Universidade Estadual de Campinas
Faculdade de Odontologia de Piracicaba

CLARICE MAIA SOARES DE ALCÂNTARA PINTO

Análise histopatológica, ultraestrutural e da composição química de partículas metálicas em amostras teciduais adjacentes a placas e parafusos de osteossíntese em cirurgia buco-maxilo-facial

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Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Clínica Odontológica – Área de Concentração em Cirurgia e Traumatologia Buco-Maxilo-Faciais

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Orientador: Prof. Dr. Marcio de Moraes

Este exemplar corresponde à versão final da tese defendida pela aluna Clarice Maia Soares de Alcântara Pinto e orientada pelo Prof. Dr. Marcio de Moraes

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A Ata da defesa com as respectivas assinaturas dos membros encontra-se no processo de vida acadêmica do aluno.

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RESUMO

O objetivo do presente estudo foi avaliar os achados histopatológicos de amostras de tecidos adjacentes a placas e parafusos de osteossíntese removidos de pacientes devido indicações clínicas, identificar a composição química das partículas metálicas encontradas nestes tecidos e relacionar os achados histopatológicos e ultraestruturais com os aspectos clínicos apresentados pelos pacientes. A população experimental incluiu 38 pacientes dos quais placas e parafusos associados foram removidos e a curetagem de um espécime de tecido mole adjacente às placas foi realizada. Para seis dos 38 pacientes, as placas e os parafusos foram removidos de dois sítios anatômicos distintos e um espécime tecidual foi obtido de cada sítio. Os prontuários dos pacientes foram avaliados e os seguintes dados foram obtidos: tipo de procedimento cirúrgico que levou à instalação das placas e parafusos; tempo decorrido entre a instalação e a remoção desses dispositivos (período de retenção); indicação para remoção das placas e parafusos e sítio anatômico da remoção. Todas as amostras de tecido obtidas foram analisadas através de microscopia óptica e os seguintes aspectos foram avaliados: presença de debris; osso vital ou desvitalizado; células gigantes multinucleadas; tecido de granulação; fibrose e inflamação. Para as amostras nas quais debris foram visualizados através da microscopia óptica, foi realizada a análise através de microscopia eletrônica de varredura (MEV). Quando as partículas metálicas foram localizadas, foi utilizada a análise de espectroscopia por energia dispersiva de raios-X (EDS) para determinação da composição química dos debris. Quarenta e quatro amostras teciduais foram obtidas. Sinais clínicos de inflamação, em associação com história de infecção pós-operatória, foram o principal motivo para a remoção das placas, correspondendo a 21 casos (47.72%). Trinta e três (75%) espécimes teciduais foram obtidos durante a remoção de placas mandibulares, oito (18.2%) espécimes foram obtidos da maxila e dois (4.5%) foram obtidos do complexo zigomático. Todos os espécimes teciduais exibiram graus variados de fibrose. Debris foram identificados em 42 (95.45%) espécimes. Áreas de inflamação foram identificadas em 34 (77.27%) amostras e células gigantes multinucleadas foram visualizadas em 15 (34.09%). As células inflamatórias e as células gigantes não estavam restritas às áreas de debris. Seis (13.63%) amostras apresentaram áreas de colônias

bacterianas. Dos 42 espécimes em que debris foram visualizados na microscopia óptica, quarenta foram avaliados utilizando MEV e EDS. Não foi possível obter um preparo adequado dos cortes histológicos para dois espécimes, para a análise no MEV. O titânio foi identificado como um dos elementos constituintes das partículas metálicas em 34 (85%) espécimes. Além do titânio, outros metais e elementos não-metálicos foram encontrados na análise por EDS. Diferentes elementos químicos identificados nos espécimes teciduais podem ser atribuídos a contaminantes resultantes do processo de manufatura das placas e parafusos ou a debris liberados durante a instalação e a remoção destes implantes. Apesar da identificação de partículas metálicas em grande parte dos espécimes teciduais avaliados, não foram visualizadas alterações histopatológicas evidentes, relacionadas a estas partículas.

Palavras-chave: Materiais biocompatíveis; Titânio; Fixação interna de fraturas; Próteses e Implantes; Remoção de dispositivo

ABSTRACT

The aim of the present study was to assess the histopathological findings of tissue samples adjacent to osteosynthesis plates and screws removed from patients due to clinical indications, as well as to identify the chemical composition of metal particles found in these tissues and to correlate the histopathological and ultrastructural findings with the clinical aspects exhibited by the patients. The experimental population comprised 38 patients from whom plates and associated screws were removed and curettage was performed to obtain a specimen of soft tissue adjacent to the plates. For six of the 38 patients, plates and screws were removed from two different anatomical sites and a tissue specimen was obtained from each site. Patient records were examined and the following data were recorded: the type of surgical procedure that led to the insertion of the plates and screws; the time period that elapsed between the insertion and removal of these devices (retention period); indications for the removal of plates and screws and the anatomic site of removal. All the tissue specimens were analyzed using optical microscopy and the following aspects were recorded: the presence of debris; vital or devitalized bone; multinucleated giant cells; granulation tissue; fibrosis and inflammation. For samples in which debris was visualized under light microscopy, scanning electron microscopy (SEM) analysis was performed. When metal particles were located, spectroscopy energy dispersive X-ray (EDS) analysis was used to determine the chemical composition of debris. Forty-four tissue samples were obtained. Clinical signs of inflammation, associated with history of postoperative infection, were the main reason for plate removal, corresponding to 21 cases (47.72%). Thirty-three (75%) tissue specimens were obtained during mandible plate removal, eight (18.2%) were taken from the maxilla and two (4.5%) were taken from the zygomatic complex. All of the tissue specimens exhibited varying degrees of fibrosis. Debris was found in 42 (95.45%) specimens. Areas of inflammation were identified in 34 (77.27%) samples and multinucleated giant cells were visualized in 15 (34.09%). Inflammatory and giant cells were not confined to areas of debris. Six (13.63%) samples exhibited areas of bacterial colonies. Of the 42 specimens in which debris was visualized in the optical microscopy, 40 were analyzed using SEM and EDS. It was not possible to obtain an appropriate preparation of histological sections for two specimens in the SEM

analysis. Titanium was identified as one of the metal particles´ constituent elements in 34 (85%) specimens. Besides titanium, other metals and non-metallic elements were found in the EDS analysis. Different chemical elements identified in the tissue specimens can be attributed to contaminants resulting from the manufacturing process of plates and screws or debris released during the insertion or removal of these implants. Despite the identification of metal particles in most of the tissue specimens, no obvious histopathological alterations related to these particles were visualized.

Keywords: Biocompatible materials; Titanium; Fracture fixation, Internal; Prostheses and Implants; Device removal

LISTA DE ABREVIATURAS E SIGLAS

Al – Alumínio

Ba – Bário

C – Carbono

Ca – Cálcio

Cl – Cloro

Co – Cobalto

Cr – Cromo

Cu – Cobre

EDS – Espectroscopia por energia dispersiva de raios-X

H – Hidrogênio

Fe – Ferro

K – Potássio

N – Nitrogênio

Ni – Níquel

Mg – Magnésio

Mn – Manganês

Mo – Molibdênio

MEV – Microscopia eletrônica de varredura/ Microscópio eletrônico de varredura

Na – Sódio

O – Oxigênio

Pb – Chumbo

Pd – Paládio

Ti –Titânio

Ti cp – Titânio comercialmente puro

Ti-6Al-4V – Liga de titânio, alumínio e vanádio

S – Enxofre

Si – Silício

V – Vanádio

Zn – Zinco

SUMÁRIO

INTRODUÇÃO

DESENVOLVIMENTO DA FIXAÇÃO INTERNA NA CIRURGIA BUCO-MAXILO-FACIAL

A traumatologia moderna teve início com o advento da osteossíntese, a qual consistiu em um dos principais avanços no campo da Cirurgia Crânio-Maxilo-Facial (Mukerji *et al.*, 2006). Buck foi o pioneiro no emprego da fixação metálica ao esqueleto crânio-maxilo-facial ao utilizar, em 1847, fio de aço para fixação de uma fratura mandibular (Rowe, 1971). A primeira utilização de placas e parafusos de aço para fixação interna no esqueleto facial é creditada a Schede, o qual descreveu seu uso para fixação de fraturas mandibulares, em 1888. As primeiras placas utilizadas para osteossíntese, contudo, eram volumosas e foram projetadas exclusivamente para uso em fraturas mandibulares (Gilardino *et al.*, 2009). As miniplacas foram introduzidas por Michelet *et al*. em 1973 (Michelet *et al.*, 1973), e posteriormente popularizadas por Champy (Champy *et al*., 1978).

O aço inoxidável, uma liga composta por cromo (Cr), níquel (Ni), molibdênio (Mo) e ferro (Fe) e, posteriormente, em 1936, o *Vitallium,* composto por cobalto (Co), Cr e Mo, pavimentaram o caminho da fixação interna. Enquanto diversos outros metais foram testados e abandonados, o aço inoxidável, o *Vitallium* e o titânio (Ti) ganharam popularidade durante a evolução da fixação interna para o esqueleto facial (Gilardino *et al*., 2009). Em 1951, Leventhal propôs a utilização do Ti como material no tratamento de fraturas, na tentativa de encontrar um material que apresentasse a inércia do *Vitallium* combinada à facilidade de utilização do aço inoxidável e, em 1967, Snell & Dott descreveram o uso de placas de Ti para o tratamento de fraturas faciais (Snell & Dott, 1967).

Atualmente, diferentes sistemas de fixação estão disponíveis, desde placas de compressão espessas para reconstrução mandibular até placas de perfil baixo para fixação no terço médio da face e placas biodegradáveis constituídas de diferentes polímeros (Mukerji *et al*., 2006; Gilardino *et al.* 2009). Sistemas de fixação interna ideais devem ser biocompatíveis, não causar prejuízos à vascularização e apresentar adequadas propriedades mecânicas durante o período de implantação. É desejável que as condições da superfície destes implantes sejam tais que apenas

uma fina camada de tecido mole com uma adequada aderência seja induzida (Ungersböck *et al.*, 1996).

Os sistemas de fixação de Ti para Cirurgia Buco-Maxilo-Facial são geralmente constituídos de Ti comercialmente puro ou de ligas de Ti (Gilardino *et al.*, 2009). Atualmente, os implantes cirúrgicos de Ti, variando desde os produzidos a partir do Ti comercialmente puro - American Society for Testing and Materials - graus 1 a 4 (ASTM F67, 2006) até as ligas à base de Ti, são considerados os mais biocompatíveis (Oshida, 2010). Desta forma, o Ti e suas ligas tornaram-se alguns dos materiais mais utilizados na confecção de implantes cirúrgicos, devido às propriedades apresentadas por este metal, dentre as quais se destacam, além da biocompatibilidade, a elevada resistência à corrosão (Haug, 1996), baixa densidade, elevada resistência elétrica específica (Niinomi, 2001) e o módulo de elasticidade mais próximo ao apresentado pelo osso cortical, quando comparado com o de outros materiais, tais como o aço inoxidável (Voggenreiter *et al.*, 2003).

IMPLANTES CIRÚRGICOS DE TITÂNIO E RESPOSTA TECIDUAL LOCAL

Após a instalação dos implantes metálicos, uma resposta inflamatória local é notada, a qual inclui o acúmulo de neutrófilos, linfócitos, plasmócitos e macrófagos. Com materiais biocompatíveis, tais como o Ti e ligas como a de Ti-6Al-4V, esta resposta inflamatória diminui com o tempo. Geralmente esses materiais tornam-se encapsulados por uma camada de tecido fibroso que os isola do restante do organismo (Haug, 1996). A espessura e a composição celular desta camada de tecido mole refletem a biocompatibilidade do material e as condições da superfície (Ungersböck *et al.*, 1996). Deste modo, os implantes produzidos a partir destes materiais podem ser mantidos indefinidamente no organismo. No entanto, a corrosão, a toxicidade e a hipersensibilidade podem alterar esta resposta. A cápsula fibrosa pode tornar-se necrótica e ser substituída por tecido de granulação ou o implante pode tornar-se rodeado por fibrina. Baseado na resposta biológica local favorável, os dispositivos de fixação de Ti parecem ser ideais como implantes permanentes (Haug, 1996).

Em implantes de Ti, uma camada de óxido forma-se espontaneamente sobre a superfície. A espessura da camada de óxido sobre os implantes não

anodizados de Ti é da ordem de 5nm. A camada de óxido pode aumentar durante o período de implantação no ambiente do corpo até 40nm e pode incorporar tanto substâncias orgânicas como inorgânicas, como fósforo (P), cálcio (Ca) e enxofre (S). A camada de óxido oferece resistência à corrosão e reduz a difusão de íons metálicos para os tecidos adjacentes (Ungersböck *et al.*, 1996).

EVIDÊNCIAS DE LIBERAÇÃO DE PARTÍCULAS METÁLICAS A PARTIR DE PLACAS E PARAFUSOS DE TITÂNIO

Apesar do Ti ser bem tolerado pelos tecidos biológicos e amplamente utilizado como material para confecção de implantes cirúrgicos (Moberg *et al*., 1989), há relatos de toxicidade e hipersensibilidade associadas a este material (Harloff *et al*., 2010). Embora ainda não existam evidências claras de danos a longo prazo causados pela retenção de implantes de Ti empregados para osteossíntese, estudos têm documentado a presença de partículas de Ti nos tecidos moles circunjacentes a placas de Ti empregadas no esqueleto maxilo-facial (Schliephake *et al*., 1993; Acero *et al*., 1999; Langford & Frame, 2002; Theologie-Lygidakis *et al*., 2007).

Os efeitos biológicos da exposição a partículas de metal parecem ocorrer não apenas no local do implante, mas também em locais distantes, como resultado da fagocitose e do transporte ativo (Ray *et al*., 1998). Devido às evidências de liberação de partículas de Ti provenientes de placas e parafusos para osteossíntese, documentadas em diferentes estudos, e à possibilidade de alterações teciduais degenerativas decorrentes da presença destas partículas (Kim *et al*., 1997), a remoção rotineira das placas após o período de reparo ósseo é recomendada por alguns autores, mesmo na ausência de sintomas (Katou *et al*., 1996; Kim *et al*., 1997; Acero *et al.,* 1999). Em contrapartida, outros estudos verificaram não haver evidências de dissolução passiva e progressiva destas partículas ao longo do tempo (Meningaud *et al*., 2001; Langford & Frame, 2002) e que a presença destas partículas nos tecidos não estava associada a um possível processo de corrosão destes implantes após a instalação (Langford & Frame, 2002; Theologie-Lygidakis *et al*., 2007). Desta forma, diferentes autores afirmam que a remoção das placas e parafusos de Ti não necessita ser realizada de forma rotineira, exceto nos casos de indicações clínicas (Mosbah *et al*., 2003; Theodossy *et al*., 2006).

COMPLICAÇÕES E POSSIBILIDADE DE REMOÇÃO DAS PLACAS E PARAFUSOS DE TITÂNIO

A fixação das fraturas maxilo-faciais com uso de placas e parafusos é um tratamento estabelecido e amplamente utilizado. Contudo, a necesidade de remoção dos dispositivos de fixação em decorrência de complicações pós-operatórias é reportada em diferentes estudos, que apresentam ampla variação das taxas de remoção destes implantes (Bhatt *et al.*, 2005; Rallis *et al*., 2006; Bakathir *et al*., 2008; Kuhlefelt *et al*., 2010; O'Connell *et al*., 2009; Thorén *et al*., 2010; Kyrgidis *et al*., 2013).

As principais razões descritas na literatura para remoção das placas e parafusos consistem de infecção, exposição do material, palpabilidade, perda de parafusos, fratura das placas, dor ou desconforto local, necessidade de um segundo procedimento cirúrgico e sensibilidade térmica (Rallis *et al*., 2006; Bakathir *et al*., 2008; Campbell & Lin, 2009; Kuhlefelt *et al*., 2010). De acordo com Gilardino *et al.* (2009), as taxas de complicação associadas ao uso de placas e parafusos de Ti são influenciadas também pela indicação cirúrgica que motivou o seu uso, como cirurgia ortognática eletiva *versus* trauma facial, e fatores como fragmentação óssea, desvitalização dos tecidos e contaminação local. Não está claro, no entanto, até que ponto as placas são removidas de forma rotineira em casos assintomáticos (Thorén *et al*., 2008).

Em grande parte dos hospitais e serviços de Cirurgia Buco-Maxilo-Facial, as placas e os parafusos não são removidos após o período de reparo ósseo, sendo a remoção destes implantes realizada apenas nos casos que apresentem indicações clínicas (Mosbah *et al*., 2003; Theodossy *et al*., 2006). Controvérsias, contudo, ainda existem quanto à questão da necessidade de remoção destes dispositivos. Muitos cirurgiões defendem a retenção dos implantes, principalmente devido à falta de evidências científicas que justifiquem sua remoção, bem como devido à morbidade associada a um novo procedimento cirúrgico (Hayes *et al*., 2010). Taxas de remoção variam em diferentes países e estudos mostram que a prática da remoção rotineira de implantes cirúrgicos é determinada muitas vezes por fatores culturais e não pelo conhecimento baseado em evidências sobre os verdadeiros resultados funcionais deste procedimento cirúrgico (Vos *et al*., 2012).

EMPREGO DA MICROSCOPIA ÓPTICA, DA MICROSCOPIA ELETRÔNICA DE VARREDURA E DA ESPECTROSCOPIA POR ENERGIA DISPERSIVA DE RAIOS-X NA ANÁLISE DE PARTÍCULAS METÁLICAS PRESENTES NOS TECIDOS

Estudos de microscopia óptica, microscopia eletrônica de transmissão e de varredura foram previamente realizados por diferentes autores para caracterização das partículas metálicas nos tecidos adjacentes a placas de Ti (Rosenberg *et al.*, 1993; Schliephake *et al*., 1993; Torgersen *et al.*, 1995; Theologie-Lygidakis *et al*., 2007). Uma desvantagem da utilização da microscopia óptica nestes estudos é o seu limite de resolução (Schliephake *et al*., 1993). Por outro lado, o microscópio eletrônico de varredura (MEV), ao utilizar feixes de elétrons no lugar de fótons, utilizados em um microscópio óptico convencional, permite solucionar o problema da resolução relacionada à fonte de luz branca (Dedavid *et al*., 2007).

A microscopia eletrônica de transmissão, por sua vez, exige o preparo de secções de tecido ultrafinas, de aproximadamente 80 nm. Desta forma, a dureza dos detritos de metal pode causar riscos e distorção das amostras durante a preparação (Schliephake *et al*., 1993). Em contraste, o MEV permite a observação dos espécimes com maior espessura (Sammons & Marquis, 1997).

A Espectroscopia por energia dispersiva de raios-X (EDS) é uma técnica eficaz, que possibilita revelar os elementos e, por conseguinte, os compostos químicos presentes em uma determinada amostra. Basicamente, a análise por EDS consiste na detecção dos raios-X característicos produzidos por cada elemento após o bombardeamento de uma amostra com elétrons de alta energia em um microscópio eletrônico. O que faz do EDS particularmente útil é que a quantidade de raios X emitida por cada elemento presente em uma amostra tem uma relação direta com a concentração do referido elemento (massa atômica ou fração). Desta forma, é possível avaliar as concentrações das várias substâncias químicas presentes em uma amostra. Todos os elementos exceto o hidrogênio e o hélio são capazes de produzir raios-X característicos (Taylor, 2014).

A hipótese-teste aventada, que justificou a realização do presente trabalho, foi a de que debris metálicos são liberados para os tecidos a partir das placas e parafusos de osteossíntese, esta liberação pode ocorrer de forma gradual

ou tempo-dependente, é capaz de induzir alterações teciduais e estar associada à sintomatologia clínica.

O objetivo do presente estudo foi avaliar os achados histopatológicos de fragmentos de tecidos adjacentes a placas e parafusos para osteossíntese removidos devido indicações clínicas, identificar a composição química das partículas metálicas encontradas nestes tecidos e relacionar os achados histopatológicos e ultraestruturais com os aspectos clínicos apresentados pelos pacientes dos quais as placas foram removidas.

CAPÍTULO 1

COULD MAXILLOFACIAL OSTEOSYNTHESIS PLATES INDUCE TISSUE RESPONSE AND CLINICAL COMPLICATIONS?

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ABSTRACT

The aim of the present study was to assess the histopathological findings of tissue specimens adjacent to osteosynthesis maxillofacial plates and screws, as well as to identify the chemical composition of the metal particles found in these specimens and to correlate the histopathological and ultrastructural findings with the patient´s clinical data. The experimental population comprised 38 patients from whom plates and associated screws were removed due to clinical indications and curettage of a soft tissue specimen was performed. Patient records were reviewed to obtain data related to surgical treatment. Tissue specimens were analyzed using optical microscopy. Specimens in which debris was found in the optical microscopy were also analyzed using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). Forty-four tissue specimens were obtained. During the optical microscopy, debris was identified in 95.45% of the specimens. Areas of inflammation were identified in 77.27% of the specimens and multinucleated giant cells were found in 34.09% of the specimens. Inflammatory and giant cells were not confined to areas of debris. Titanium (Ti) was identified in 85% of the tissue specimens analyzed using SEM and EDS. Besides Ti, other metals and non-metallic elements were found. Many metal elements identified in the tissue specimens can be attributed to contaminants resulting from the manufacture of plates and screws or debris released during the insertion or removal of implants. However, no obvious histopathological alterations were associated with debris and no correlation was found between debris and the plate retention period.

Keywords: Biocompatible materials; Titanium; Osteosynthesis plates; Implants

INTRODUCTION

Titanium (Ti) is widely used as an implant material, due to its proven biocompatibility and the high corrosion resistance of commercially pure titanium and its alloys[1]. Despite the excellent clinical performance of titanium plates in maxillofacial surgery, doubts have emerged about their long-term behavior in tissues and their potential local and systemic side effects [2].

Titanium plates and screws, which are resistant to corrosion due to the surface oxide film, have been classified as “harmless” in many studies and as such, their removal after bone healing in the maxillofacial area is not considered mandatory[3,4]. However, one concern about leaving metal plates and screws in tissues is the possibility of corrosion[5]. There are also reports of hypersensitive reactions to titanium[6].

Although Ti is widely acknowledged to be well tolerated by living tissues, causing minimal local reactions, a number of studies have shown that Ti and Ti alloy implants release metal ions and particles into surrounding tissues[3,6-9]. Several mechanisms, including mechanical wear and electrochemical corrosion, could be the reason for this local metal release and potential dissemination[10]. The clinical significance and long-term side-effects of trace amounts of titanium in the tissues around plates is still unknown[6].

Thus, the scientific literature on oral and maxillofacial surgery contains much opinion but little data related to the removal of internal fixation devices and consequently, the long-term management of these devices remains controversial[11]. In most cases, titanium plates are not removed following bone union unless a clinical indication is provided[1].

The aim of the present study was to assess the histopathological findings of tissue specimens adjacent to osteosynthesis maxillofacial plates and screws removed from patients due to clinical indications, as well as to identify the chemical composition of metal particles found in these tissues and to correlate the histopathological and ultrastructural findings with the clinical data of the patients from whom the plates and associated screws were removed.

MATERIALS AND METHODS

This study was approved by the human research ethics committee of Piracicaba Dental School, under the number 019/2013. All patients signed a statement of informed consent prior to the procedure.

The experimental population for this observational retrospective study comprised 38 patients from whom plates and associated screws were removed (Figure 1) over a 8 year period (2007 to 2014) and curettage of a soft tissue

specimen was performed during the removal of implants. In all cases, the removal of plates and associated screws was performed due to postoperative complications or clinical indications. The surgeons involved in the present study do not usually remove osteosynthesis implants under asymptomatic conditions or without clinical indications. Plates and screws were removed from two different anatomical sites in six patients, and a soft tissue specimen was curetted from each site.

Patient records were examined and the following data were recorded: the type of surgical procedure that led to the insertion of the plates and screws; the time that elapsed between the insertion and removal of these devices (retention period); the indication for the removal of plates and screws and the anatomic site of removal.

During the retrieval of plates and screws, tissue specimens were excised from the overlying soft tissue or the underlying bone around the screw holes. These tissue samples were immediately fixed in 10% formaldehyde. Subsequently, the specimens were embedded in paraffin wax, sectioned with a microtome (RM 2165, Leica Microsystems, Wetzlar, Germany) and stained with hematoxylin and eosin in preparation for the light microscopy examination.

HISTOPATHOLOGICAL ANALYSIS - OPTICAL MICROSCOPY

All tissue specimens were studied using a light microscope (AX10, Carl Zeiss, Göttingen, Germany). The following aspects were recorded: deposits of debris; vital or devitalized bone; multinucleated giant cells; granulation tissue; fibrosis and inflammation. Inflammation, when found, was classified as acute, chronic or mixed, according to the type of cells found, as well as mild, moderate or severe, based on its intensity. Inflammation was defined as acute when polymorph nuclear granulocytes were predominant, chronic when sparse lymphocytes and plasma cells were found and mixed when polymorph nuclear granulocytes were also present.

Specimens in which debris was found in the light microscopy analysis were then prepared for the scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) analyses.

SCANNING ELECTRON MICROSCOPY AND ENERGY DISPERSIVE X-RAY SPECTROSCOPY ANALYSES

Sections of 5-7 µm (thickness) were cut from the resulting blocks using the RM 2165 microtome (Leica Microsystems, Wetzlar, Germany). Subsequently, the specimen sections were placed onto glass slides and maintained in a hot air oven (Soc FABBE Ltda., São Paulo, Brazil) at 55°C for 24 hours. For the deparaffinization process, the specimens were embedded in Xylol for 20 minutes, during which time the solution was changed twice, and in alcohol (99.5%) for 10 minutes, also with two changes of the solution during this period, at room temperature.

After deparaffinization, each microscope slide with the specimen sections was fixed to an aluminum stub with a carbon strip and sputter coated with carbon in a Denton Vacuum LLC Desk II (Denton Vacuum Inc, Moorestown, NJ, USA) for five minutes to achieve an adequate topographic contrast, thereby enhancing the detail as required for the analysis. The specimens were analyzed using a JSM 5600 LV scanning electron microscope (JEOL, Tokyo, Japan) to locate metal particles, based on backscattered electron emission. When metal particles were located, secondary electron emission was used to confirm the presence of particles in the tissue section, rather than on it.

During the energy dispersive X-ray spectroscopy analysis, the chemical composition of particles was determined by a Vantage digital microanalysis system (C10001 model, Noran Instruments, Middleton, USA). Backscattered SEM photomicrographs were also analyzed, using ImageJ software (National Institute of Health, Maryland, USA) to measure the diameter of particles.

SPSS software (version 18.0) was used to conduct the chi-squared (x^2) and the non-parametric Mann-Whitney tests during the statistical analysis of the histopathological, ultrastructural and clinical findings. The results were considered statistically significant when $p < 0.05$.

RESULTS

Forty-four soft tissue specimens were obtained from 38 patients who had maxillofacial plates and associated screws removed. In six of these 38 patients, the

plates and screws were removed from two different anatomical sites, resulting in two different soft tissue specimens for each patient. There were 29 male and nine female patients, with an age range of 13 to 61 years (mean age of 34.64 years). The plates had been *in situ* for two months to 10 years (mean of 18.41 months).

Records were not available for four patients. Thus, the data related to the type of surgical procedure that led to the insertion of the plates and screws, the retention period and the indication for their removal could not be collected for these individuals. Fracture repair in the maxillofacial area was the main reason for the insertion of plates/screws (59.10% of the cases). The mandible was the most common anatomical site of plate removal (75% of the tissue specimens). Table 1 displays the data collected from patient records relating to indications for plate/screw insertion/removal and the anatomical site of removal.

Table 1. Indications for plate and screw insertion, reasons for implant removal and anatomical site of removal

Indication for plate/screw insertion	**Specimens (n)**	**Percentage**
Fracture repair	26	59.10%
Orthognathic surgery	11	25%
Pathology/Reconstructive surgery	2	4.54%
Not available	5	11.36%
Reason for plate/screw removal		
Inflammation/ Infection	21	47.72%
Elective secondary procedures	9	20.45%
Non-union	2	4.54%
Other (Palpability, discomfort, skeletal development)	5	11.36%
Not available	7	15.90%
Anatomical site of plate/screw removal		
Mandible	33	75%
Maxilla	8	18.18%
Zygomatic bone	2	4.54%
Not available	1	2.27%

Clinical signs of inflammation, associated with history of postoperative infection, were the main reason for plate removal, corresponding to 21 cases (47.72%). In these cases, signs of inflammation (redness, pain, swelling) were clinically evident, and a history of postoperative infection, previously treated with the aid of antibiotics or under treatment while the surgical procedure for plate removal

was performed, was described in the charts. Other reasons included the need for a second surgical procedure due to non-union (4.54%), local discomfort due to the use of a dental prosthesis (2.27%), plate palpability (4.54%), skeletal development (2.27%) and elective secondary procedures (20.45%), such as bone graft surgery and the insertion of dental implants. The reason for plate removal was not available for seven cases (15.90%).

All the soft tissue specimens exhibited varying degrees of fibrosis (Figure 2A). Debris was located in the optical microscopy for 42 (95.45%) of the 44 specimens and comprised granular deposits and larger irregular particles. The debris was extracellular and was dispersed between collagen fibers (Figure 2B-D).

Areas of inflammation were identified in 34 (77.27%) specimens, of which 15 (44.11%) were classified as mild inflammation, 12 (35.29%) were classified as moderate and 7 (20.58%) were classified as severe. Chronic inflammation was observed in 28 (82.35%) specimens, while mixed inflammation was found in 6 (17.64%) specimens. Ten specimens did not exhibit areas of inflammation in the optical microscopy. Areas of granulation tissue were confirmed in three (6.81%) specimens and giant cells were found in 15 (34.09%) specimens (Figure 2E). The inflammatory cells and multinucleated giant cell reactions were not confined to areas of metal impregnation. No statistical significance was found between the presence of debris and multinucleated giant cells in the optical microscopy (x^2= 0.23; p= 0.627).

Fragments of bone were observed in 35 (79.54%) specimens. Of these, six (17.14%) contained both vital and devitalized bone fragments, 23 (65.71%) only contained devitalized bone and six (17.14%) specimens only contained vital bone. These bone fragments may have detached during plate removal. Dystrophic calcifications were observed in five (11.36%) specimens (Figure 2F). Six (13.63%) specimens exhibited areas of bacterial colonies (Figure 2G-H). Four (66.66%) of these six tissue specimens were obtained from patients in whom clinical signs of inflammation associated with history of infection were the reason for plate removal. The other two specimens were obtained from the same patient, whose record was not available.

No statistically significant differences were found between the presence of clinical signs of inflammation in patients with history of postoperative infection, the main reason for plate removal, and the histopathological findings related to the

presence of multinucleated giant cells, intense inflammation, chronic inflammation and devitalized bone. A statistically significant difference was found between clinical inflammation with history of infection and the presence of bacteria colonies in the optical microscopy (x^2= 4.40; p= 0.03). Of the 42 specimens in which deposits of debris were visualized in the optical microscopy, forty (95.23%) were analyzed using SEM and EDS. It was not possible to obtain an appropriate preparation from the existing blocks for two of the specimens.

Metal debris, ranging from 2 to 90 µm in diameter, was identified in the SEM analysis (Figure 3). The chemical composition of the debris was confirmed in the EDS analysis (Figure 4). Ti was identified as one of the metal particles´ constituent elements in 34 (85%) specimens. The chemical composition of the metal particles found in the six specimens that did not contain Ti are displayed in Table 2. In specimens in which Ti was found, other metals were also identified in the constitution of the particles. Of the thirty-four specimens containing Ti, nine (26.47%) also exhibited particles composed of other metals in which Ti was not found.

Besides titanium, the following elements were identified in the EDS analysis: iron (Fe); aluminum (Al); vanadium (V); nickel (Ni); barium (Ba); calcium (Ca); cobalt (Co); copper (Cu); lead (Pb); chlorine (Cl); chromium (Cr); sulfur (S); potassium (K); magnesium (Mg); manganese (Mn); molybdenum (Mo); sodium (Na); silicon (Si) and zinc (Zn). Table 3 shows the number of specimens in which each element was identified. The Ti value ranged between 6.51% and 93.53%, when considering the element weight value (Wt%). Nevertheless, this value should not be used to express the total amount of Ti per specimen, given that each sample only represented a section of the soft tissue curettage.

No statistically significant differences were found between the reason for plate insertion and the presence of particles in the optical microscopy analysis (x^2= 0.51/ p= 0.474 for fracture repair; x^2= 0.40/ p= 0.525 for orthognathic surgery) or between the reason for plate insertion and the presence of Ti in the EDS analysis (x^2= 2.9/ p= 0.08 for fracture repair; x^2= 2.55/ p= 0.110 for orthognathic surgery). No statistically significant differences were found between the presence of the elements Al, Ba, Co, Cr, Cu, Fe, Mo, Ni, Pb, V and Zn and the histopathological findings. When considering inflammation intensity and the presence of debris in the optical microscopy analysis, as well as inflammation intensity x identification of Ti in the EDS

analysis, statistically significant differences were found between severe inflammation and the presence of debris (x^2= 3.97; p= 0.04) and between severe inflammation and the presence of Ti (x^2= 3.89; p= 0.04), respectively.

No statistically significant differences were found between the implant retention period and inflammation, the presence of Ti, indications for implant insertion or the reason for implant removal (Mann-Whitney test).

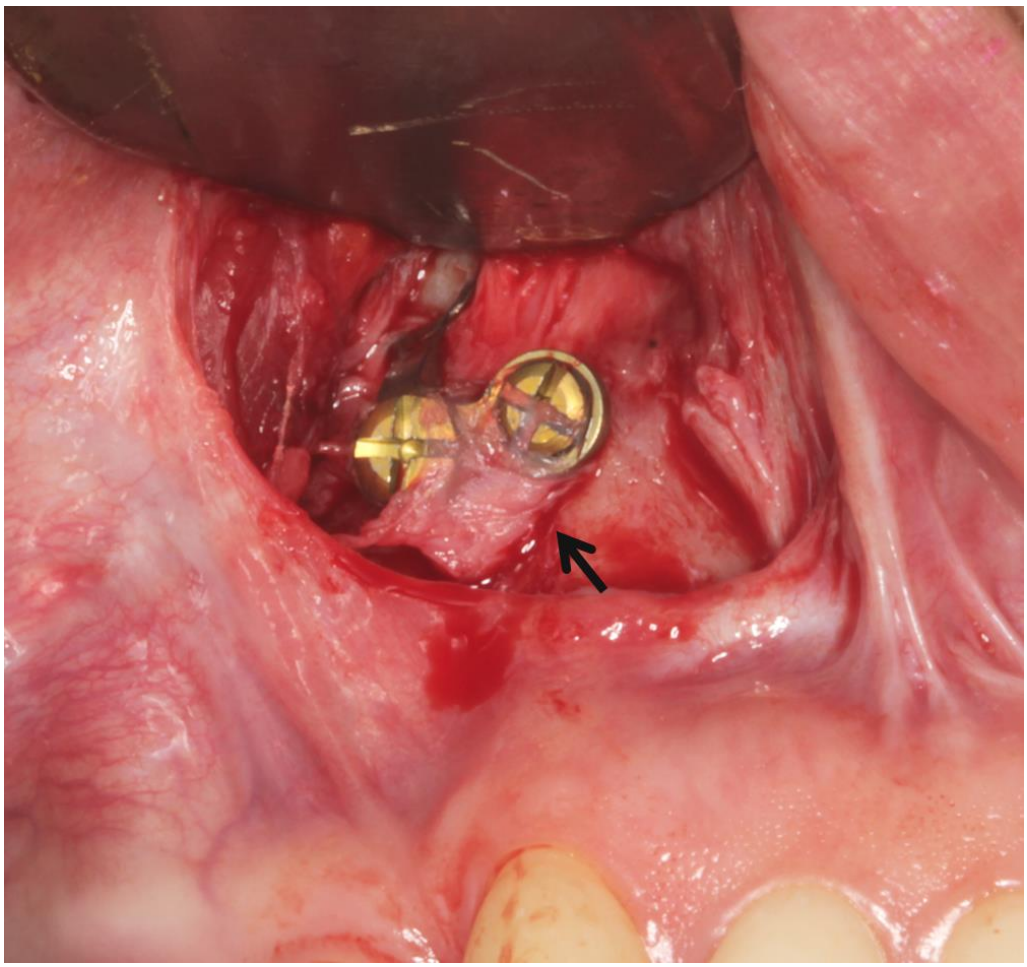

Figure 1. Surgical maxillary plate and screw exposure before removal. Soft tissue visualized over the implants before curettage (black arrow).

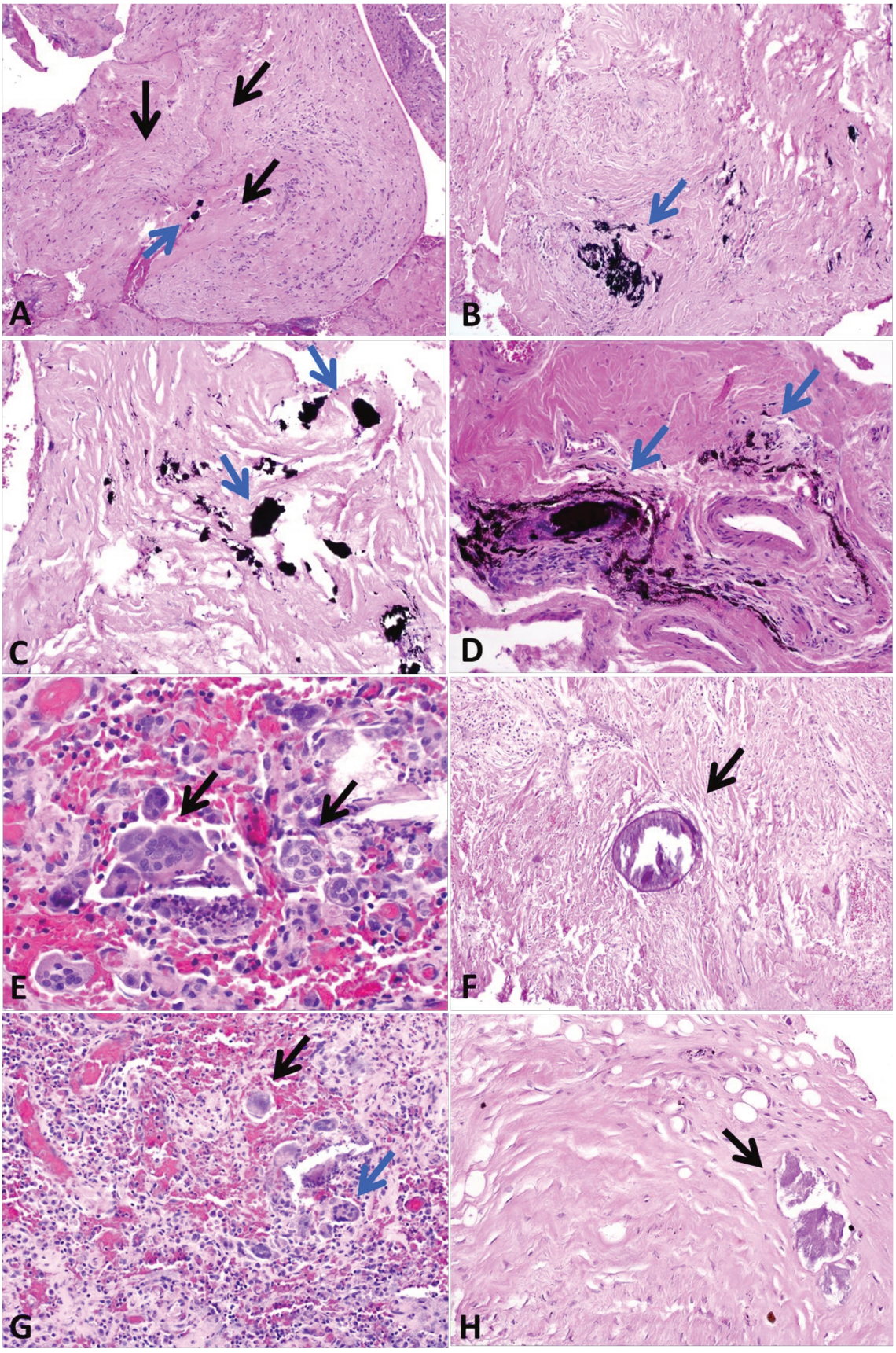

Figure 2 (A-H). Photomicrographs of the histopathological findings: A. Metal particles (blue arrow) and fibrosis (black arrows) in a tissue specimen (HE 100x); B-D. Metal

particles in different tissue specimens (blue arrows) - HE 100X in B and HE 200x in C-D; E. Area containing multiple giant cells (HE 400x); F. Dystrophic calcification in a tissue specimen; G. Area of inflammatory infiltrate containing bacteria (black arrow) in association with giant cells (blue arrow) (HE 200X); H. Bacterial colony (black arrow) observed in a tissue specimen.

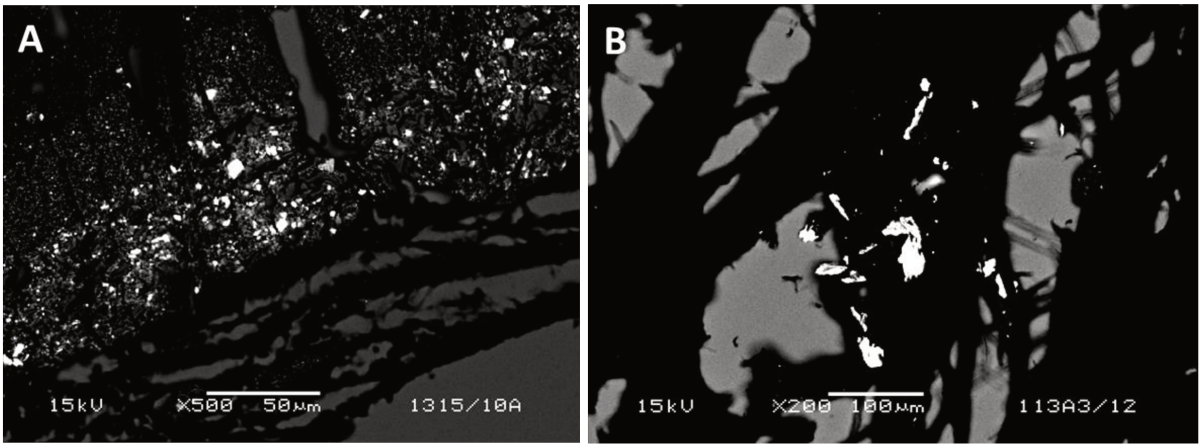

Figure 3 (A-B). Backscattered SEM photomicrographs showing the metal particles in two different tissue specimens.

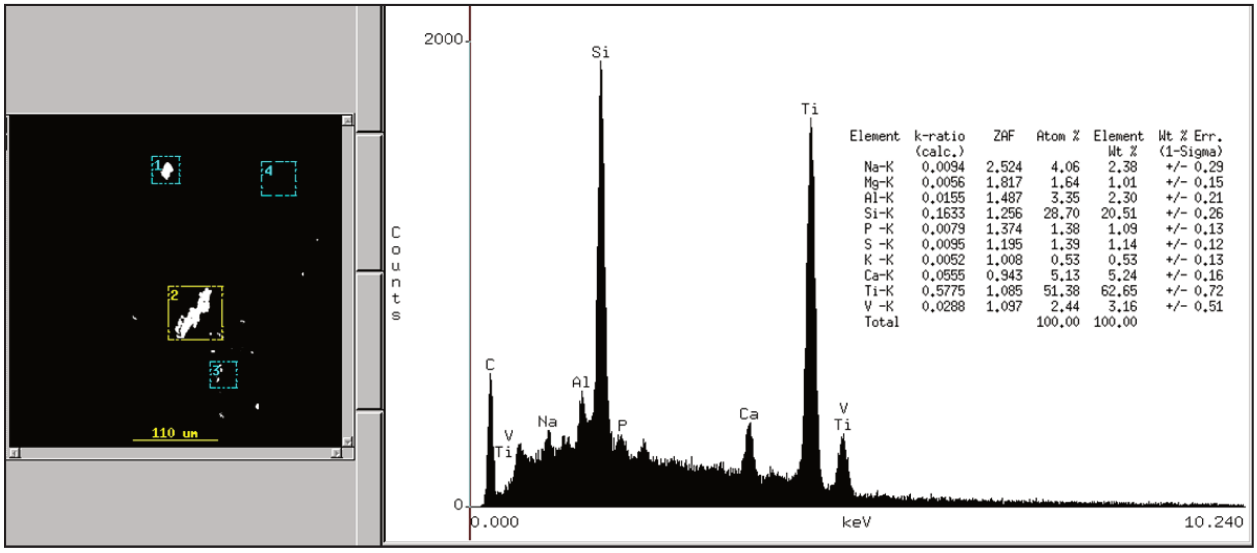

Figure 4. EDS analysis of a tissue specimen containing titanium in the composition of metal particles.

Table 2. Metal elements identified in the metal particles of the six specimens that did not contain Ti in the EDS analysis.

Specimen	Elements			
1	Fe			
2	Fe	Al	Cr	
3	Fe	Cr	Mg	
4	Fe	Al	Mg	
5	Fe	Cr	Mg	
6	Al	Cu	Mg	Zn

Table 3. Chemical elements identified in the EDS analysis and the number/percentage of specimens in which the elements were identified.

Element	Ti	Fe	Al	V	Ni	Ba	Ca	Cl	Co	Cr	Cu	K	Mg	Mn	Mo	Na	P	Pb	S	Si	Zn
Number of specimens (n)	34	21	29	6	3	1	40	3	1	10	1	38	36	2	2	39	13	2	35	40	2
Percentage of specimens (%)	85	52,5	72,5	15	7,5	2,5	100	7,5	2,5	25	2,5	95	90	5	5	97,5	32,5	5	87,5	100	5

DISCUSSION

Deposits of metal debris in tissues around titanium maxillofacial plates have been previously reported in a number of studies[3,6-8,12-14]. Many of these studies were motivated by different questions, including the following: Would the release of particles from internal fixation systems be gradual or time-dependent? Could the particles released induce a tissue response or be associated with local clinical complications? Could this evidence justify plate and screw removal after the bone healing period, even in the absence of symptoms? Similar questions also motivated the present study.

Plate damage secondary to either surgical manipulation or movement whilst *in situ* has been suggested as an important cause of titanium release[12,15]. Ray

et al.[16] identified finishing defects such as rough metal edges, protuberances and metal fragments on unused titanium and stainless steel miniplates and screws. According to the authors, these fragments can subsequently become detached during manipulation and metal debris can be deposited within the tissues at the implantation site.

Theologie-Lygidakis *et al.*[3] found deformation of the metal in 65% of 60 titanium plates retrieved from patients, at the contour of the screw holes. Surface defects were also identified on retrieved and control plates by Langford & Frame[5]. Their study supports the claim that the anodizing process helps reduce surface contamination, given that increased surface layer damage and a significantly increased frequency of surface contamination were found on the plain plates, when compared with the anodized plates. The authors stated that surface defects are most likely the result of the manufacturing process, which involves rolling, stamping, milling and polishing.

Acero *et al.*[7] also performed SEM analysis on removed miniplates and screws, citing defects and irregularities with hole-like appearances. The authors supposed that such defects may have occurred due to corrosion, with titanium particles released to surrounding tissues. Despite the titanium debris found in soft tissues adjacent to the removed plates, as well as the defects on the surface of the plates, no signs of corrosion or surface deterioration were observed on the retrieved plates and screws throughout the retention period in the studies conducted by Langford & Frame[6] and Theologie-Lygidakis *et al.*[3].

Besides titanium plate defects and irregularities, other possible sources of metal released during the osteosynthesis procedure are the following: contact between the rotating drill and the miniplate countersink during the preparation of the screw hole; the friction between the screw head and the screwdriver tip; or the friction between the self-tapping thread of the screw and the bone during screw insertion. According to Meningaud *et al.*[17], the release of metal from titanium plates used in maxillofacial surgery is usually mechanically-induced during the surgical process of osteosynthesis. The amount of Ti released is small and accumulates preferentially in local tissues, tending not to increase over time.

Although metal particles have been observed in tissue samples removed with Ti plates in several studies, the authors have not reported correlations between

inflammation and the presence of metal particles in the tissues analyzed[3], the amount of debris and the retention period of the implants[6,17], or the presence of metal debris and clinical symptoms[6,7]. In the present study, the authors found a correlation between severe inflammation and the presence of debris and severe inflammation and the presence of Ti in the tissues analyzed. However, the inflammatory cells were not limited to areas with metal particles and the inflammatory reaction was not more intense in these areas. These cells were scattered in different areas throughout the histological sections.

Schliephake *et al.*[12] observed metal particles located extracellularly within the collagenous tissue and a weak phagocytic activity with no evident inflammatory reactions 5-8 months after the plates and screws insertion. According to the authors, these findings are related to the mechanism of cellular uptake and transport, and suggest that the amount of metal particles in the tissues represents a stable state and it seems unlikely that all metal particles would be removed from the site of implantation 5-8 months after insertion of titanium plates and screws.

Unlike the study of Schliephake *et al.*[12], in the present study a phagocytic activity was not observed in the tissue specimens evaluated, even in the cases in which the retention period of the osteosynthesis plates was inferior to 5-8 months. Furthermore, in many cases the implants remained *in situ* for years and metal particles were visualized in these tissue samples, dispersed between collagen fibers, even in the case in which the period of retention was 10 years. Thus, considering the histopathological findings observed, it seems that the phagocytic activity and the mechanism of cellular transport may not be associated with the degradation of the metal particles over time, either during the first few months after installation of the plates, or during the subsequent years.

In the optical microscopy, metal particles were observed in 95.45% of the tissue specimens analyzed in the present study. This is a high percentage when compared to other studies that performed similar analysis on tissues adjacent to maxillofacial plates that had been[6,7,18]. However, Ti was only identified in 84.6% of the specimens analyzed using EDS. Other elements were also identified in the EDS analysis. The presence of Si in all of the specimens must be due to the glass slides used as support for the histological sections, while other elements such as Na, K, Cl and Ca could be related to biological tissues (bone and soft tissue), in which the

particles were inserted. The elements Ba, Co, Cu, Pb, S, Mg, Mn and Zn, which were found in a number of specimens, could be the result of contamination during the manufacturing process of plate and screw implants.

The presence of Fe, Al and V could be correlated with the composition of the titanium alloy used in internal fixation systems, such as the Ti-6Al-4V alloy, usually used in the manufacture of fixation screws. The presence of Fe, Ni, Cr and Mo could be attributed to the release of fragments and debris from stainless steel instruments used during the insertion and removal of plates and screws. Besides surgical instruments, drills for internal fixation systems and the disposable microtome blades used to cut the specimens are also manufactured from stainless steel and as such, represent a possible source of contamination.

Krischak *et al.*[19] stated that the surgical instruments used for implant removal are generally made of stainless steel, which can be a source of trace metal analysis errors and contamination during sample collection and the processing of biological tissues. In order to minimize contamination, the authors used instruments made of ceramics (zirconium dioxide) for the retrieval of commercially pure titanium (cp Ti) and stainless steel plates. Despite the use of zirconium dioxide instruments for implant removal, the authors found substantially higher concentrations of Fe, Cr, Mo and Ni in the tissue adjacent to the cp Ti plates than in the controls (tissues before treatment). Considering the composition of the plates, the increased concentration of these elements was attributed to the wear of the stainless steel instruments during implant insertion[19].

In a study by Theologie-Lygidakis *et al.*[3], deposits were found on 87% of the plates, consisting of organic remnants of proteinaceous origin. The presence of carbon, oxygen, nitrogen, sulphur, phosphorus and iron was confirmed in most of the deposits, whereas calcium, phosphorus, magnesium and strontium were also identified in bone-integrated regions. Si and Al were also detected and described as possible tissue contaminants. According to the authors, the deposits of debris detected in tissues under light microscopy were initially attributed to the presence of titanium particles. However, elemental analysis of the debris deposits did not confirm this. The authors concluded that the incidence of titanium deposits may have been overestimated.

Al and Si contaminants were also identified in the EDX surface analysis of control and retrieved plates and screws in studies by Langford & Frame[5] and Matthew *et al.*[14], both of which analyzed titanium and stainless steel plates using EDX and identified deposits of Al and Si on the surface of both types of plates. Previous studies have also identified Al and Si on the surfaces of titanium plates and in the soft tissues adjacent to titanium miniplates[20,21]. According to Langford & Frame[5], the contaminants are often found embedded in surface irregularities and could occur during manufacture as a result of polishing with aluminum oxide or silica carbide abrasives. In order to exclude the possibility that the Al detected in the EDX analysis could have been due to the backscatter from the aluminum base plate used to mount the specimens in the SEM, the authors studied two of the samples which were positive for Al contamination on both aluminum and graphite base plates. No differences were found in the frequency of Al contamination detection. According to the authors, other possible sources of Al contamination include atmospheric dust or plates autoclaving prior to clinical use.

Langford & Frame[6] examined 35 tissue specimens that were retrieved with titanium plates. Debris containing titanium was identified in 25 of the specimens. The authors observed different degrees of fibrosis in all the soft tissues and identified areas of inflammation, although there was no evidence of an inflammatory response or a giant cell reaction adjacent to the debris. Their findings are similar to those observed in the present study. In their study, no evidence of tissue degeneration was observed around the titanium particles. In other studies, degenerative tissue changes were also not recorded around metal particles, although Kim *et al.*[15] reported irregular deposition and degenerative tissue changes around the particles.

The fibrosis observed in all tissue specimens in the present study, as well as in other similar studies, appears to be associated with surgical trauma or instability in the fixation system. Acero *et al.*[7] found a statistically significant correlation between screw or plate mobility and fibrous tissue in the bone-titanium interface during the ultrastructural analysis.

Similarly, the inflammatory reactions observed in different areas along the histological sections could be correlated with other factors, regardless of the presence of metal debris in the tissues, such as the loss of screws. Inflammation may be associated with the presence of the fixing material, loose screw reactions or

implant exposure caused by suture dehiscence. Dehiscence leads to the exposure of plates and screws and may also result in biofilm-related implant infections[22]. Several studies have cited infection as the main reason for the symptomatic removal of titanium plates and screws[6,22-24]. The same was observed in the present study. However, no correlation was found between clinical signs of inflammation associated with history of infection as the main reason for implant removal and the histopathological findings, with the exception of the presence of bacteria in the optical microscopy.

The incidence of removal of titanium osteosynthesis plates and screws in oral and maxillofacial surgery varies widely in the literature. In many reported studies, postoperative complications and other clinical indications are the main reasons for these implants removal. The reported incidence of osteosynthesis implants removal in oral and maxillofacial surgery over the last fourteen years (2002 – 2015) can be seen in Table 4. Table 5 displays a review of the studies that evaluated tissue samples adjacent to titanium plates in oral and maxillofacial surgery, in human patients.

Factors that can possibly induce a major biological injury during the osteosynthesis procedure should be considered and minimized if possible. Care must be taken during the drilling procedure. If the heat generated when drilling is conducted without appropriate cooling, bone necrosis can increase around the screw, which may cause loosening in the post-operative period.

Katou *et al.*[13] studied the immuno-inflammatory responses in tissues surrounding titanium miniplates and found that the titanium particles released induce and maintain chronic inflammation and fibrous encapsulation. The authors confirmed the presence of CD68+ and CD11c+ macrophages containing titanium particles in the lysosomes and the lymphocytes CD4+ and CD8+ in tissue from patients more than 6 months after implant insertion. This suggests that chronic inflammation had continued. The authors stated that non-functioning plates and screws should be removed after bone healing.

Table 4. Review of the reported incidence of osteosynthesis metal implants removal in oral and maxillofacial surgery over the last fourteen years (2002 – 2015).

Authors	Country	Year of publication	Type of surgery	Patients (n)	Plates inserted (n)	Period of plates retention	Patients (n)/ removal rate (%)	Plates (n)/ removal rate (%)	Reasons for removal
Islamoglu *et al.*[25]	Turkey	2002	Maxillofacial trauma	66	296	----------	18 (27.27%)	21 (7.1%)	Clinical indications
Velich *et al.*[26]	Hungary	2002	- Maxillofacial trauma - Orthognathic surgery	452	1,396	Mean of 10.1 months	54 (11.95%)	108 (7.73%)	Clinical indications
Bhatt & Langford[27] †	England	2003	- Maxillofacial trauma - Orthognathic surgery - Reconstructive surgery	153	308	----------	28 (18.3%)	51 (16.55%)	Clinical indications
Mosbah *et al* [28] .§	United Kingdom	2003	- Maxillofacial trauma - Orthognathic surgery	658	----------	----------	65 (10%)	----------	Clinical indications
Bhatt *et al.*[1] §	United Kingdom	2005	- Maxillofacial trauma - Orthognathic surgery - Reconstructive surgery	153	308	----------	21 (13.7%)	32 (10.4%)	Clinical indications
Murthy & Lehman[29] §	United States	2005	Maxillofacial trauma	76	163	----------	5 (6.57%)	6 (3.68%)	Clinical indications
Nagase *et al.*[30]	Canada	2005	Maxillofacial trauma	135	497	----------	45 (33.3%)	135 (27.2%)	Clinical indications
Alpha *et al.*[31]	United States	2006	Orthognathic surgery: sagittal split osteotomy	533	1,066	----------	54 (10%)	70 (6.5%)	Clinical indications
Rallis *et al.*[32]	Greece	2006	Maxillofacial trauma	280	599	0.5 - 36 months (mean of 11.5 months)	27 (9.64%)	37 (6.17%)	Clinical indications
Theodossy *et al.*[33]	United Kingdom	2006	Orthognathic surgery: sagittal split osteotomy	80	160	2–12 months (mea of 5.5 months)	16 (20%)	25 (15.6%)	Clinical indications
Bakathir *et al.*[11]	Oman	2008	Maxillofacial trauma	465	----------	- Pediatric patients: 2- 10 months (mean of 3.2 months) - Adult patients:1-60 months (mean of 8.5 months)	109 (23.4%)* (53.2% of pediatric patients; 46.8% adult patients) * 12.9% for adult patients alone (excluding pediatric patients)	----------	- Pediatric patients (<16 yrs): routine removal - Adult patients (>16 yrs): Clinical indications
Haraji *et al.*[34]	Iran	2009	Orthognathic surgery: Le Fort I	142	----------	4-18 months	15 (10.6%)	----------	Clinical indications
O'Connell *et al.*[2]	Ireland	2009	- Maxillofacial trauma - Orthognathic surgery	535	1247	2.5–68 months (mean of 19 months)	30 (5.6%)	32 (2.56%)	Clinical indications

† Bhatt & Langford [27]: The authors affirmed that the plates removed were mostly titanium, although stainless steel, chrome-cobalt, and other unknown metals were encountered among the early cases.

§ The authors do not describe if the plates installed/ removed were made from titanium.

Table 4. Continuation

Authors	Country	Year of publication	Type of surgery	Patients (n)	Plates inserted (n)	Period of plates retention	Patients (n)/ removal rate	Plates (n)/ removal rate	Reasons for removal
Kuhlefelt *et al.*[35]	Finland	2010	Orthognathic surgery: sagittal split osteotomy	153	308	97-1154 days	29 (18.6%)	56 (18.2%)* *Plate removal was bilateral in 28 cases, although the reason for removal was unilateral in 14 of them	Clinical indications
Thorén *et al.*[23] §	Finland	2010	Maxillofacial trauma	238	436	29 days -2.7 years (mean of 8.8 months)	48 (20.2%)	76 (17.4%)	Clinical indications
Falter *et al.*[24]	Belgium	2011	Orthognathic surgery	570	3,197	2-65 months (mean of 9.9 months)	157 (27.5%)	622 (19.5%)	Clinical indications
Hanson *et al.*[36] §	United States	2011	Maxillofacial trauma	4,879	----------	----------	246 (5%)	----------	Clinical indications
Rauso *et al.*[37]	Italy	2011	- Maxillofacial trauma - Orthognathic surgery	164	660	----------	53 (32.3%)	103 (15.6%)	- Pediatric patients (<16 y): routine removal - Adult patients (>16 y): clinical indications
Kubota *et al.*[38]	Japan	2012	Maxillofacial trauma	138	345	101 - 371 days (median of 211 days)	96 (69.56%)	----------	Clinical indications
Kang *et al.*[39]	Korea	2014	Maxillofacial trauma: midfacial fractures	56	125	----------	5 (9%)	----------	Clinical indications
Verweij *et al.*[40]	The Netherlands	2014	Orthognathic surgery: sagittal split osteotomy	248* *Bicortical screws or plates	496 sites* *486 sites – bicortical screws; 10 sites – plates	----------	14 (5.64%)* 12 – bicortical screws; 2 – plates)	17 sites* *14 sites- bicortical screws; 3 sites - plates	Clinical indications
Little *et al.*[41]	United Kingdom	2015	Orthognathic surgery	202	854	84 – 1071 days (mean of 281 days)	21 (10.4%)	27 (3.2%)	Clinical indications
Llandro & Langford [42]	United Kingdom	2015	Maxillofacial trauma: orbitozygomatc complex fractures	216	307	111 – 972 days	6 (2.78%)	8 (2.6%)	Clinical indications

Table 5. Review of the studies that analyzed tissue samples adjacent to titanium plates in oral and maxillofacial surgery, in human patients

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Authors	Country	Year of publication	Type of surgery	Period of plates retention	Samples/ Patients (n)	Results of tissue samples analyses
Rosenberg *et al.*[18]	Switzerland	1993	- Maxillofacial trauma - Orthognathic surgery - Reconstructive surgery	3 – 32 months (mean of 8 months)	39 samples (24 patients)	Debris detected in 71.8% of the samples. The composition of debris was evaluated for one sample and consisted of Ti
Schliephake *et al.*[12]	Germany	1993	- Maxillofacial trauma	5-8 months	10 samples (10 patients)	Debris of Ti detected in the samples
Katou *et al.*[13]	Japan	1996	- Maxillofacial trauma	6-24 months (mean of 9.2 months)	17 samples (12 patients)	Debris detected in all the specimens. Ti identified in the debris
Kim *et al.*[15]	Korea	1997	- Maxillofacial trauma - Reconstructive surgery	2-18 months (mean of 7.1 months)	16 samples (14 patients)	Debris detected in all the specimens. The composition of the debris was not assessed
Acero *et al.*[7]	Spain	1999	- Maxillofacial trauma - Orthognathic surgery	1-40 months	20 samples (20 patients)	Debris detected in 80% of the tissue samples. The composition of the debris was not assessed
Meningaud *et al.*[17]	France	2001	- Maxillofacial trauma - Orthognathic surgery	15 days – 36 months (mean of 8 months)	51 samples (51 patients)	Ti identified in all the specimens
Langford & Frame[6]	United Kingdom	2002	- Maxillofacial trauma - Orthognathic surgery	1 month-13 years (mean of 21.8 months)	35 samples (31 patients)	Debris found in 71.4% of specimens. Ti detected in the debris
Theologie-Lygidakis *et al.*[3]	Greece	2007	- Maxillofacial trauma - Orthognathic surgery - Reconstructive surgery	4 - 36 months (mean of 7.4 months)	60 samples (44 patients)	Debris detected in 68% of the tissue specimens. Ti identified in debris only in few cases
Alcântara-Pinto *et al.*	Brazil	Present study	Maxillofacial trauma - Orthognathic surgery - Reconstructive surgery	2 - 120 months (mean of 18.41 months)	44 samples (38 patients)	Debris detected in 95.45% of the tissue specimens. Ti identified in 34 samples.

According to Langford & Frame[6], tissue responses in the vicinity of plates and screws can be caused by factors other than the biocompatibility of the implant material. Tissue trauma, hematoma, tissue healing and mechanical instability between the implant and the surrounding tissues may be at least partly responsible for the histological alterations observed in the tissues. The same authors stated that the presence of intracellular particles suggests that at least some of the metal is removed from the tissues by phagocytosis and it is possible that some small extracellular particles may have been phagocytosed and subsequently left in place after the necrosis of these phagocytic cells.

The correlation between metal debris in tissues adjacent to titanium plates and the duration of the plate retention period has not been confirmed in many studies[6,17]. The same is true for the correlation between these metal particles and the clinical symptoms exhibited by patients. Although particles were found in most of the specimens in the present study, regardless of whether the plates had been retained for months or years, no correlation was found between the implant retention period and clinical, histopathological or ultrastructural findings.

Although Ti and other metal elements were identified in most of the tissue specimens in the present study, a foreign body reaction was not observed in association with the metal particles identified in the optical microscopy analysis. Similarly, no degenerative alterations surrounding the metal particles were observed in the histological analysis. Therefore, the histological findings suggest that Ti does not promote the induction of an inflammatory response or a foreign body reaction *per se*. Similar findings have been described by other authors[3,6,7,8,12].

In a previous study conducted by the present authors, titanium plates and screws retrieved from patients due to clinical indications were analyzed in terms of metallographic properties and chemical composition. No correlation was found between the microscopic structure and chemical composition of titanium plates and screws and the need for their removal or their period of retention[4]. Thus, considering the analysis of retrieved titanium plates and screws and the results obtained from the study of tissues adjacent to these osteosynthesis implants, the authors of the present study do not see the need for the routine removal of osteosynthesis plates and screws after a bone healing period, except in cases of complaints from patients or clinical indications, due to infections, pain, dehiscence or screw loosening.

Furthermore, implant removal can be a complicated procedure[43]. The risk of a second surgical procedure, nerve injuries, discomfort, anesthesia, hematoma and the risk of releasing more metal during the removal of plates must be considered. Other aspects such as days lost from work, and the additional cost to society should also be considered[17].

CONCLUSION

Based on the results obtained in the present study, it can be concluded that:

1. Many metal elements identified in tissue specimens can be attributed to contaminants resulting from the manufacturing process of plates and screws or debris released during the insertion or removal of plates and screws.

2. Despite the identification of metal particles in the tissue samples assessed, obvious histopathological alterations related to these particles were not visualized.

3. No correlation was found between debris in tissue specimens adjacent to titanium plates and screws and the retention period of these implants, which suggests that particles are not released over time.

4. The findings of the present study suggest that there is no reason for the routine removal of titanium osteosynthesis plates and screws after the bone healing period.

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CONCLUSÃO

A partir dos resultados obtidos, pode-se concluir que:

1. A presença de debris não foi dependente do período de retenção das placas e parafusos para osteossíntese;

2. Apesar da identificação de partículas metálicas em grande parte dos espécimes, não foram visualizadas alterações histopatológicas evidentes relacionadas às partículas;

3. Desta forma, não foram verificados indícios que justifiquem a remoção rotineira destes implantes.

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APÊNDICE

DESCRIÇÃO DA METODOLOGIA

OBTENÇÃO DOS ESPÉCIMES TECIDUAIS E PREPARO DOS CORTES HISTOLÓGICOS PARA MICROSCOPIA ÓPTICA

Durante a remoção das placas e dos parafusos de osteossíntese, as amostras teciduais foram obtidas a partir do tecido mole sobrejacente às placas e aos parafusos, ou do tecido subjacente aos mesmos, visualizado após a remoção dos implantes.

Após obtidas, as amostras teciduais foram imediatamente fixadas em formol a 10%. Subsequentemente, as amostras foram incluídas em parafina líquida, para confecção dos blocos histológicos. Após o resfriamento e a solidificação da parafina, os blocos foram seccionados para confecção dos cortes histológicos, com emprego do micrótomo RM 2165 (Leica Microsystems, Wetzlar, Alemanha - Figura 1). Os cortes obtidos foram fixados individualmente em lâminas de vidro e corados com hematoxilina e eosina. Foram preparadas duas lâminas contendo os cortes histológicos a partir de cada bloco, para estudo através da microscopia óptica.

ANÁLISE HISTOPATOLÓGICA – MICROSCOPIA ÓPTICA

Todas as amostras teciduais foram estudadas utilizando o microscópio óptico AX10 (Carl Zeiss, Göttingen, Alemanha). Os seguintes aspectos foram avaliados: depósitos de detritos; presença de osso vital ou desvitalizado; células gigantes multinucleadas; tecido de granulação; fibrose e inflamação. A inflamação, quando encontrada, foi classificada como aguda, crónica ou mista, de acordo com o tipo de células encontradas, bem como leve, moderada ou intensa, de acordo com a intensidade. A inflamação foi definida como aguda quando granulócitos polimorfonucleares eram as células predominantes, crônica quando os linfócitos e plasmócitos foram identificados, e mista quando granulócitos polimorfonucleares também estavam presentes, além dos linfócitos e plasmócitos.

No caso das amostras em que foram visualizados debris na análise de microscopia óptica, foram confeccionados novos cortes histológicos e preparadas

novas lâminas para análise através da microscopia eletrônica de varredura (MEV) e da espectroscopia por energia dispersiva de raios-X (EDS).

Figura 1. Confecção do corte histológico para preparo das lâminas. Imagem mostrando o bloco contendo o tecido incluído em parafina e posicionado no micrótomo RM 2165 (Leica Microsystems, Wetzlar, Alemanha).

MICROSCOPIA ELETRÔNICA DE VARREDURA E ESPECTROSCOPIA POR ENERGIA DISPERSIVA DE RAIOS-X

Cortes de 5-7 µm de espessura foram obtidos a partir dos mesmos blocos histológicos utilizados para a confecção dos cortes estudados através da microscopia óptica. Para isso, foi utilizado também o micrótomo RM 2165 (Leica Microsystems, Wetzlar, Alemanha – Figura 1). Os cortes foram posicionados em lâminas de vidro e mantidos em estufa (Soe Fabbe Ltda., São Paulo, Brasil) a 55 ° C durante 24 horas. Subsequentemente, foram submetidos ao processo de desparafinização, para remoção da parafina e preparo para análise por MEV.

Para a desparafinização, as amostras foram embebidas em uma solução de xilol (Chemco Indústria e Comércio Ltda., São Paulo, Brasil) durante 20 minutos, tempo durante o qual a solução foi trocada duas vezes, e em álcool etílico absoluto P.A. - A.C.S.- 99,5% (Synth, São Paulo, Brasil) durante 10 minutos, também com duas trocas da solução durante este período, à temperatura ambiente.

Após a desparafinização, cada lâmina de vidro contendo as secções das amostras foi submetida ao processo de cobertura com carbono em uma metalizadora modelo Denton Vacuum LLC Desk II (Denton Vacuum Inc, New Jersey, EUA – Figura 2). Para isso, cada lâmina foi posicionada sobre um *stub* de alumínio e fixada a este com fitas de carbono nas margens laterais da lâmina (Figura 2B). O stub de alumínio contendo a lâmina foi então posicionado na plataforma da metalizadora (Figura 2A-B), o cilindro de vidro foi instalado (Figura 2C) e realizou-se o posicionamento do fio de carbono nos terminais do cabeçote da metalizadora. O cabeçote foi posicionado sobre o cilindro de vidro e o processo de cobertura com carbono foi realizado durante um período de cinco minutos.

Após o término do processo de cobertura com carbono, cada amostra foi analisada utilizando um microcópio eletrônico de varredura modelo JSM 5600 LV (JEOL, Tóquio, Japão – Figura 3A-C). O *stub* de alumínio contendo a lâmina com a amostra foi inicialmente fixado a um porta-*stub* e este foi posicionado no disco central do estágio do MEV (Figura 3B). A voltagem de aceleração empregada para a análise das amostras no MEV foi de 15kV.

As amostras foram inicialmente analisadas no MEV utilizando a emissão de elétrons retroespalhados, para visualização das partículas de metal (Figura 3C). Uma vez que as partículas eram localizadas, a emissão de elétrons secundários era utilizada para visualização da superfície da amostra, a fim de verificar se as partículas visualizadas encontravam-se realmente inseridas na amostra tecidual, ou se as partículas apresentavam-se superficialmente sobre esta. Caso fosse visualizado que as partículas apresentavam-se superficialmente sobre o tecido, a emissão de elétrons retroespalhados era novamente empregada para estudo de outra área da amostra e visualização de novas partículas que, uma vez localizadas, eram da mesma forma avaliadas também através da emissão de elétrons secundários.

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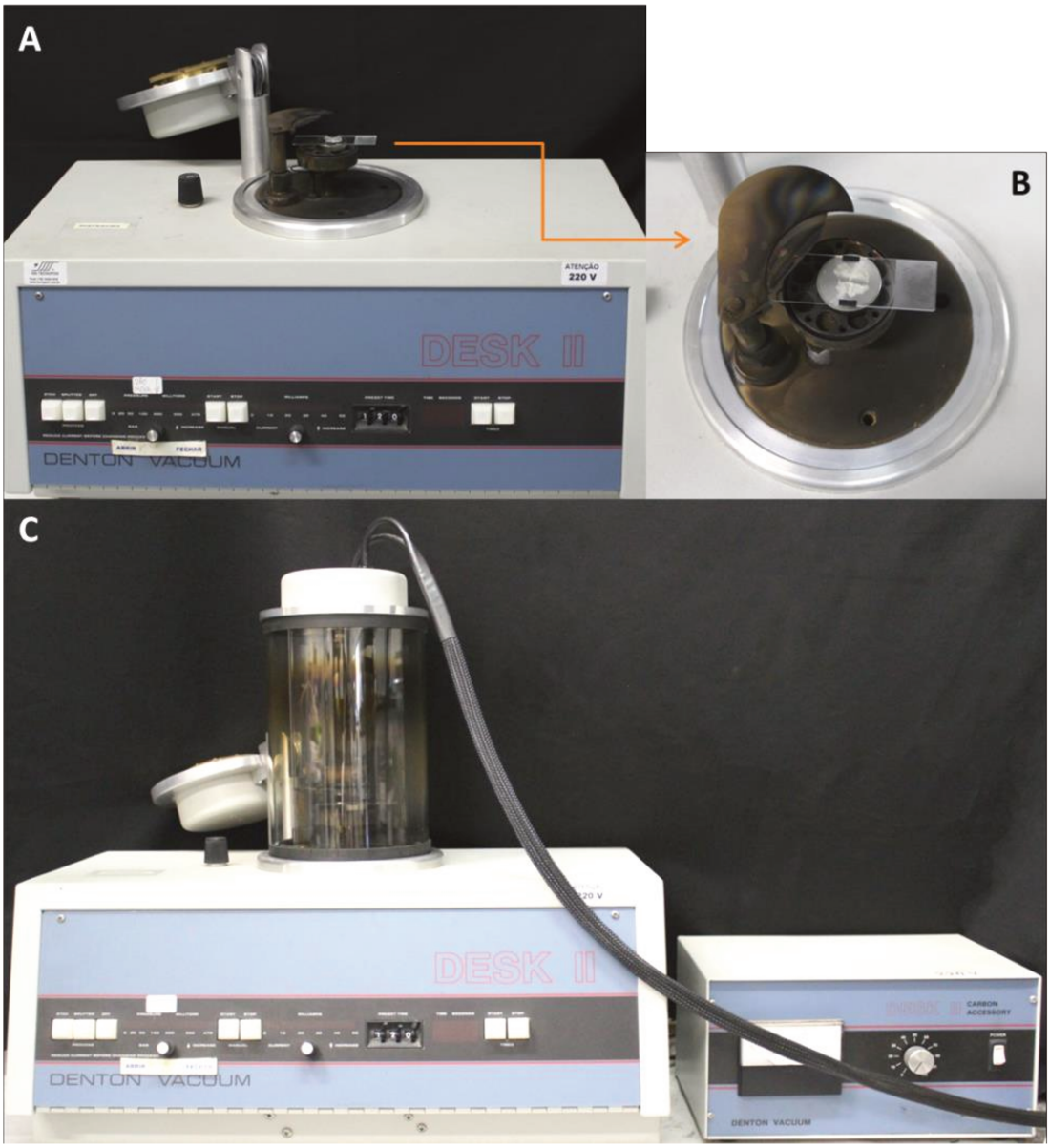

Figura 2. A. Lâmina de vidro posicionada na plataforma do Metalizador Denton Vacuum LLC Desk II (Denton Vacuum Inc, New Jersey, EUA); B. Detalhe da lâmina posicionada sobre o *stub* de alumínio na plataforma; C. Processo de cobertura da amostra com carbono, com o cilindro e o cabeçote em posição.

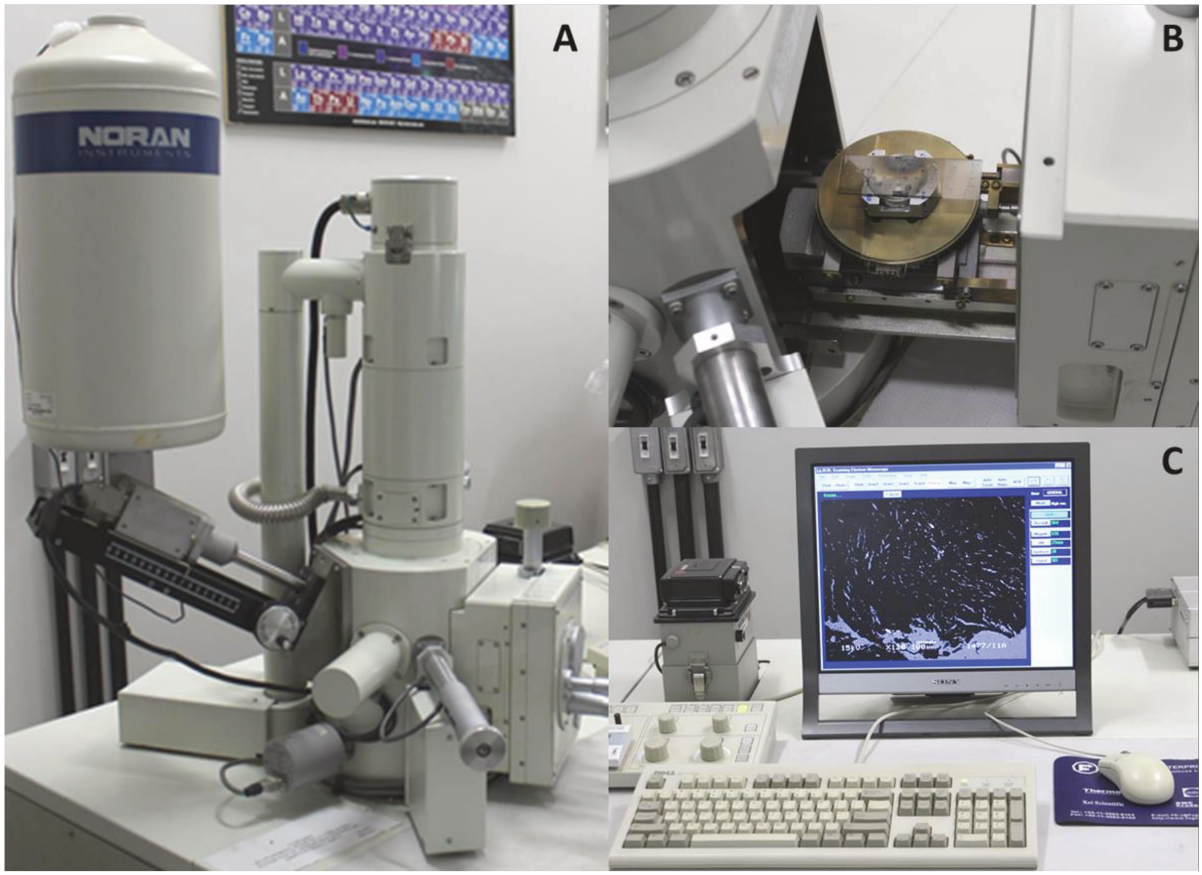

Figura 3. A. Microscópio eletrônico de varredura JSM 5600 LV (JEOL, Tóquio, Japão); B. Detalhe da lâmina de vidro posicionada sobre o *stub*, no estágio do MEV; C. Análise das imagens geradas através do estudo do espécime no MEV.

Ao estudar a superície da amostra e verificar, utilizando a emissão de elétrons secundários, que as partículas se apresentavam inseridas no tecido, e não sobre este, a emissão de elétrons retroespalhados era novamente empregada para avaliação da amostra e emprego da análise de espectroscopia por energia dispersiva de raios X (EDS).

Para a análise por EDS, empregada para determinação da composição química das partículas metálicas visualizadas, foi utilizado o Vantage digital microanalysis system, modelo C10001 (Noran Instruments, Middleton, EUA). Para tanto, uma a quatro áreas contendo as partículas foram delimitadas em um mesmo campo de visualização da amostra, de acordo com a disposição das partículas. Uma área que não continha partículas também foi delimitada dentro do mesmo campo e utilizada como área-controle (Figura 4). A partir da análise por EDS, os elementos

químicos contituintes das partículas, bem como do tecido em que estas estavam inseridas, em cada área delimitada dentro do campo de análise na amostra, foram identificados (Figura 4).

Fotomicrografias das áreas contendo as partículas nas amostras, obtidas no MEV durante a emissão de elétrons retroespalhados, foram também analisadas utilizando o software ImageJ (Instituto Nacional de Saúde, Maryland, EUA), a fim de se obter a mensuração do diâmetro das partículas

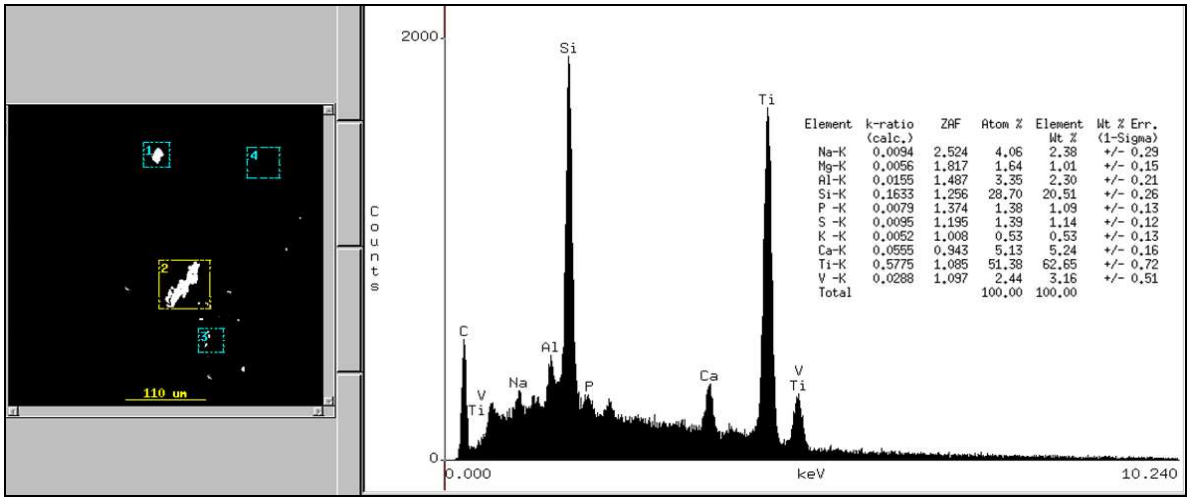

Figura 4. Análise por EDS de uma amostra tecidual, mostrando a delimitação das partículas a serem avaliadas e a identificação dos elementos químicos constituintes. No exemplo em questão, a área 2, contendo a maior partícula no campo, foi avaliada e os elementos químicos identificados podem ser visualizados do lado direito da imagem. A área 4 é o controle.

ANEXO 1

Certificado do Comitê de Ética em Pesquisa

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Análise metalográfica e análise de gases de placas e parafusos para osteossíntese e de implantes dentários removidos dos pacientes em casos de indicação clínica"**, protocolo nº 019/2013, dos pesquisadores Clarice Maia Soares de Alcântara Pinto e Marcio de Moraes, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 27/05/2013.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project **"Metallographic analysis and gases analysis of plates and screws for osteosynthesis and dental implants removed from patients in cases of clinical indication"**, register number 019/2013, of Clarice Maia Soares de Alcântara Pinto and Marcio de Moraes, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 05/27/2013.

Prof. Dr. Felippe Bevilacqua Prado
Secretário
CEP/FOP/UNICAMP

Profa. Dra. Lívia Maria Andaló Tenuta
Coordenadora
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

ANEXO 2

Declaração de não infração de direitos autorais.

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Processo_____
Rubrica_____

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DECLARAÇÃO

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Tese de Doutorado intitulada "ANÁLISE HISTOPATOLÓGICA, ULTRAESTRUTURAL E DA COMPOSIÇÃO QUÍMICA DE PARTÍCULAS METÁLICAS EM AMOSTRAS TECIDUAIS ADJACENTES A PLACAS E PARAFUSOS DE OSTEOSSÍNTESE ", não infringem os dispositivos da Lei nº 9.610/98, nem o direito autoral de qualquer editora.

Piracicaba, 11 de Setembro de 2015.

Clarice M. S. de A. Pinto
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ANEXO 3

Comprovação da submissão do artigo para publicação.

Elsevier Editorial System(tm) for
International Journal of Oral & Maxillofacial Surgery
Manuscript Draft

Manuscript Number:

Title: Histopathological, ultrastructural and chemical analyses of soft tissue adjacent to maxillofacial osteosynthesis plates

Article Type: Research Paper

Keywords: titanium; osteosynthesis plates; implants; biocompatible materials

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Abstract: The aim of the present study was to assess the histopathological findings of tissue specimens adjacent to osteosynthesis maxillofacial plates and screws, as well as to identify the chemical composition of the metal particles found in these specimens and to correlate the histopathological and ultrastructural findings with the patient´s clinical data. The experimental population comprised 38 patients from whom plates and associated screws were removed due to clinical indications and curettage of a tissue specimen was performed. Tissue specimens were analyzed using optical microscopy. Specimens in which debris was found were also analyzed using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). Forty-four tissue specimens were obtained. Debris was identified in 95.45% of the specimens. Areas of inflammation were identified in 77.27% of the specimens and were not confined to areas of debris. Titanium (Ti) was identified in 85% of the specimens analyzed using SEM and EDS. Besides Ti, other metals and non-metallic elements were found. Many metal elements can be attributed to contaminants resulting from the manufacture of plates/screws or debris released during the insertion/removal of implants. No obvious histopathological alterations were associated with debris and no correlation was found between debris and the plate