Philadelphia, v. 63, n.11, p. 1634-1641, 2005.

REQUENA, L. et al. Adverse reactions to injectable soft tissue fillers. **Journal of the American Academy of Dermatology**, St. Louis, MO, v. 64, n. 1, p. 1-34, 2011.

RESENDE, V. C. L. et al. Avaliação tardia de 1366 casos de preenchimento dérmico com implante de ácido hialurônico em face. **Revista da Sociedade Brasileira de Cirurgia Plástica**, São Paulo, v. 23, p. 34, 2008. Suplemento 3.

ROHRICH, R. J. et al. Soft-tissue filler complications: the important role of biofilms. **Plastic and Reconstructive Surgery**, Baltimore, v. 125, n. 4, p. 1250-1256, 2010.

ROMAGNOLI, M; BELMONTESI, M. Hyaluronic acid-based fillers: theory and practice. **Clinics in dermatology**, New York, v. 26, n. 2, p. 123-159, 2008.

ROSA, S. C. **Estudo Experimental da Reação Tissular Frente a Substâncias Remodeladoras Cutâneas Utilizadas em Cirurgia Plástica Estética e Reparadora**. 2001. 129 f. Dissertação (Mestrado em Medicina) — Universidade de Brasília, Brasília, 2001.

ROSA, S. C.; DE MACEDO, J. L. S. Materiais injetáveis para aumento de partes moles. **Revista da Sociedade Brasileira de Cirurgia Plástica**, São Paulo, v. 22, n. 2, p. 116-121, 2007.

ROSA, S. C.; DE MACEDO, J. L. S. Reações adversas a substâncias de preenchimento subcutâneo. **Revista da Sociedade Brasileira de Cirurgia Plástica**, São Paulo, v. 20, n. 4, p. 248-252, 2005.

ROUSSO, J. J.; PITMAN, M. J. Enterococcus faecalis complicating dermal filler injection: a case of virulent facial abscesses. **Dermatologic Surgery**, New York, v. 36, n. 10, p. 1638-1641, 2010.

SAGE, R. J.; CHAFFINS, M. L.; KOUBA, D. J. Granulomatous foreign body reaction to hyaluronic acid: report of a case after melolabial fold augmentation and review of management. **Dermatologic Surgery**, New York, v. 35, p. 1696-1700, 2009.

SANCHIS-BIELSA, J. M. et al. Foreign body granulomatous reactions to cosmetic fillers: a clinical study of 15 cases. **Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics**, St. Louis, MO, v. 108, n. 2, p. 237-241, 2009.

SAYLAN, Z. Facial fillers and their complications. **Aesthetic Surgery Journal**, New York, v. 23, n. 3, p. 221-224, 2003.

SHANZ, S. et al. Arterial embolization caused by injection of hyaluronic acid (Restylane). **The British Journal of Dermatology**, Oxford, v. 146, n. 5, p. 928-929, 2002.

SMITH, K. C. Reversible vs. nonreversible fillers in facial aesthetics: concerns and considerations. **Dermatology Online Journal**, Davis, v. 14, n. 8, p. 3, 2008.

SOUSA, E. M. et al. Resposta Inflamatória e Deposição de Colágeno Após Implante Intramuscular com Polimetilmetacrilato em Camundongos Balb/c. **Acta Scientiae Veterinarie**, Porto Alegre, v. 36, n. 1, p. 13-19, 2008.

TOY, B. R.; FRANK, P. J. Outbreak of Mycobacterium abscessus infection after soft tissue augmentation. **Dermatologic Surgery**, New York, v. 29, n. 9, p. 971-973, 2003.

VARGAS, K. F. et al. Use of polymethylmethacrylate as permanent filling agent in the jaw, mouth and face regions - Implications on dental practice. **Gerodontology**, Oxford, [2011]. No prelo.

VARGAS, A. F.; DE AMORIM, N. G.; PITANGUY, I. Complicações tardias dos preechimentos permanentes. **Revista da Sociedade Brasileira de Cirurgia Plástica**, São Paulo, v. 24, n. 1, p. 71-81, 2009.

VEDAMURTHY, M. Soft tissue augmentation - Use of hyauronic acid as dermal filler. **Indian Journal of Dermatology, Venereology and Leprology**, Vellore, v. 70, n. 6, p. 383-387, 2004.

VIER-PELISSER, F. V. et al. The effect of head-fractioned teletherapy on pulp tissue. **International Endodontic Journal**, Oxford, v. 40, n. 11, p. 859-865, 2007.

ZIELKE, H. et al. Risk profiles of different injectable fillers: results from the Injectable Filler Safety Study (IFS Study). **Dermatologic Surgery**, New York, v. 34, n. 3, p. 326-335, 2008.

ZIMMERMANN, U. S.; CLERICI, T. J. The histological aspects of fillers complications. **Seminars in Cutaneous Medicine and Surgery**, Philadelphia, v. 23, n. 4, p. 241-250, 2004.

YEOM, J. et al. Effect of cross-linking reagents for hyaluronic acid hydrogel dermal fillers on tissue augmentation and regeneration. **Bioconjugate Chemistry**, Washington, DC, v. 21, n. 2, p. 240-247, 2010.

YOON, E. S.; HAN, S. K.; KIM, W. K. Advantages of the presence of living dermal fibroblasts within Restylane for soft tissue augmentation. **Annals of Plastic Surgery**, Boston, v. 51, n. 6, p. 587-592, 2003.

ANEXOS

ANEXO A

Gerodontology

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International authors who are not completely fluent in the English language should seek help in the preparation of their manuscripts. Such assistance will enhance the review, improve the chance of acceptance, and greatly reduce the time until publication if the article is accepted.

If your manuscript is accepted, the Editors reserve the right to determine whether it will be published in the print edition or solely in the Internet edition of the Journal. Articles accepted for publication are subject to editorial revision.

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Duality of Interests. Any commercial or other associations that might create a duality of interests in connection with a submitted manuscript must be disclosed. All sources of external funds supporting the work must be indicated in a footnote, as should all corporate affiliations of the authors including author(s) relationship with a corporate entity involved with the subject of the research or product being espoused in the submission. A cover letter at the time of submission should inform the Editor of pertinent consultancies, stock ownership or other equity interests, or patent licensing arrangements. All information will remain confidential while the paper is being reviewed and will not influence the editorial decision. If the manuscript is accepted, the Editor will communicate with the authors how best to disclose the relevant information.

Publication Standards of Ethical Conduct. Submitting manuscripts for publication that contain elements of fabrication, falsification, or plagiarism constitutes a major violation of the universally accepted standards of ethical and scientific conduct.

Articles falling into the following categories are invited for submission:

Review manuscripts. Manuscripts that review the current status of a given topic, diagnosis, or treatment are encouraged. These manuscripts should not be an exhaustive review of the literature, but rather should be a review of contemporary thought with respect to the topic. Likewise, the bibliography should not necessarily be all-inclusive but rather include only seminal, pertinent, and contemporary references deemed to be most important by the author.

Clinicopathologic Conference. Papers submitted for the Clinicopathologic Conference (CPC) should present interesting, challenging, or unusual cases. The presentation should simulate clinical work-up, including a differential diagnosis. The complete diagnostic evaluation, management, and follow-up must be included. CPC articles will be organized into five parts: *Clinical presentation*-describe the clinical and imagining characteristics of the lesion. Use clinical photographs and radiographs as appropriate. *Differential diagnosis*-list and discuss lesions to be considered as reasonable diagnostic possibilities. *Diagnosis*-histopathologic findings illustrated with photomicrographs.*Management*-describe the treatment of the patient and response to treatment. *Discussion*-concentrate on the most interesting aspect(s) of the case.

Medical Management and Pharmacology Update. The Medical Management and Pharmacology Update (MMPU) is intended to provide concise, current reviews of medical problems and how they relate to dentistry. Manuscripts should include a good review of the

clinical aspects of the disease, stressing the impact of the disease on the dental management and dental treatment of the patient. Emphasis should be placed on new developments, new research, or new approaches to therapy or management. Manuscripts should not be an exhaustive review of the literature, but rather a review of contemporary thought with respect to the topic. Likewise, the bibliography need not be all inclusive but rather should include only seminal, contemporary references deemed by the author to be most pertinent. The desired format for manuscripts submitted for the MMPU would include: an abstract; topic introduction/overview; epidemiology/demographics; etiology and pathogenesis; clinical presentation/physical findings; diagnosis (laboratory tests, diagnostic imaging, etc.); medical management and treatment; complications; prognosis; oral manifestations/dental implications and significance; and dental management (of patients with the disease). Manuscripts should not exceed 12 pages in 12 point, double-spaced Times New Roman (Tables and Figures count toward the 12-page limit).

Pharmacology Update is a component section of MMPU that offers the reader the opportunity to obtain concise information regarding drugs used in the practice of medicine, clinical dentistry and dental specialties. Papers submitted should present clearly and concisely background information regarding the disease or condition that is managed, the indications, rational and approved uses of the specific drugs or class of drugs, the advantages and benefits of the drug or drug class over previous drugs, mechanism of action, criteria for selection, usual dosage, pharmacokinetics, adverse effects, drug interactions, and oral health and dental management considerations. Emphasis should be placed on new developments, effectiveness in clinical trials, therapeutic outcomes and safety. Manuscripts should reflect the contemporary thought with respect to the topic. Use of figures to illustrate the mechanism of action, and tables to presents therapeutic outcomes, drug interactions, and adverse effects are encouraged. Manuscripts should utilize the above mentioned categories for formatting the paper. Papers should not exceed 3000 words. The recommended font is 12 point, double spaced Times New Roman. A maximum of 50 references is recommended.

Clinical Notes. The Clinical Notes feature is intended to provide a forum for brief communications of a technical nature.They are not scientific papers; they may report a new instrument, technique, procedure, or, in rare situations, an interesting case report.

Preparation of manuscripts. Only original manuscripts that have not been published in other forms will be considered for publication. Correct preparation of the manuscript by the author will expedite the reviewing and publication procedures. Manuscripts should be word processed double-spaced. Please note the following requirements and the instructions for online submission at http://ees.elsevier.com/tripleo.

The article, including all tables, should be formatted in the latest version of Microsoft Word. The use of appropriate subheadings throughout the body of the text (Methods, Results, and Discussion sections) is required. Legends for figures and tables should appear after the reference list. If an illustration has been taken from published material, the legend must give full credit to the original source. Illustrations must also be submitted electronically as separate files (not embedded). File specifications are listed below in "Illustrations." Tables should be submitted as separate files (in Microsoft Word (*.doc) format.)

Routine case reports add little to our knowledge, but good case reports may occasionally be

published if they meet certain criteria: (1) are of rare or unusual lesions that need documentation, (2) are well documented cases showing unusual or "atypical" clinical or microscopic features or behavior, or (3) are cases showing good long-term follow-up information, particularly in areas in which good statistics on results of treatment are needed.

Title Page. The title page of the manuscript should include the title of the article, the full name of the author(s), academic degrees, positions, and institutional affiliations. Listed authors should include only those individuals who have made a significant creative contribution. The corresponding author's address, business and home telephone numbers, fax number and e-mail address should be given.

Authorship. All persons who are identified as authors must have made substantial contribution to the manuscript through significantly contributing to the conception, design, analysis or interpretation of data; drafting or significantly revising the manuscript; and providing final approval of the manuscript. All three of these conditions must be met by each author. Persons who contribute to the effort in supporting roles should not be included as authors; rather they should be acknowledged at the end of the paper.

Abstract. An abstract of no more than 150 words, typewritten double-spaced, should precede the introduction to the article and must accompany each manuscript.

Structured abstract. A structured abstract limited to 150 words must be used for data-based research articles. The structured abstract is to contain the following major headings:*Objective(s); Study Design; Results; and Conclusion(s).* The*Objective(s)* reflects the purpose of the study, that is, the hypothesis that is being tested. The *Study Design* should include the setting for the study, the subjects (number and type), the treatment or intervention, and the type of statistical analysis. The*Results* include the outcome of the study and statistical significance if appropriate. The *Conclusion(s)* states the significance of the results.

Methods. The methods section should describe in adequate detail the experimental subjects, their important characteristics, and the methods, apparatus, and procedures used so that other researchers can reproduce the experiment. When the paper reports experiments on human subjects, the methods section must indicate that the protocol was reviewed by the appropriate institutional review board (IRB) and that each subject in the project signed a detailed informed consent form.

Animals. Please indicate that protocols were reviewed by the appropriate institutional committee with respect to the humane care and treatment of animals used in the study.

References. References should be cited selectively. Personal communications and unpublished data are not to be cited as references; instead, are to be cited in parentheses at the appropriate place in the text. Make sure all references have been verified and are cited consecutively in the text (not including tables) by superscript numbers. Reference list format must conform to that set forth in "Uniform Requirement for Manuscripts Submitted to Biomedical Journals" (Ann Intern Med 1997;126:36-47). A copy of these Requirements may be viewed/printed online at www.icmje.org. References to articles in press must include authors' surnames and initials, title of article, and name of journal. The reference list should be typed double-spaced on a separate page and numbered in order as the reference citations

appear in the text. For journal citations, include surnames and initials of authors, complete title of article, name of journal (abbreviated according to the Cumulated Index Medicus), year of publication, volume, number, and inclusive page numbers. For book citations, surnames and initials of authors, chapter title (if applicable), editors' surnames and initials, book title, volume number (if applicable), edition number (if applicable), city and full name of publisher, year of publication, and inclusive page numbers of citation.

EXAMPLES (if six or fewer authors, list all; if seven or more list first six and add *et al*):

Format for periodical references: Pullon PA, McGivney J. Computer utilization in an oral biopsy service. Int J Oral Surg 1977;6:251-5.

Format for book references: Seakins J, Saunders R, editors. Treatment of inborn errors of metabolism. London: Churchill Livingstone: 1973; p. 51-6.

Format for chapter references: Hudson FB, Hawcroft J. Duration of treatment in phenylketonuria. In: Seakins J, Saunders R, editors. Treatment of inborn errors of metabolism. London: Churchill Livingstone; 1973. p. 51-6.

Journal article on the Internet: Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Illustrations. Illustrations should be numbered and provided with suitable legends.

A reasonable number of halftone illustrations or line drawings will be reproduced at no cost to the author, but special arrangements must be made with the Editor-in-Chief for color plates, elaborate tables, or extra illustrations. Typewritten or freehand lettering on illustrations is not acceptable. All lettering must be done professionally, and letters should be in proportion to the drawings or photographs on which they appears.

Illustrations must be submitted in electronic format. All images should be at least 5 inches wide. Images should be provided in TIF or EPS format, per the instruction for online submission at http://ees.elsevier.com/tripleo. Macintosh or PC is acceptable. Graphics software such as Photoshop and Illustrator (not presentation software such as PowerPoint, CorelDraw, or Harvard Graphics) should be used in the creation of the art. Color images need to be CMYK, at least 300 DPI, and be accompanied by a digital color proof, not a color laser print or color photocopy. Note: This proof will be used at press for color reproduction. Gray scale images should be at least 300 DPI accompanied by a proof. Combinations of gray scale and line art should be at least 1200 DPI accompanied by a proof. Line art (black and white or color) should be at least 1200 DPI with a proof.

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Legends to illustrations. Each illustration must be accompanied by a legend. These should be typed double-spaced on a separate page. If an illustration has been taken from published material, the legend must give full credit to the original source.

Tables. The tables should be typewritten double-spaced, including column heads, data and footnotes, and submitted on separate pages. Tables should be self-explanatory and should supplement, not duplicate, the text. All table reference citations should be repeats of numbers assigned within the text, not initial citations. A concise title should be supplied for each table. All columns should carry concise headings describing the data therein. Type all footnotes immediately below the table and define abbreviations. If a table or any data therein have been previously published, a footnote to the table must give full credit to the original source.

Video and Computer Graphics. Authors are encouraged to submit videos and computer-generated graphics; eg, a slide presentation with or without animation and sound. An author who wishes to supply such material should notify the editors in the cover letter and note this intention in the Author Comments area of the online submission. Although the publisher will not edit any video or computer graphic, editors and reviewers may suggest changes. All patient-identifying information must be removed or masked.

The maximum length of a video or computer graphic is 8 minutes. Longer submissions may be divided into smaller clips, each of which should be identified at the beginning of the section (eg, Video Clip 1, Graphic 1). A concise legend for each video clip or computer graphic presentation must be included with the manuscript. Videos are to be submitted in MGEG-1 or MPEG-2 (*.mpg) or QuickTime (*.mov) format. More detailed instructions can be found at http://www.elsevier.com/artwork. Videos and computer graphics accompanying a manuscript declined for publication will not be accepted separately. If the manuscript is accepted for publication, the presentation will be archived at www.mosby.com/tripleo.

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Checklist for authors

__Letter of submission
__Title page
    __Title of article
    __Full names(s), academic degree(s), affiliation(s) and titles of author(s)
    __Author to whom correspondence, galleys, and reprint request are to be sent, including address and business and home telephone numbers, fax number, and e-mail address
__Structured abstract (double-spaced)
__Article proper (double-spaced)
(Figures and tables should not be part of the text of the manuscript but added as separate files)
__Statement of IRB review (stated in manuscript)
__References (double-spaced on a separate page)
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__Legends (double-spaced, on a separate page)
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__Video/computer graphics, properly formatted (as separate files)
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__If this paper was presented at a meeting identification of organization, city, and year (on a separate page)

Updated March 2010

ANEXO D

De: ees.tripleo.0.107d1b.548ce4ff@eesmail.elsevier.com em nome de OOOOE (Triple O) journal
Enviada: qui 14/4/2011 19:48
Para: Maria Antonia Z de Figueiredo
Assunto: Acceptance of your submission TRIPLEO-D-11-00384R1

Ms. Ref. No.: TRIPLEO-D-11-00384R1
Title: CLINICAL AND HISTOLOGICAL EVALUATION OF EFFECTS OF HYALURONIC ACID IN RAT TONGUE
Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology

Dear Dr. Zancanaro de Figueiredo,

I am pleased to inform you that your manuscript, "CLINICAL AND HISTOLOGICAL EVALUATION OF EFFECTS OF HYALURONIC ACID IN RAT TONGUE," which you recently submitted to Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology has been carefully reviewed and has been accepted for publication.

Edited proofs will be e-mailed to you in approximately four weeks. Before publication, you will receive the proof of your manuscript and further information from the publisher, Elsevier, Inc. Please read the proof carefully and contact the publisher if anything is unclear or incorrect; the authors have final responsibility for the accuracy of the publication.

Thank you for preparing this informative article for the readership of the Journal. I hope you found the review process informative and helpful and will also consider us again in the future.

Sincerely,

David Precious, CM DDS MSc FRCDC FRCS
Section Editor, Oral & Maxillofacial Surgery
Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology
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ANEXO E

Comissão Científica e de Ética
Faculdade da Odontologia da PUCRS

Porto Alegre 10 de março de 2010

O Projeto de: Dissertação

Protocolado sob nº:	0007/10
Intitulado:	Estudo experimental em ratos submetidos à injeção submoca de ácido hialurônico em distintas concentrações avaliação clínica e histológica
Pesquisador Responsável:	Profa. Maria Antonia Zancanaro de Figueiredo
Pesquisadores Associados	Ruchielli Loureiro Borghetti; Karlon Fróes de Vargas e Sabrina Ponzatti Moure
Nível:	Mestrado

Foi ***aprovado*** pela Comissão Científica e de Ética da Faculdade de Odontologia da PUCRS em *10 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

ANEXO F

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

Ofício 029/10 – CEUA Porto Alegre, 18 de março de 2010.

Senhora Pesquisadora:

O Comitê de Ética para o Uso de Animais apreciou e aprovou seu protocolo de pesquisa, registro CEUA 10/00151, intitulado: **"Estudo experimental em ratos submetidos à injeção submucosa de ácido hialurônico em distintas concentrações: avaliação clínica e histológica".**

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
N/Universidade

APÊNDICES

APÊNDICE A

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO EM ESTOMATOLOGIA CLÍNICA
FICHA DE AVALIAÇÃO CLÍNICA

IDENTIFICAÇÃO

Rato nº: __ **Peso inicial:** _____g

Substância Injetada:	**Tempo:**
□ Grupo 1 (Ácido hialurônico 5,5mg/mL)	□ Subgrupo A (7 dias)
□ Grupo 2 (Ácido hialurônico 25mg/mL)	□ Subgrupo B (60 dias)
□ Grupo 3 (NaCl 0,9%)	□ Subgrupo C (90 dias)

AVALIAÇÃO CLÍNICA LOCAL

□ Sinais inflamatórios:

( ) eritema ( ) edema

□ Lesão nodular

Sinais secundários: □ sim □ não

Especifique (sangramento, supuração, abscesso, úlcera...):

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

Fotos: _____

Data da avaliação: __/__/____.

APÊNDICE B

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO EM ESTOMATOLOGIA CLÍNICA

FICHA DE AVALIAÇÃO HISTOLÓGICA

IDENTIFICAÇÃO

Rato nº:_____ Peso inicial: _____

Lâmina nº: _____

Substância injetada:

□ Grupo 1 (Ácido hialurônico 5,5mg/mL)
□ Grupo 2 (Ácido hialurônico 25mg/mL)
□ Grupo 3 (NaCl 0,9%)

Tempo:

□ Subgrupo A (7 dias)
□ Subgrupo B (60 dias)
□ Subgrupo C (90 dias)

AVALIAÇÃO HISTOLÓGICA

HE:

VARIÁVEL	ESCORE
□ Linfócitos	□ 0- Sem inflamação
□ Plasmócitos	□ 1- Leve: células mononucleares esparsas
□ Macrófagos	□ 2- Moderado: infiltrado mononuclear e/ou neutrófilos e eosinófilos esparsos
□ Eosinófilos	
□ Neutrófilos	□ 3- Severo: infiltrado polimorfonuclear de neutrófilos e eosinófilos
□ Células Gigantes	
□ Fibroplasia	
□ Edema	
□ Hiperemia	

Migração

Rim

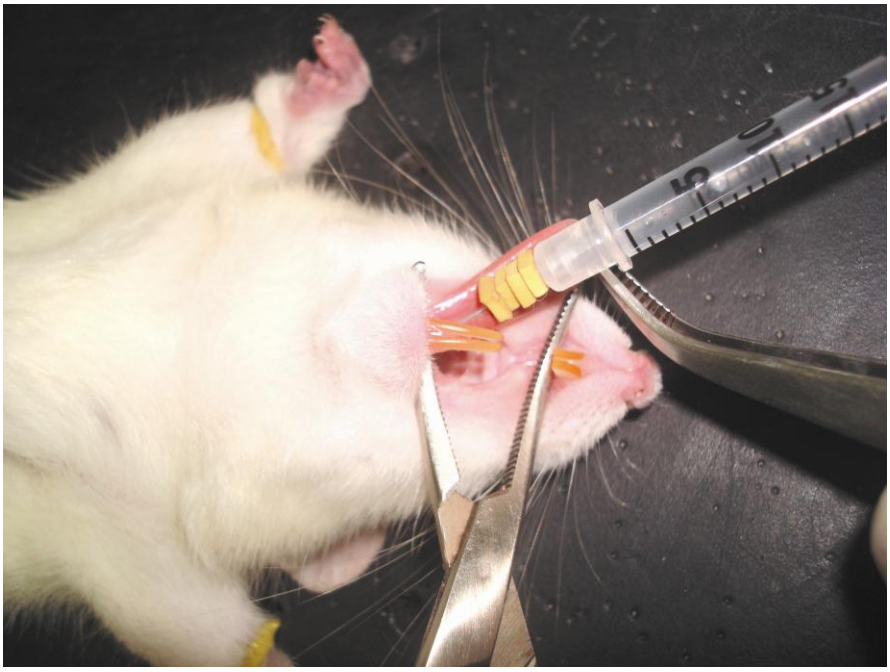
□ Ausência de material
□ Presença de material

□ Ausência de resposta inflamatória
□ Presença de resposta inflamatória

Observações: _____

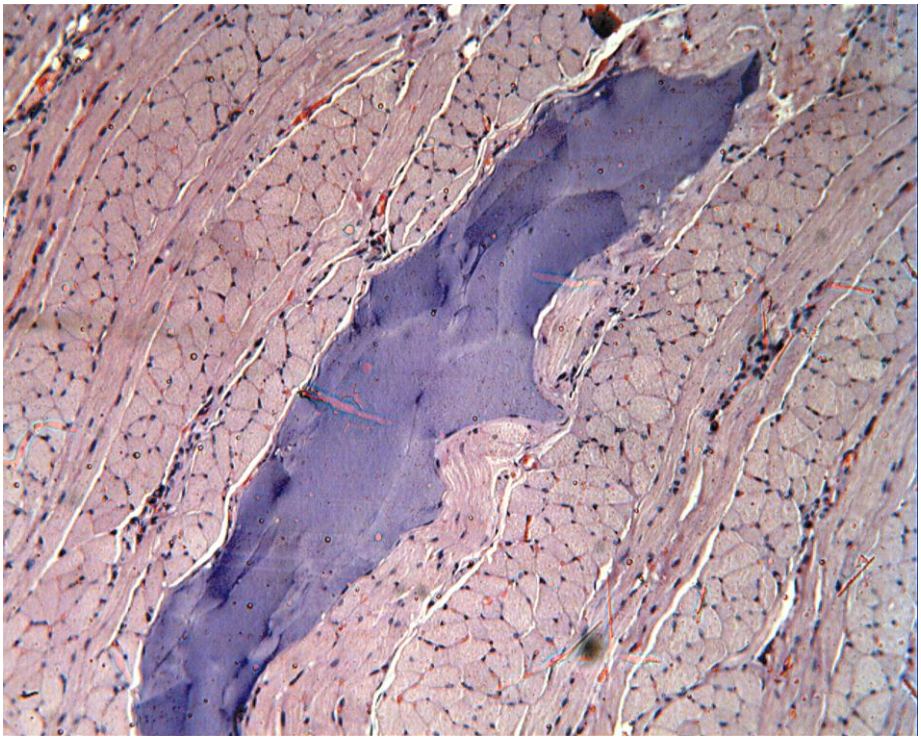
Fotos:_____ Data da avaliação: __/__/__.

APÊNDICE C

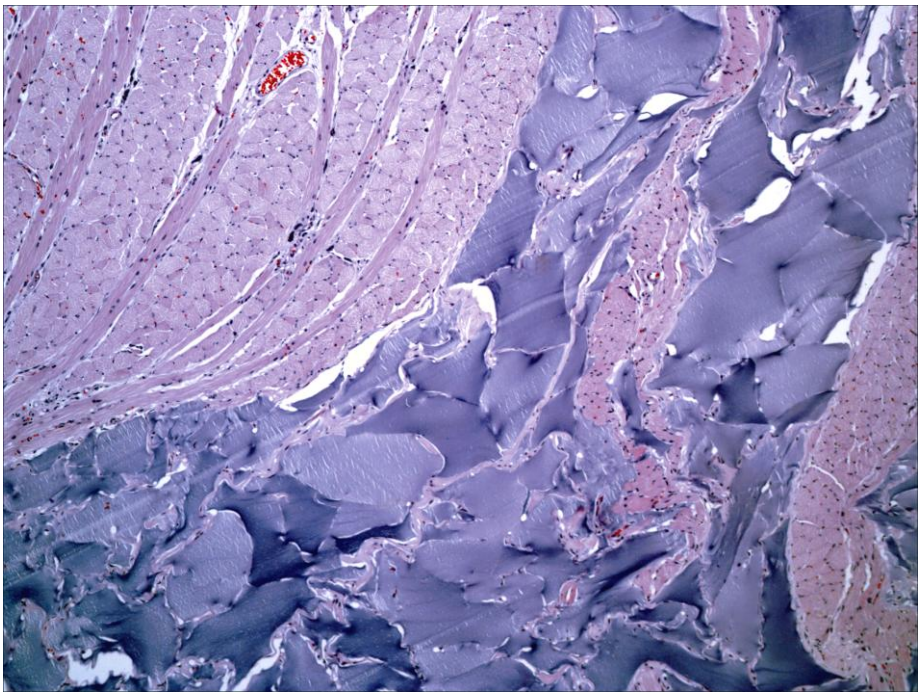


Aplicação do material de preenchimento em ventre lingual, lado esquerdo.

APÊNDICE D

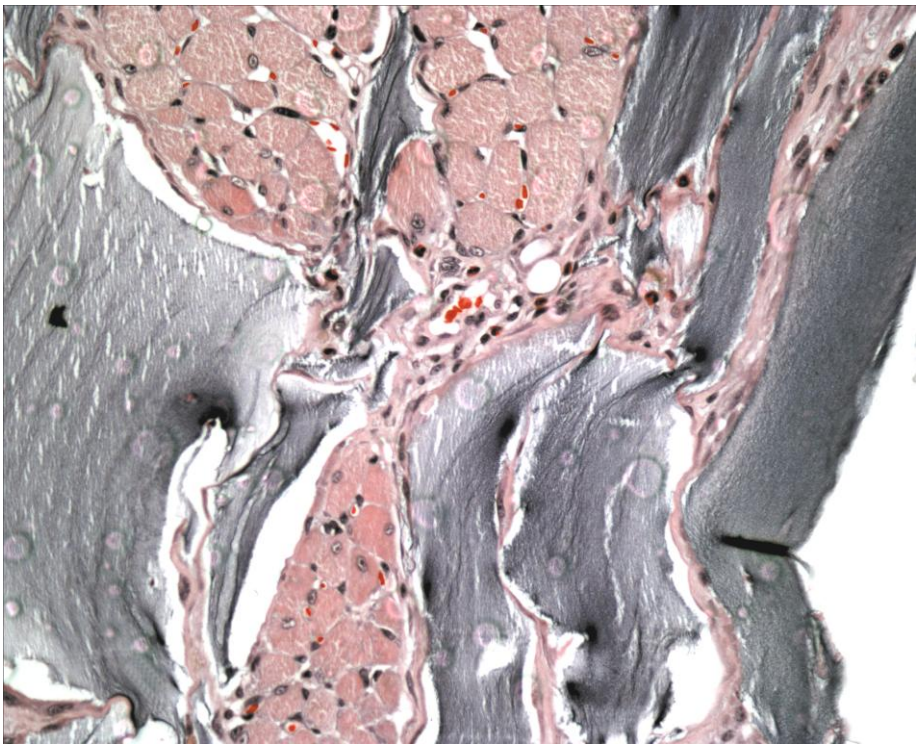


Ausência de inflamação (escore 0) adjacente ao AH 5,5mg/mL em 60 dias. (HE, aumento aproximado 100x).

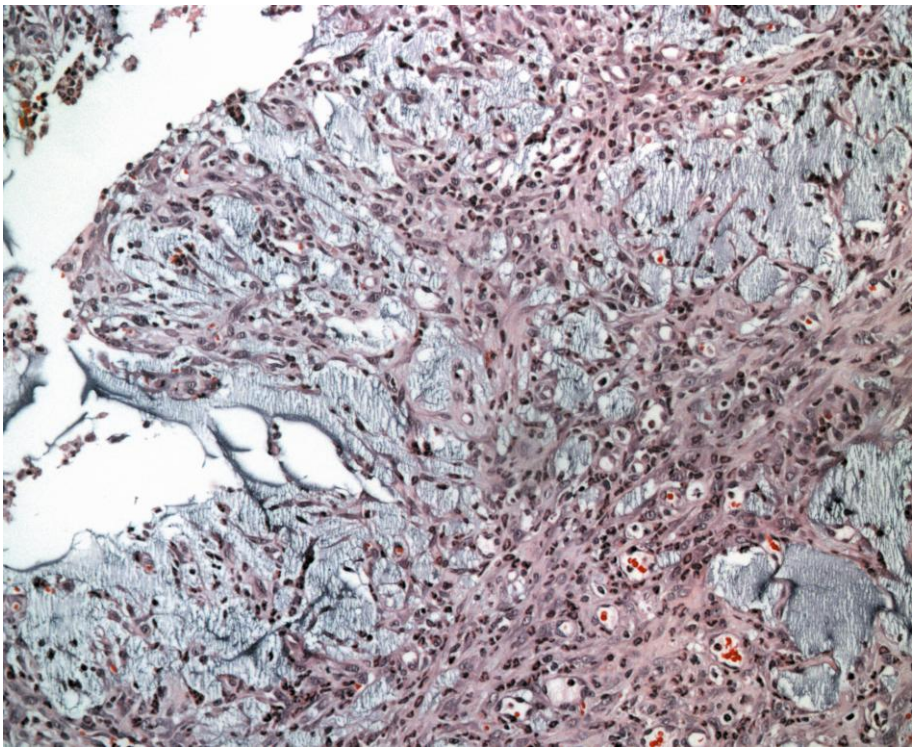


Inflamação leve (escore 1) adjacente ao AH 5,5mg/mL, em 90 dias. (HE, aumento aproximado 100x).

APÊNDICE E

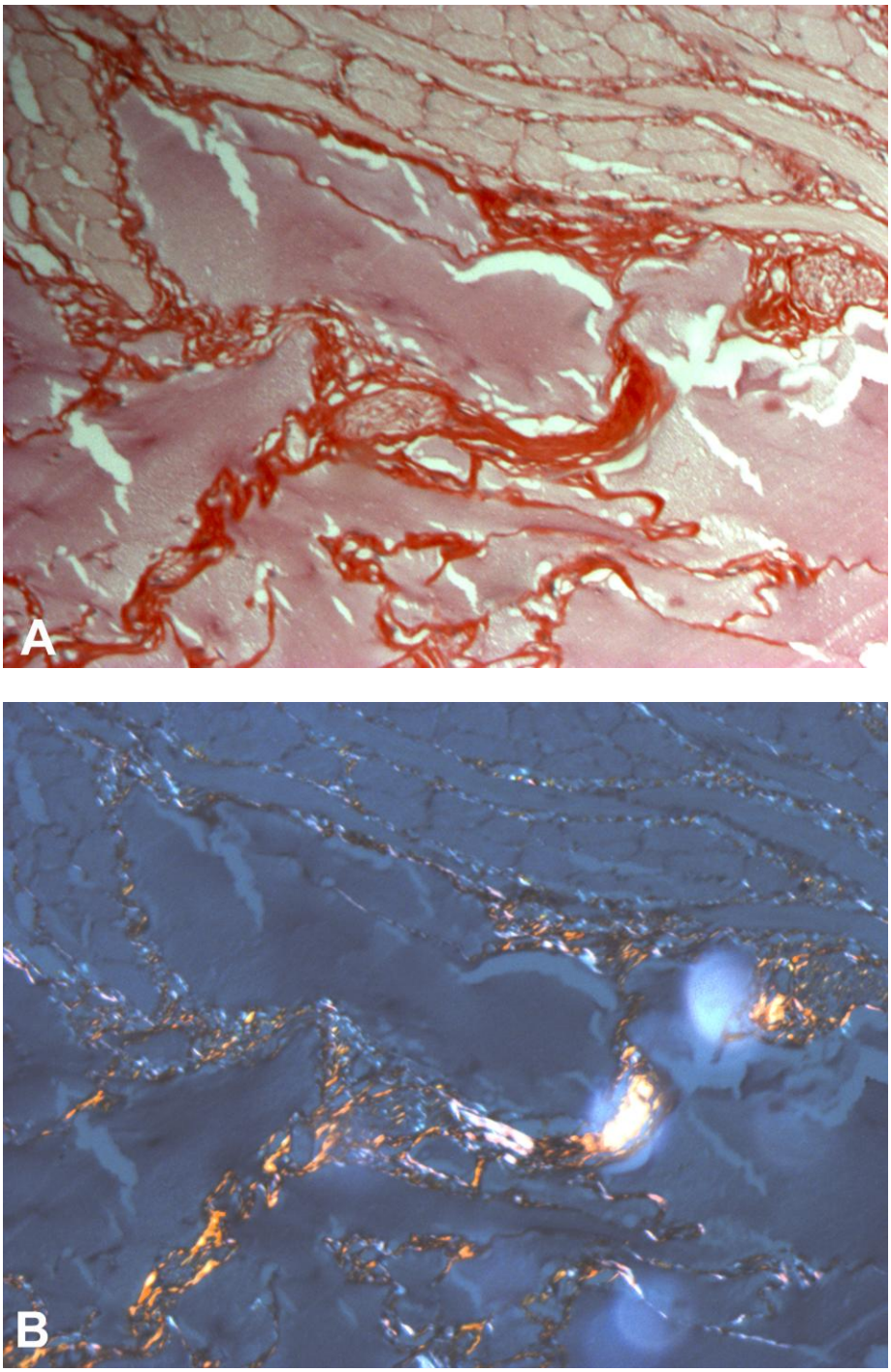


Inflamação moderada (escore 2) adjacente ao AH 25mg/mL aos 7 dias. (HE, aumento aproximado 400x).



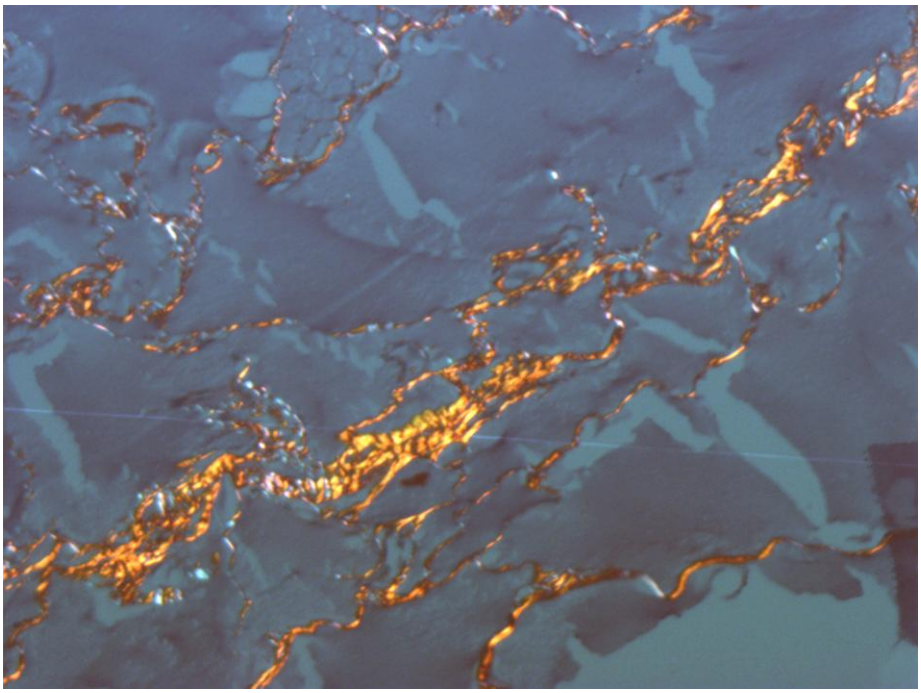
Inflamação intensa (escore 3) circunjacente ao AH 25mg/mL, aos 7 dias. (HE, aumento aproximado 200x).

APÊNDICE F



Marcação das fibras colágenas próximas ao AH 25mg/mL (90 dias) através da microscopia de luz tradicional (A) e polarizada (B). (Picrosírius, aumento aproximado 100x).

APÊNDICE G



Fibras colágenas, circunjacente ao AH 25mg/mL aos 90 dias, evidenciadas pela luz polarizada. (Picrosírius, aumento aproximado 100x).