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ALEXANDRE RODRIGUES FREIRE

MECANOBIOLOGIA DO TECIDO ÓSSEO ALVEOLAR NA REGIÃO DOS MOLARES EM RATOS COM TRAUMA OCLUSAL DENTAL

MECHANOBIOLOGY OF ALVEOLAR BONE TISSUE IN THE MOLAR REGION IN RATS WITH DENTAL OCCLUSAL STRESS

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Universidade Estadual de Campinas

Faculdade de Odontologia de Piracicaba

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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 Doutor em Biologia Buco-Dental, Área de Anatomia.

Thesis presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Dental Biology, in Anatomy area.

Orientador: Prof. Dr. Paulo Henrique Ferreira Caria

Co-orientador: Prof. Dr. Felippe Bevilacqua Prado

Este exemplar corresponde à versão final da tese defendida pelo aluno Alexandre Rodrigues Freire e orientada pelo Prof. Dr. Paulo Henrique Ferreira Caria

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RESUMO

Os fenômenos mecanobiológicos envolvem as características mecânicas do tecido, a nível microscópico, relacionadas às mudanças de metabolismo, apresentando mudanças estruturais e fisiológicas. Os estímulos mecânicos na estrutura óssea implicam na presença de tensões e deformações que resultam alterações na remodelação óssea. Nos tecidos dentoalveolares, especialmente no periodonto de suporte, são conhecidas as alterações estruturais resultantes da perda do equilíbrio da oclusão, especialmente no trauma oclusal. Para entender como ocorrem tais alterações mecânicas e respostas biológicas específicas nestes locais, estudos recentes propõem a aplicação da teoria do mecanostato associada à simulação computacional por análise de elementos finitos. Foram apresentados dois estudos para demonstrar as alterações nos estímulos mecânicos computacionalmente e relacionar com as respostas biológicas que alteraram estruturalmente o osso alveolar de suporte na região dos molares. No primeiro estudo foram utilizados animais que se submeteram à cirurgia de extração do segundo e terceiros molares inferiores, unilateralmente, permanecendo o primeiro molar em oclusal isoladamente, o qual ficou sujeito a um trauma oclusal. A oclusão no primeiro molar foi simulada por análise de elementos finitos e os resultados foram comparados com resultados em análise histológica. O estudo conclui que as regiões com aumento de compressão mecânica devido ao dente estar isolado foram compatíveis com áreas de reabsorção observadas histologicamente. O segundo estudo apresentou um modelo experimental de trauma oclusal em animais com a cimentação de resina sobre a superfície oclusal dos molares superiores, unilateralmente. A mordida posterior foi simulada em análise de elementos finitos para observar os estímulos mecânicos no osso alveolar de suporte tanto no primeiro molar superior quanto no inferior do lado com o trauma. Em comparação foi realizada microtomografia computadorizada para avaliar os efeitos biológicos resultando em alteração estrutura, na qual o volume da crista óssea alveolar foi mensurado. O estudo conclui que aumento da compressão observada computacionalmente, possibilita entender a causa da redução de volume óssea na região de interesse, sendo essa redução maior no molar superior, ou seja, com a presença do material cimentado.

Palavras-chave: Oclusão Dentária Traumática; Análise de Elementos Finitos; Perda do Osso Alveolar.

ABSTRACT

The mechanobiology phenomena involve the mechanical characteristics of tissue in microscopic level, which is related to changes of metabolism and presenting structural and physiological alterations. The mechanical stimuli in bone structure imply in stresses and strain in the tissue which results in changes in bone remodeling. In dentoalveolar tissues, mainly in the supportive periodontium, the structural changes resulted by the loss of occlusal equilibrium are known. To understand how these mechanical changes occurs and its consequent biological responses, recent studies proposed the application of mechanostat theory associated to computational simulation by finite element analysis. Two studies were presented to demonstrate by computational method the changes in mechanical stimuli and relate to biological responses that resulted in structural changes in the alveolar bone support in molar region. In the first study, animals were submitted to extraction of second and third lower molars, unilaterally, and the first lower molar was kept, which was subject to occlusal stress. The molar occlusion was simulated by finite element analysis and the results were compared with histological analysis. The study concluded that the regions with increase of mechanical compression in isolated molar were compatible with resorption areas in histological observation. The second study presented an experimental model of occlusal stress in animals, where a resin block was cemented on the occlusal surface of upper molars, unilaterally. The posterior occlusion was simulated by finite element analysis to observe the mechanical stimuli both first upper and lower molars, in the same side of occlusal stress. In comparison, the micro-CT was performed to evaluate the biological effect resulting in structural changes, in which the alveolar bone crest volume was measured. The study conclude that the increase of compression, observed in computational analysis, gives the possibility to understand the cause of bone volume reduction in the region of interest, being this reduction was major in the upper molar support tissue, i.e. with the presence of cemented material.

Keywords: Dental Occlusion, Traumatic. Finite Element Analysis. Alveolar Bone Loss

SUMÁRIO

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“O único lugar onde o sucesso vem antes do trabalho é no dicionário”

— Albert Einstein

INTRODUÇÃO

A descrição dos fenômenos mecanobiológicos no tecido ósseo envolve características mecânicas relacionadas às mudanças de metabolismo, assim como alterações nos sistemas locais, como as alterações na mastigação. Tais características são descritas por alterações estruturais e fisiológicas. A mecanosensibilidade é compreendida em multiescala, tanto em nível microscópico quanto em nível macroscópico. Assim, a mecânica é traduzida progressivamente e seus resultados tornam-se evidentes nas mudanças de forma e função dos órgãos, mudanças essas que interferem na função mecânica (estímulos mecânicos), que por sua vez interferem na mecanosensibilidade dos tecidos, formando um ciclo de relação estímulo/resposta (Jacobs et al., 2010).

Do ponto de vista mecânico, as cargas produzidas pelos sistemas, em nível macroscópico, produzem tensões e deformações teciduais as quais produzem mudanças complexas na matriz extracelular, que por sua vez estimulam as células. Do ponto de vista biológico, os sinais intracelulares contribuem para alterar o metabolismo celular, os sinais entre as células e a resposta tecidual. Pode-se definir que os sinais mecânicos são convertidos em sinais bioquímicos, ocorrendo mudanças nas moléculas. (Stoltz & Wang, 2002; Henneman et al., 2008). De acordo com Wolff (1892) as alterações mecânicas definem a conformação da arquitetura óssea uma vez que cada alteração na forma e função do osso, ou na sua função apenas, é seguida por alterações definidas na arquitetura interna e definem igualmente a alteração na sua estrutura externa de acordo com as leis matemáticas.

Questões sobre como ocorrem as alterações no esqueleto humano frente aos estímulos mecânicos permitem estudar o comportamento mecânico do osso em nível tecidual, conforme demonstrado em estudos recentes (Boccaccio et al., 2011; Betts & Müller, 2014; Wang et al., 2014; Oftadeh et al., 2015). Entender a mecanobiologia do tecido ósseo implica em determinar as deformações a nível tecidual e seus efeitos biofísicos em nível celular. A partir desta perspectiva, pode-se considerar cada ciclo de estímulos/respostas com foco nas forças mecânicas que ocorrem nas diversas atividades diárias.

A remodelação óssea que ocorre no esqueleto através de estímulos mecânicos é regulada basicamente por células que agem na formação e na reabsorção óssea, ou seja, osteoblastos e osteoclastos, respectivamente. A regulação deste processo ocorre com a atividade dos osteoblastos, que possuem uma interação complexa com os osteoclastos pelo eixo “RANKL-RANK-OPG” (Simonet et al., 1997; Khosla, 2001; Kobayashi et al., 2009; Luvizuto et al., 2010). Este eixo é um mecanismo de sinalização importante no sistema esquelético durante a remodelação óssea. O RANKL (receptor activator for nuclear factor K B ligant) é expresso por osteoblastos e ativa células T, enquanto que o RANK (receptor activator for nuclear factor K) é expresso em osteoclastos. O RANKL livremente secretado, assim como o presente na superfície dos osteblastos se liga à RANK, inibindo a diferenciação das células precursoras de osteoclastos em osteoclastos maduros. Assim, durante a formação e remodelação óssea, a maturação osteoclástica e o aumento da capacidade de reabsorção são estimulados pelo aumento da expressão da RANKL osteoblástica ligando-se à RANK nos osteoclastos e inibidas pela Osteoprogeterina (OPG) (Khosla, 2001; Jayakumar & DiSilvio, 2010).

As proteínas das células do tecido ósseo são mecanosensitivas devido ao fato de suas funções primárias serem estruturais. Deste modo, as forças que se distribuem no tecido são diretamente traduzidas em proteínas do citoesqueleto (Jacobs et al., 2010). Neste processo estudos mostram que os osteócitos possuem papel fundamental. Diferentes tipos de estímulos determinam toda a mecânica celular e pericelular dos osteócitos na matriz óssea, sendo eles o campo eletromagnético, as deformações celulares diretas e o fluxo de substância extracelular (Owan et al., 1997; Vander et al., 2000; You et al., 2000). Estes sinais ativam principalmente sinais como IGF, TGF-beta, PGE2, e também o sistema RANKL-OPG (Owan et al., 1997). Entender os processos relacionados com a função mecânica ainda é um desafio para a interpretação das respostas celulares considerando a interação entre o meio extra e intracelular. Nos últimos anos ocorreu maior aproximação entre estudos aplicados à mecânica da estrutura óssea e aplicados ao entendimento da remodelação óssea (Oftadeh et al., 2015).

Dentre as ações mecânicas que o esqueleto é submetido, a mastigação é uma que interfere nas relações estímulo/resposta por um sistema envolvendo três estruturas anatômicas importantes, principalmente do ponto de vista mecânico: dentes, ligamento periodontal (LPD) e osso alveolar. O processo resulta na estimulação mecânica que é distribuída para os dentes, em seguida LPD e osso alveolar. Os estímulos mecânicos distribuídos pelo LPD, sob forças funcionais têm sido estudados (Ona & Wakabayashi, 2006; Field et al., 2009; Poiate et al., 2009). As respostas biológicas do LPD a partir da aplicação de forças oclusais foram pesquisadas microscopicamente em modelos animais envolvendo força unidirecional (Naveh et al., 2012) ou multidirecional (Meitner, 2008).

A função mecânica do LPD ocorre pela absorção e distribuição das forças produzidas durante a função mastigatória e outros contatos dentais (Lindhe et al., 2003). Adaptações mecânicas dos tecidos dentais de suporte permitem o espessamento do LPD e a mobilidade dental (Milne et al., 2009). Estas adaptações estão envolvidas com o equilíbrio entre as forças de tração/compressão e as resultantes das atividades ósseas anabólicas-catabólicas, que geram respostas teciduais representadas por alterações vasculares, celulares e da matriz do tecido conjuntivo (Ericsson & Lindhe, 1982; McNeill, 2000).

O equilíbrio obtido na função mastigatória ocorre pela ação correta das forças musculares, movimento mandibular e contato oclusal. O desequilíbrio na função destas estruturas resulta em alterações estruturais dentoalveolares, especialmente no LPD e osso alveolar, e podem ser caracterizadas como hipofunção e hiperfunção mastigatórias. A hipofunção mastigatória ocorre a partir da diminuição parcial ou total das forças mastigatórias, ou a ausência de contato oclusal causado por perdas dentais (Denes et al., 2013). A resposta biológica causada pela diminuição da estimulação mecânica é caracterizada pelo estreitamento do espaço periodontal, vasoconstrição e deformações nas estruturas dos mecanoreceptores (Muramoto et al., 2000; Iwasaki-Hayashi et al., 2001). Outra característica importante é a perda óssea devido ao aumento dos osteoclastos (Enokida et al., 2005; Shimomoto et al., 2007).

A hiperfunção mastigatória é definida como o aumento das ações mecânicas podendo ser de sobrecarga muscular (Parker, 1990) ou o aumento da magnitude de cargas oclusais sobre os dentes (Nozaki et al. 2010). Estudos (Kaku et al., 2005; Goto et al., 2011; Oliveira-Diniz et al., 2012; Campos et al., 2014) consideram o aumento da carga oclusal como um desequilíbrio provocado por alterações oclusais como perdas dentais, mudanças de posição e os contatos prematuros pelo aumento da superfície oclusal no sentido cervico-oclusal (Beckmann et al., 1998; Usami et al., 2003). Neste último, a resultante da pressão sobre os dentes resulta num conjunto de cargas mecânicas alteradas em direção, sentido, localização e magnitude, considerando assim um trauma oclusal (Nozaki et al., 2010).

Estudos mostram que o trauma oclusal ocorre quando o peridonto ultrapassa seu limiar de adaptação funcional, ou seja, ocorre transmissão de forças excessivas para as estruturas adjacentes (Poiate et al., 2009). Estes efeitos podem ser primários, quando forças excessivas são aplicadas ao dente e o periodonto apresenta características estruturais normais, ou secundários, quando há alterações morfológicas significativas, principalmente com a perda dos tecidos de suporte (Nogueira-Filho et al., 2004; Feitosa et al., 2009). Os sinais clínicos já descritos em casos de trauma oclusal incluem o aumento da mobilidade dental e fraturas dentais (Pihlstrom et al., 1986). Radiograficamente, os sinais observados são o aumento do espaço periodontal e a reabsorção óssea (Kobayashi et al., 1998). Histologicamente, o osso alveolar apresenta locais de reabsorção e dependendo do tipo de trauma, o LPD pode apresentar processo inflamatório (Kaku et al., 2005; Goto et al., 2011; Oliveira-Diniz et al., 2012).

O modelo experimental para o estudo do trauma oclusal e seus efeitos sobre os tecidos periodontais de suporte têm sido a cimentação de materiais rígidos sobre a superfície oclusal dos molares de ratos (Kaku et al., 2005; Goto et al., 2011; Oliveira-Diniz et al., 2012; Campos et al., 2014). Recentemente o trauma também foi simulado experimentalmente através de dispositivo de aplicação de cargas *in vivo* (Nozaki et al., 2010). Numerosos estudos mostraram que, em dados qualitativos e estatísticos em análises histológicas, o resultado nas primeiras semanas com o trauma é a perda óssea da crista óssea alveolar, local referido ao ápice do septo interradicular até ao nível do terço cervical da raiz dos molares (Nozaki et al., 2010; Furfaro et al., 2014). Isso ocorre associado às alterações locais, como casos de processos inflamatórios, e alterações sistêmicas, como o diabetes. Consequentemente, também ocorre o aumento do espaço periodontal (Oliveira-Diniz et al., 2012). Estes estudos propõem que tais alterações acontecem devido às mudanças na remodelação óssea, cuja resposta está associada a mudanças mecânicas dos tecidos.

A microtomografia computadorizada (micro-CT) tem sido utilizada em modelos experimentais de estudo da remodelação óssea no septo interradicular dos primeiros molares de ratos (Naveh et al., 2012; Furfaro et al., 2014) possibilitando a obtenção de dados morfométricos tridimensionais, como o volume ósseo e o espaço trabecular, assim como a densidade óssea aparente, ou seja, de imagem. Tais dados são importantes para auxiliar na determinação das características mecânicas da estrutura óssea com as ações das forças oclusais, que podem ser simuladas computacionalmente utilizando a análise de elementos finitos (AEF) (Milne et al., 2009; Boccaccio et al., 2011).

A teoria do mecanostato tem sido discutida com a aplicação da AEF, pois os resultados computacionais são associados aos resultados laboratoriais. As mudanças nas tensões mecânicas no osso alveolar podem ser determinadas com as simulações tridimensionais a partir de cargas oclusais, onde as dissipações de energia, avaliadas nas tensões equivalentes de von-Mises foram verificadas tanto nas lâminas ósseas vestibular e lingual quanto no septo interradicular (Milne et al., 2009). Entretanto, os modelos mecanobiológicos utilizados em AEF podem apresentar limitações ao serem simplificados, mesmo não ocorrendo a perda dos resultados mais relevantes. Devido às tecnologias computacionais atuais, tais limitações podem ser corrigidas considerando a hipótese a ser testada. Assim, os algoritmos aplicados à mecanorregulação na estrutura óssea alveolar podem estar próximos à interpretação das respostas biológicas, porém as variações nas respostas biológicas sugerem cautela nas conclusões, nas quais ainda é um desafio na mecanobiologia computacional.

Alterações oclusais resultam em mudanças na distribuição de tensões e na estimulação mecânica para o osso alveolar. Estudos que envolveram este tipo de alteração biomecânica e sua associação às mudanças morfológicas do tecido ósseo são escassos na literatura. Esta tese abordou a associação acima descrita, por meio das análises histológicas, análise de micro-CT, e análise de elementos finitos.

CAPÍTULO 1:

MECHANOBIOLOGY OF MORPHOLOGICAL CHANGES IN ALVEOLAR BONE CAUSED BY OCCLUSAL STRESS AFTER TEETH LOSS. PRELIMINARY STUDY IN RATS*

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ABSTRACT

Morphological changes of alveolar bone through parafunctional factors have been widely studied using the rat as biological model, which the loss of dose/response equilibrium were stimulated by the occlusal surface raising. The purpose of this study was to evaluate the relationship between the mechanical stimuli and biological response in alveolar bone support of isolated tooth after extraction, using finite element analysis and histological characterization. Five rats were divided into two groups: a control group where the mandibular teeth were kept and an experimental group with isolated first molar after extraction of second and third molars. The animals were subjected in this condition during two weeks and, then, they were killed. The samples were scanned in microCT and were processed for histological analysis. From microCT images, were built the geometries of bony structure, dental and periodontal structures for finite element analysis. The finite element analysis was performed to simulate the occlusal force according the in vivo occlusal condition. The stress in alveolar bone was evaluated for mechanical behavior analysis and the bone architecture was evaluated in histological analysis, both at the alveolar crest. The results in the alveolar crest of isolated tooth showed major mechanical stimuli due to increase of compressive stress and occurrence of bone loss by bone resorption. The region of changes occurred similarly in both experimental and computational analyses. In conclusion, the occlusal stress condition increased the compressive stress resulted in bone loss, where can be established the different stress response in computational simulation and the experimental results of morphological changes.

Keywords: finite element analysis, rat, occlusal stress, bone resorption.

1. INTRODUCTION

Mechanical stimuli affect the bone formation (Bourrin et al., 1995). These stimuli regulate the bone mass and architecture. Due to its high turnover, in response to normal stimuli in occlusion, the alveolar bone is properly to study the mechanical stimuli/bone remodeling relationship (Vignery and Baron, 1980).

In dentistry, changes in occlusion result in changes in stimuli/response equilibrium and in alveolar bone remodeling (Henneman et al., 2008). The morphological changes of alveolar bone through parafunctional factors have been widely studied using the rat as biological model, which the loss of stimuli/response equilibrium were stimulated by the occlusal surface raising (Kaku et al., 2005; Shimomoto et al., 2007; Goto et al.; 2011), resulting in an excessive mechanical stress. These studies reported that the alveolar bone remodeling occurs mainly at the interradicular septum, in first molar bifurcation. These results show the significant importance and influence of mechanical stimuli in alveolar bone remodeling. However, it is a feel known the relationship between the features, quantity and kind of mechanical stimuli (stress/strain) and the alveolar bone tissue response (resorption/apposition).

In finite element analysis (FEA), it is possible to establish the relationship between the stress and the strain quantification after occlusal changes by tooth loss and bone remodeling associated to clinical data (Field et al., 2008). The same relation can be observed in animal model *in vivo* through the results in histologic analysis associated to FEA in orthodontic forces stimulation (Milne et al., 2009). The computational results in FEA are important due to the wide variation in mechanical systems that can be applied to biological model. Moreover, it can solve questions about the computational result interpretation, when the biological response is interest.

This study evaluated the relationship between mechanical stress and biological response in the alveolar bone tissue, whose relationship was established through FEA and the histologic analysis.

2. MATERIALS AND METHODS

2.1 Sample characterization

Five male Wistar rats (*Rattus norvegicus albinus*), weighing about 200–250 g, obtained from Multidisciplinary Center for Biological Research (CEMIB) at the State University of Campinas (Campinas, São Paulo, Brazil), were kept in separated cages, with controlled temperature (22 ± 2°C) and light-dark cycle (12/12 h) and free access to water and feed. All experiments were conducted in accordance to the guidelines of National Council for Control of Animal Experimentation (CONCEA). The animals were randomly divided into two groups, according described in the Figure 1. The experimental group (E) was composed by left mandibles, where the first molar was isolated by extractions of second and third molars. The control group (C) was composed by the right mandibles (without extractions). This study was approved by the Committee for Ethics in Animal Experimentation (CEUA/UNICAMP, Brazil. number 2570-1).

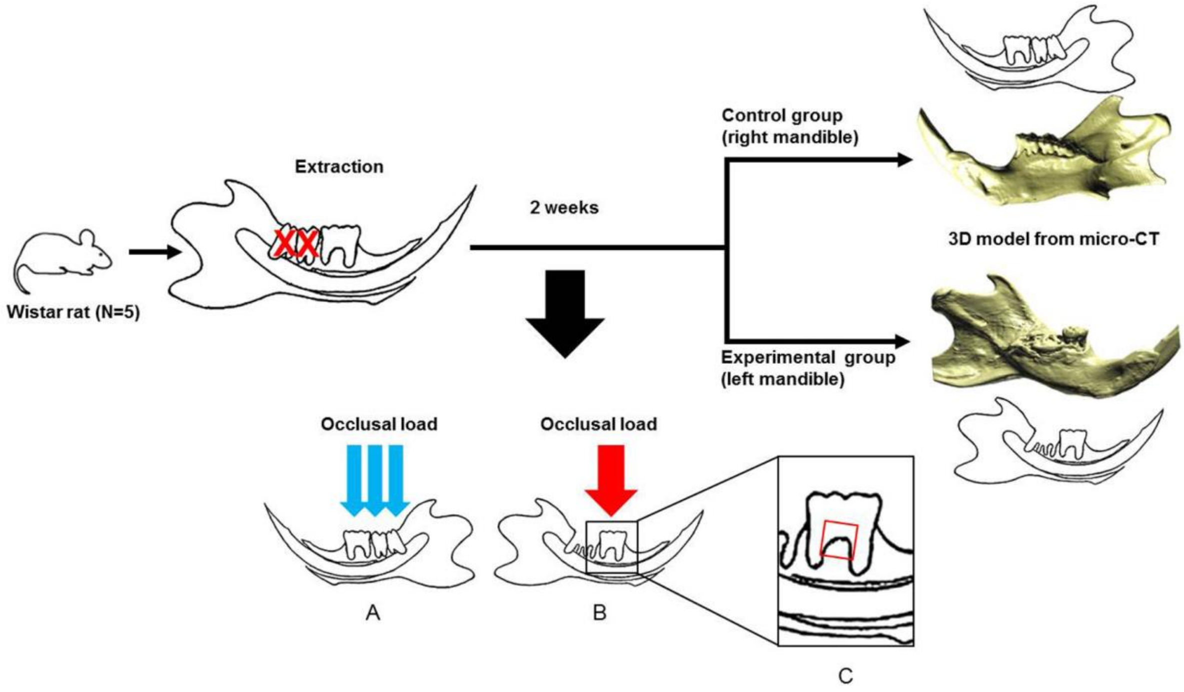

Figure 1. Study design included the sample, teeth extraction, post-extraction period and the group division according the side of the mandible. (A) Control group: the teeth were kept; (B) Experimental group: with isolated tooth (1st molar) during post-extraction period. (C) Region of interest: was evaluated the bone remodeling in the histological and FEA.

2.2 Experiment design

Occlusal stress was induced on the inferior first molar by extractions of second and third molar in the left mandibles under general anesthesia using injection of ketamine hydrochloride (40-87 mg/kg) (Dopalen®, Agribrands Brazil Ltda., Paulínia, São Paulo, Brasil) and muscle relaxant xylazine hydrochloride (5-13 mg/kg) (Anasedan®, Sespo Ind. Co. Ltda, Paulínia, São Paulo, Brasil), by intramuscular injection.

After anesthesia, the animals were positioned according Doku et al. (1966) method. The second and third molars were extracted using a Hollemback 3s (SSWhite/Duflex, Rio de Janeiro, Brazil), which was performed the syndesmotomy, dislocation and extraction at the left side. The sockets were sutured. The rats were kept in separated cages, with normal feed, at the post-surgery period.

After 2 weeks, the animals were killed by excessive anesthesia. The mandible were removed and fixed in para-formaldehyde (10%) during 24h.

2.3 MicroCT images acquisition

One sample of each group was scanned in micro-CT using the micro-tomography SkyScan 1174 (SkyScan, Belgium) with 50 Kv and 800 mA. The scanned images were configured with high resolution (1024 x 1024 pixels) and 30 µm pixel size. The images were imported in the NRecon software (SkyScan, Belgium), where the images were converted into grayscale and, then, were converted into DICOM format (Digital Imaging and Communications in Medicine).

2.4 Histological analysis

After fixation the samples were decalcified in EDTA 10% solution (pH 7.2). Then, the samples were included in paraffin and the slices were performed using a Leica® RM 2155 microtome (Leica Instruments, Germany) with 6 µm thickness and longitudinal section. The longitudinal sections allow the microscopy evaluation at the root bifurcation and alveolar crest (Kaku et al., 2005; Nozaki et al., 2010; Goto et al., 2011) (Figure 1). The selected sections located at the central region in bucco-lingual direction. The slides were counterstained with hematoxylin and eosin.

The sections were observed using a light microscope (Leica Instruments, Germany) and digitalized for qualitative evaluation at the region of interest (ROI), i.e. the region of bifurcation, in which the morphology and cellular characterization was observed.

2.5 Construction of three-dimensional geometry of rat mandible and dentoalveolar structures

From micro-CT images, the pixels of teeth, bone and periodontal ligament (PDL) were marked and converted into three-dimensional (3D) triangulated surface featured as stereolithographic format (STL) (Figure 2), using the InVesalius 3.0b software (Center for Information Technology “Renato Archer”, Campinas, Brazil. The region of interest was directed to molar region and, thus, the mandible was sectioned.

The CAD geometry was concluded by reverse engineering using the Rhinoceros 3D 5.0 software (McNeel & Associates, USA) (Figure 2). The final geometries presented the features according the groups of the study. The C group geometry presented a mandibular block containing the three molars and the E group presented the isolated first molar.

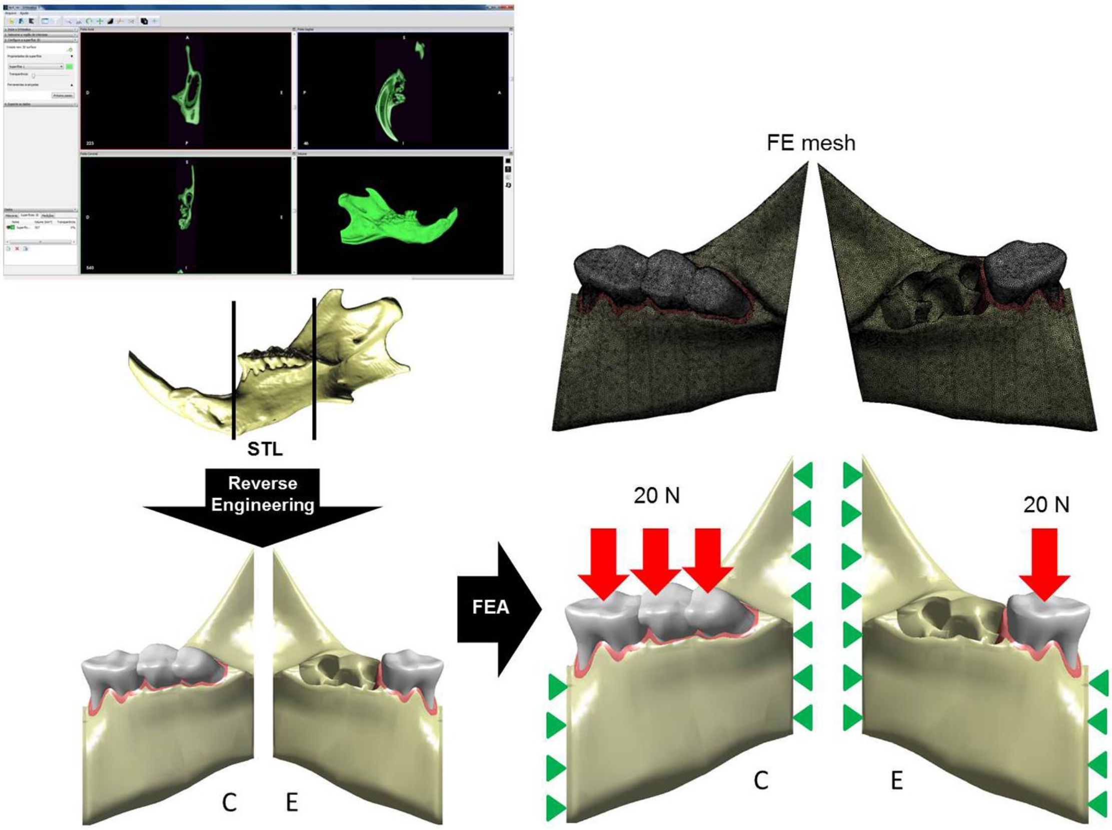

Figure 2. Geometry modeling by reverse engineering, showing the initial 3D triangulated surface (STL) and the final solids with NURBS surfaces. The figure also shows the finite element mesh (FE mesh) and the boundary condition through restraints (green triangles) and load conditions with 20 N loads (red arrows). C: control group. E: experimental group.

2.6 Simulation of masticatory loads using FEA

FEA was performed using Ansys v14 software (Ansys Inc., USA). The geometries were assigned according its mechanical properties (Table 1). Three structures were considered: bone, PDL and the molar teeth, where the rat skull have no significant trabecular bone (Chamoli et al., 2011). Thus, the mechanical properties values were assigned as linear elastic and isotropic, following these parameters (Cox et al., 2011; Cox et al., 2012).

Table 1. Mechanical properties of anatomical structures.

Anatomical structures	Young's module (MPa)	Poisson ratio
Bone	19920	0.3
Molar teeth	30000	0.3
PDL	50	0.4

The finite element mesh was composed by tetrahedral elements (Figure 2) and global size by 0.06 mm. The mesh presented 1.61 million of elements and 2.37 million of nodes for C group and 1.27 million of elements and 1.86 million of nodes for E group.

To simulate the molar chewing, the masticatory force was applied according the experimental study determined by Nozaki et al. (2010), where the force magnitude was obtained for molar chewing, being a mean force of 20 N. The force was applied in the long axis of each tooth (Figure 2). To avoid any free displacements of mandibular segment during simulation, the cut boundary of geometry were restrained.

The von Mises stress (VM) and principal stress (MP) (maximum component) were evaluated. VM is defined as stress caused by energy flow along the material which is under one load (von Mises, 1913). MP calculated the positive (tensile stress) and negative (compressive stress) stress components.

3. RESULTS

3.1 Histologic analysis

After 2 post-extraction weeks, the microscopic aspect of bone tissue at ROI showed changes in the bone matrix organization. In qualitative comparison of C and E groups, it was observed in E group that the bone tissue presented bone remodeling with predominance of bone resorption areas, mainly at the mesial contact between the mesial root and PDL (Figure 3). At the superior segment of interradicular septum the bone resorption also occurred. At the distal surface of distal root, in E group the bone tissue morphology was similar to the C group. However, an intense inflammatory process in the PDL with thickening of collagen fibers occurred (Figure 3).

At the regions with bone resorption, it was observed an increase of periodontal space at the mesial side. In this region was observed osteoclasts and resorption gaps (Figure 3).

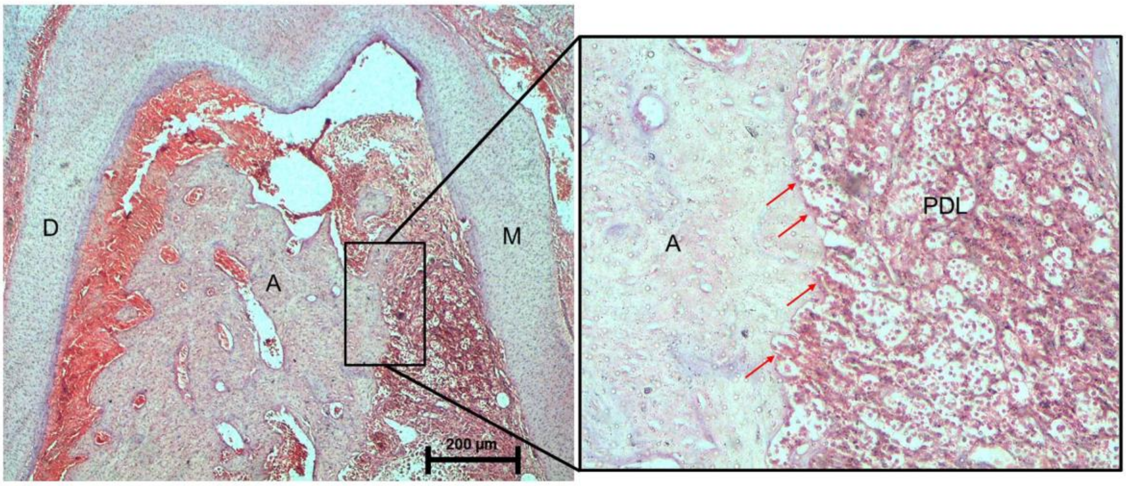

Figure 3. Microscopic view of bone tissue stained with HE and 5x mag, showing the E group where occurred bone resorption. The square shows a 20x mag of regions with osteoclasts and resorption gaps (red arrows). (A) alveolar bone crest. (M) mesial root. (D) distal root. (PDL) periodontal ligament.

3.2 FEA

In comparison between C and E groups, the E group presented higher VM at the interradicular septum (Figures 4 and 5). Thus, it was observed high load dissipation to the septum and, consequently, MP stress result presented high compressive values (ranging from -2.63 to -4.45 MPa) (Figure 5). These areas located mainly at the superior and mesial sides of the septum. In the C group, the stresses are distributed with focused compression at the superior surface of the septum (raging from -0.37 to -0.79 MPa), and tensile stress at the mesial and distal sides (ranging 0.07 to 0.7 MPa) (Figure 4).

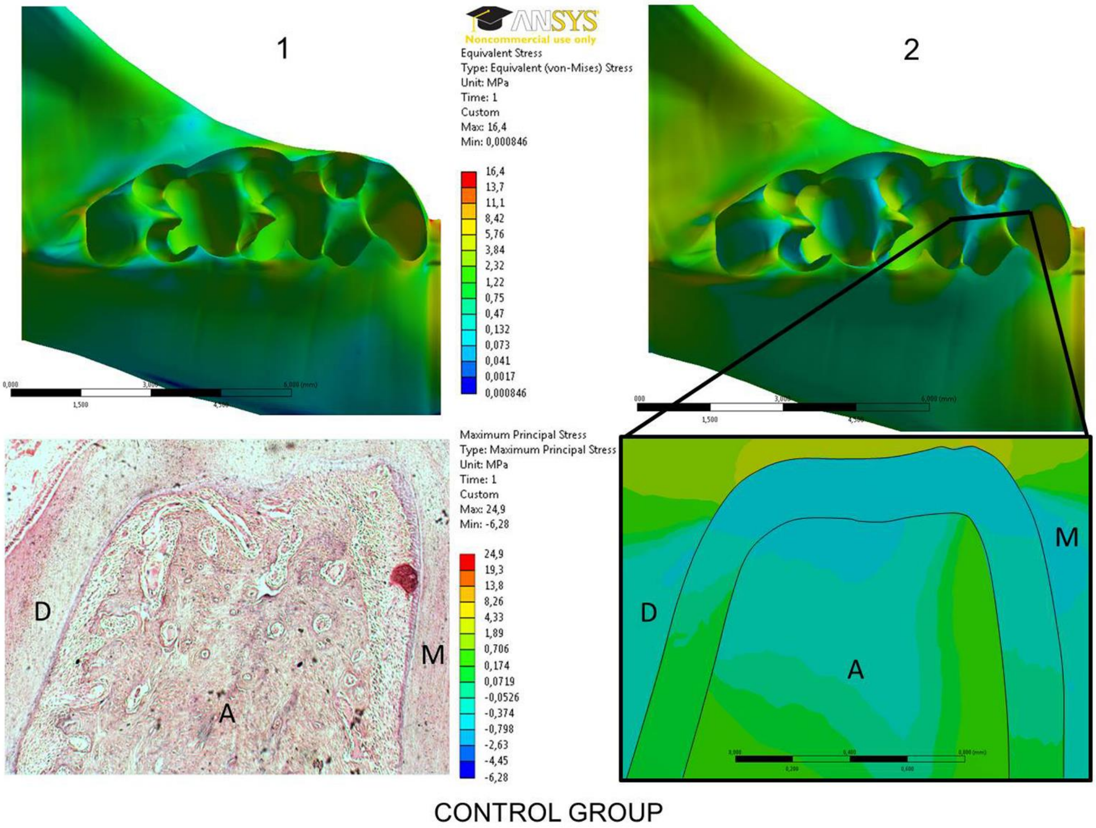

Figure 4. Relation between FEA and histological analysis in C group. The square shows a cut plane of region of interest, where the PDL was marked by black contour. (1) von Mises stress. (2) maximum principal stress. (A) alveolar bone crest. (M) mesial root. (D) distal root.

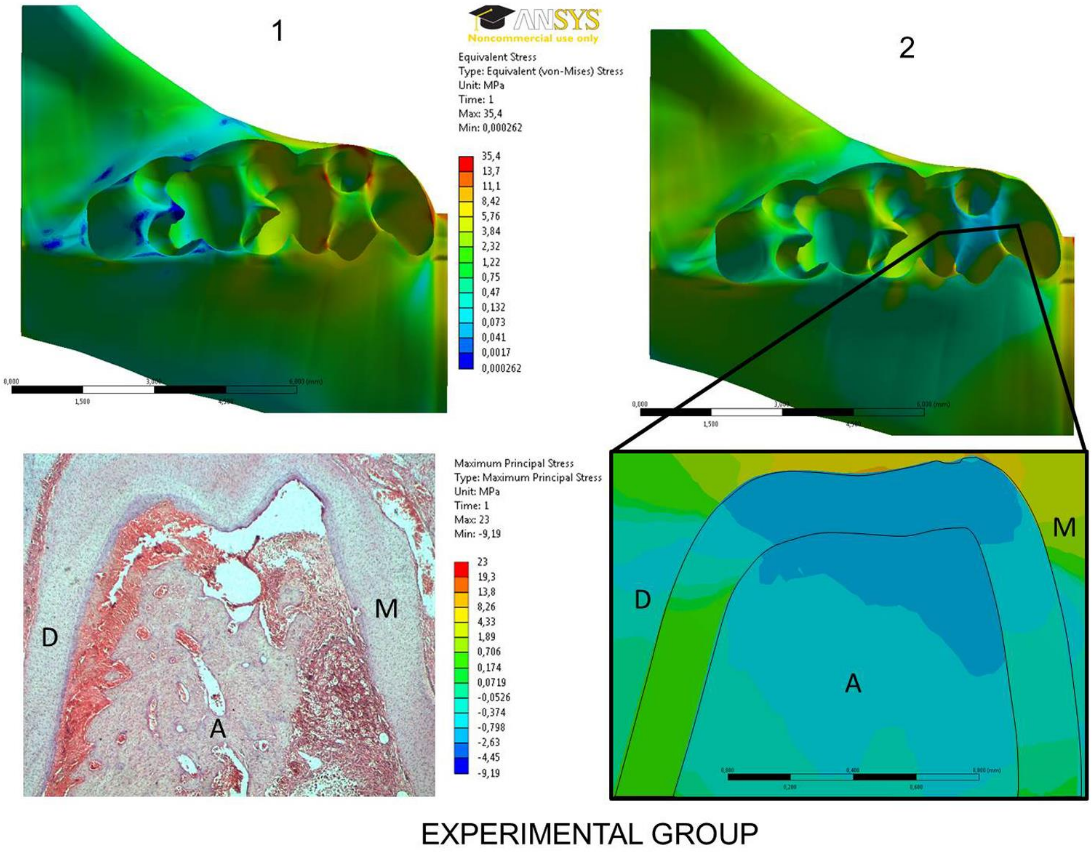

Figure 5. Relation between FEA and histological analysis in E group. The square shows a cut plane of region of interest, where the PDL was marked by black contour. (1) von Mises stress. (2) maximum principal stress. (A) alveolar bone crest. (M) mesial root. (D) distal root.

4. DISCUSSION

Compressive occlusal loads on rat molar was studied by Nozaki et al. (2010), using physical experimental simulation, showed bone resorption. This increase was based in mechanical test, being considered the functional load from 20 N. In fact, the occlusal overload determination on rat molars is complex, once the numerical values of muscular action and its relation to occlusal force resultant were calculated mathematically (Cox et al., 2011; Cox et al., 2012), i.e., without *in vivo* mensuration. However, the value of 20 N suggest to be compatible, once the incisor bite force, which is higher than molar force, was measured with an average of 24,3 N by Robins (1977). Thus, after feed crack the force have less requirement, obtained the maximum performance pattern of molar clenching (Morikawa, 1994).

Therefore the occlusal surface raise is the most used for study of occlusal stress condition (Kaku et al., 2005; Shimomoto et al., 2007; Goto et al., 2011), and have showed similar biological response, both resorption process and the area of occurrence. Similarly, the results in our study showed that the masticatory function on the isolated molar changed the alveolar crest morphology by bone remodeling with resorption (Figure 3). Our results presented a different overload condition, characterized by resultant of load concentration in an isolated tooth, compared to the normal condition where these loads were distributed on three molars in rats.

In our study, the computational simulation by FEA, the isolated molar allowed higher load transfer and, consequently, the increase of stress concentration in the alveolar bone, as presented by VM stress. Besides this increase of stress concentration at the alveolar crest, in MP stress there was an increase of compressive stress. Studies related the alveolar bone compression instability in this region during the first two weeks; both the increase of compressive load magnitude (Nozaki et al., 2010) and occlusal surface raise (Goto et al., 2011), with significant response at the superior region of alveolar bone crest. In our study, at two weeks post-extraction, we observed response in this region and observed in the mesial surface of interradicular septum (Figure 3). This result is according Naveh et al. (2012), in which was observed the contact between the tooth surface and the interradicular septum in mesial and bifurcation region, after high occlusal overload through mechanical test. Although a mechanical machine performed the experimental overload model, it is likely that our results related to mechanical hyper-stimuli in these regions, with excessive compressive stress, causing bone-remodeling instability.

The relationship between *in vivo* tests and computational simulations in rats involving the occlusal stress has been little studied. The simulations performed by Milne et al. (2009) showed the increase of VM stress and compressive stress in periradicular region during bone stimuli under masticatory and orthodontic loads, simultaneously. These results were correlated with histological analysis, where observed resorption areas. On the isolated tooth in our study, the condition of compressive stress distribution in the alveolar crest is related with histological analysis, mainly through the similar qualitative results.

Since these results were observed in areas with presence of changes in mechanical and biological response. On the other hand, this relationship will be a challenge for establish numerical values from computer simulations in dose/response relationship of these structures, when is considered the complexity to represent mechanical characteristics as stiffness, endurance and longevity, which are limiting in FEA.

In conclusion, tooth extraction caused oclusal stress on the isolated first molar increasing the mechanical compression on the alveolar bone at region of bifurcation and changed the bone remodeling showing resorption areas. The stimuli/response relationship allowed the association experimental and computational results interpretation, considering the limitations of FEA for biological structures. This study shows favorable results to open ways for better interpretation in FEA results applied to alveolar bone mechanobiology.

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CAPÍTULO 2:

FINITE ELEMENT ANALYSIS AND BONE VOLUME 3D OF THE ALVEOLAR BONE CREST OF RATS UNDER OCCLUSAL STRESS*

***Artigo submetido no periódico Journal of Periodontology* (Anexo 3).**

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ABSTRACT

Background: Loss of occlusal equilibrium affects the stimuli/response relation in dental and periodontal supportive tissues. Computational analysis is important to understand mechanical stimulation of periodontal structures, but is difficult its mechanobiological interpretation. This study hypothesized that the presence of premature contact stimulate mechanically the alveolar bone crest and can be associated to known bony biological responses. This association was performed by simulation of mastication under occlusal stress in rats. The aim of this study was investigate the mechanical and structural effects in the maxillary and mandibular alveolar bone crest of first molar of rats under experimental condition of occlusal stress.

Methods: Two groups of rats (n=25) were used: occlusal stress by premature contact (E group) during 7 days (n=5), 14 days (n=5), 21 days (n=5), 28 days (n=5) and control without stress (C group) (n=5). Micro-CT scanning was performed in entire head and focused at alveolar bone crest of upper and lower first molar. Computer simulation by finite element analysis and a 3D bone volume was performed to evaluate mechanical distribution the structural changes, respectively.

Results: Alveolar bone crest of upper and lower first molar presented bone volume reduction and increase of mechanical stress dissipation associated with increased compressive strains. The upper bone structure was more affect than lower, presenting major volume reduction and stress/strain effects.

Conclusion: The computational mechanics and morphometric results presented important association between mechanical stress/strain stimulation and biological response by structural alterations.

Keywords: alveolar bone; micro-CT; finite element analysis; mechanobiology; occlusal stress.

INTRODUCTION

Mastication occurs through movements, which performed by a group of anatomical structures including teeth, temporomandibular joint and masticatory muscles. Mechanical changes of the mandibular movements during mastication occurred by occlusal trauma or by overloads through partial teeth loss results in changes in alveolar bone stress distribution and, consequently, changes in bone tissue strain (Huja et al., 2008).

Mechanical stimuli affect the bone formation, maintenance and remodeling. These stimuli regulate bone mass and architecture. Due to the high turnover in response to the occlusion, the alveolar bone is the most appropriate to study the relation between mechanical stimuli and bone remodeling (Skerry, 2008). Occlusion alterations result in changes in the stimulus/response equilibrium and remodeling in alveolar bone (Henneman et al., 2008). The main alterations in the masticatory mechanics are the morphological changes in the occlusal surfaces, mainly in the posterior teeth (McCulloch et al., 2000; Harrel, 2003). Consequently, it can induces bone resorption followed by bone apposition and following of an adaptation period (Harrel, 2003).

Morphological changes in alveolar bone due to functional factors have been widely researched in biological models involving rats. The loss of stimulus/response equilibrium was experimentally induced through the occlusal stress by cementation of rigid material on the occlusal surface (Kaku et al., 2005; Shimomoto et al., 2007; Goto et al., 2011) or experimental model using the occlusal force increase (Nozaki et al., 2010) in rats. These studies showed that the bone remodeling occurs mainly in the alveolar bone crest support of the first molar.

Therefore, the association between computation simulations and morphological analysis was suitable due to use of micro-CT, in which allowed the construction virtual models involving animals, as rats (Koizumi et al., 2010). The study of Milne et al., (2009) showed that the association between finite element analysis (FEA) and histological analysis in rats, after orthodontic forces resulted in increase of compressive stress and resorption areas in the experimental group. Our study tested the hypothesis that the presence of premature contact stimulate mechanically the alveolar bone crest and can be associated to known bony biological responses. This association was performed by simulation of mastication under occlusal stress in rats. The aim of this study was investigate the mechanical and structural effects in the maxillary and mandibular alveolar bone crest of first molar of rats under experimental condition of occlusal stress.

MATERIAL AND METHODS

Ethical Approval

This research was approved by the Committee for Ethics in Animal Experimentation (CEUA/UNICAMP, Brazil. number 2570-1) (Anexo 2).

Sample

Twenty-five male Wistar rats (*Rattus norvegicus albinus*), 2 months old, 200-250g weight, from Multidisciplinary Center for Biological Research (CEMIB) at the State University of Campinas (Campinas, São Paulo, Brazil), were used. The rats were kept in plastic cages with controlled temperature (22 ± 2°C), controlled light cycle (12/12h) and access to water and food *ad libitum*.

The sample were calculated through statistical power test (GraphPad StatMate 2.00, GraphPad Software, San Diego, California USA) and showed that five animals allowed 90% of power, considering a significance level (α) in 5%, mean standard deviance in 10% and difference between the means close to 20%.

Induction of Occlusal Stress

The rats were subjected to occlusal stress under general anesthesia by intraperitoneal injection of ketamine (40-87 mg/kg, Dopalen®, Agribrands Brazil Ltda., Paulínia, São Paulo, Brasil) and xylasine (5-13 mg/kg, Anasedan®, Sespo Ind. Co. Ltda, Paulínia, São Paulo, Brasil). The rats were positioned according proposed by Doku et al., (1966). A portion of photopolymerized resin (Fill Magic – Vigodent, Brasil) was cemented on the occlusal surface of right upper molars (Kumasawa et al., 1995).

Experimental Design

The rats were randomly divided into two groups (figure 1):

- Experimental group (E group) (n= 20): The induction of occlusal stress was performed on the occlusal surface of right upper molars (unilaterally). Then these animals were divided into four subgroups according the period with occlusal stress, followed by euthanasia in 7 (n= 5), 14 (n= 5), 21 (n= 5) and 28 (n= 5) days.
- Control group (C group): The teeth were kept without occlusal stress. The animals were euthanized with 28 days after started the experiment.

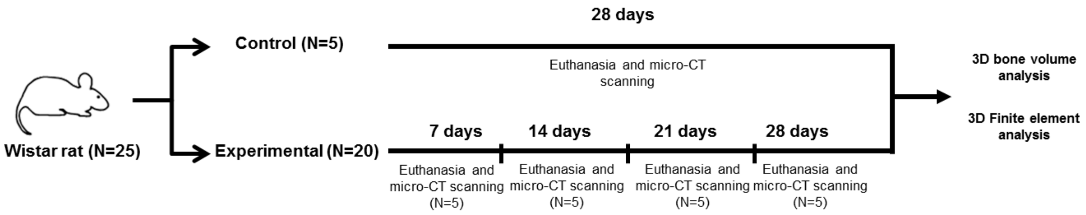


Figure 1. Illustration of experimental design

Euthanasia and Material Collection

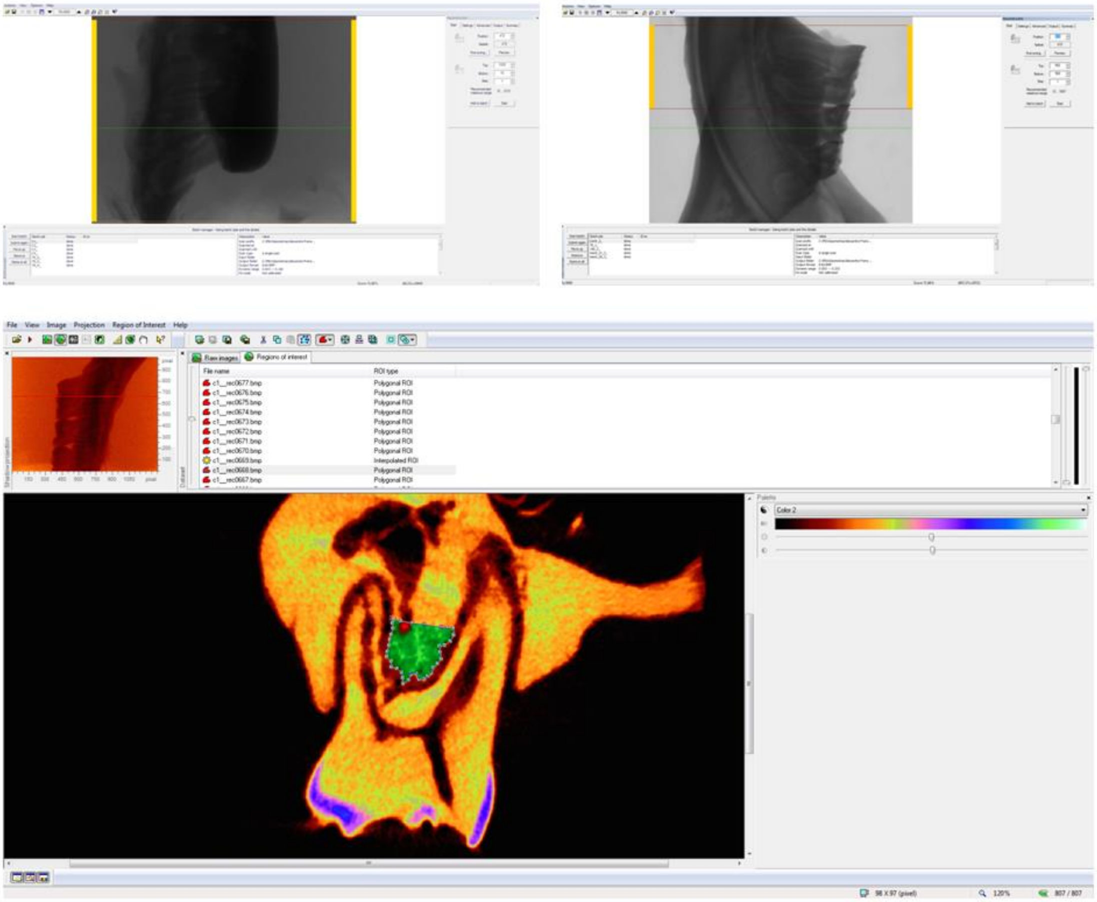
The euthanasia was performed according the proposed periods (7, 14, 21 and 28 days after occlusal stress induction) for subgroups of E group and 28 days for C group. The euthanasia was performed with an excessive dose of anesthetics. After dissection, the heads were fixed in formalin 10%, during 24h (figure 1).

Image Scanning by Micro-CT

The samples were scanned by micro-CT using a SkyScan 1174 (SkyScan, Leuven, Belgium) with voltage in 50kV and amperage in 800 mA. Two scanning were performed in each sample: the first involving the entire head, with sequential images with 30 µm of pixel size to obtain a three-dimensional geometry of maxilo-mandibular complex, which was used for FEA; the second scanning was performed after section and reduction in bone blocks composed by upper and lower molars. The sequential images were obtained with 7.7 µm, focused in the alveolar bone crest of upper and lower first molars, which were used for bone volume 3D analysis. After scanning, the images were exported to the software NRecon Reconstruction (SkyScan, Leuven, Belgium), where the images were reconstructed presenting the grayscale according x-ray attenuation coefficient related to the bone structure.

Bone Volume 3D of Alveolar Crest

The sequential images from scanned bone blocks of maxilla and mandible were used for bone volume calculation according the volume of interest VOI, using the software CT-Analyzer (SkyScan, Leuven, Belgium). A VOI protocol was determined to involve the specific dimension of alveolar bone crest of upper and lower first molar (figure 2). The slices were count 100 images from the most cervical point of root bifurcation, in apical direction following the longitudinal tooth axis. The VOI dimension was conclude with adaptation of geometric shape applied to all slices. To eliminate the dental structure, the slices were segmented through the removal of dental pixels based on grayscale.



File name	ROI type
c1__rec0677.bmp	Polygonal ROI
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c1__rec0675.bmp	Polygonal ROI
c1__rec0674.bmp	Polygonal ROI
c1__rec0673.bmp	Polygonal ROI
c1__rec0672.bmp	Polygonal ROI
c1__rec0671.bmp	Polygonal ROI
c1__rec0670.bmp	Polygonal ROI
c1__rec0669.bmp	Interpolated ROI
c1__rec0668.bmp	Polygonal ROI
c1__rec0667.bmp	Polygonal ROI

Figure 2. Bone volume 3D extraction using CTAnalyzer software of upper and lower blocks containing molar and periodontal supportive structures. The VOI was highlighted (marked by green).

Statistical Analyses

The data were analyzed with GraphPad Prism 3.2 software (GraphPad Software, Inc .; La Jolla, CA, USA). The Shapiro-Wilk test determined that the measures had a normal distribution. First, the unpaired t test was performed to verify the volume changes according the periods in the upper and lower first molar. Two-way ANOVA was calculated to compare the volume changes in each period between the upper and lower first molar. A value of P <0.05 was considered.

Finite Element Analysis

FEA was performed to evaluate, qualitatively, the mechanical effects on the alveolar bone crest under occlusal stress to perform the mechanical interpretation of morphological changes after bone tissue response. Moreover, FEA shows numerical intervals that represents the variations of mechanical response in the geometry (Panagiotopoulou et al., 2009).

Geometry construction

Sequential images from micro-CT of entire head (figure 3) were converted into DICOM format (Digital Imaging and Communications in Medicine) using Dicom converter software (SkyScan, Leuven, Belgium) and were imported to software InVesalius 3.0b (Center for Information Technology, Campinas, Brazil). In this software, the pixels of anatomical structures were marked according their different densities (figure 3). The marked pixels were converted in three-dimensional surface and optimized using the MeshLab software v1.3.2 (Visual Computing Lab, ISTI-CNR, Italy).

The surfaces were converted into CAD geometries (Computer Aided-Design) using the Rhinoceros 3D 5.0 software (McNeel & Associates, USA), which were composed by maxilla, mandible, upper and lower teeth. The geometry of the periodontal ligament (PDL) was formed by offset of root surfaces value equal to 0.2 mm (Cox et al., 2012). In the E group, the geometry of cemented resin block on the surface of upper molars was built, respecting the thickness used in the experiment (1 mm) (figure 3).

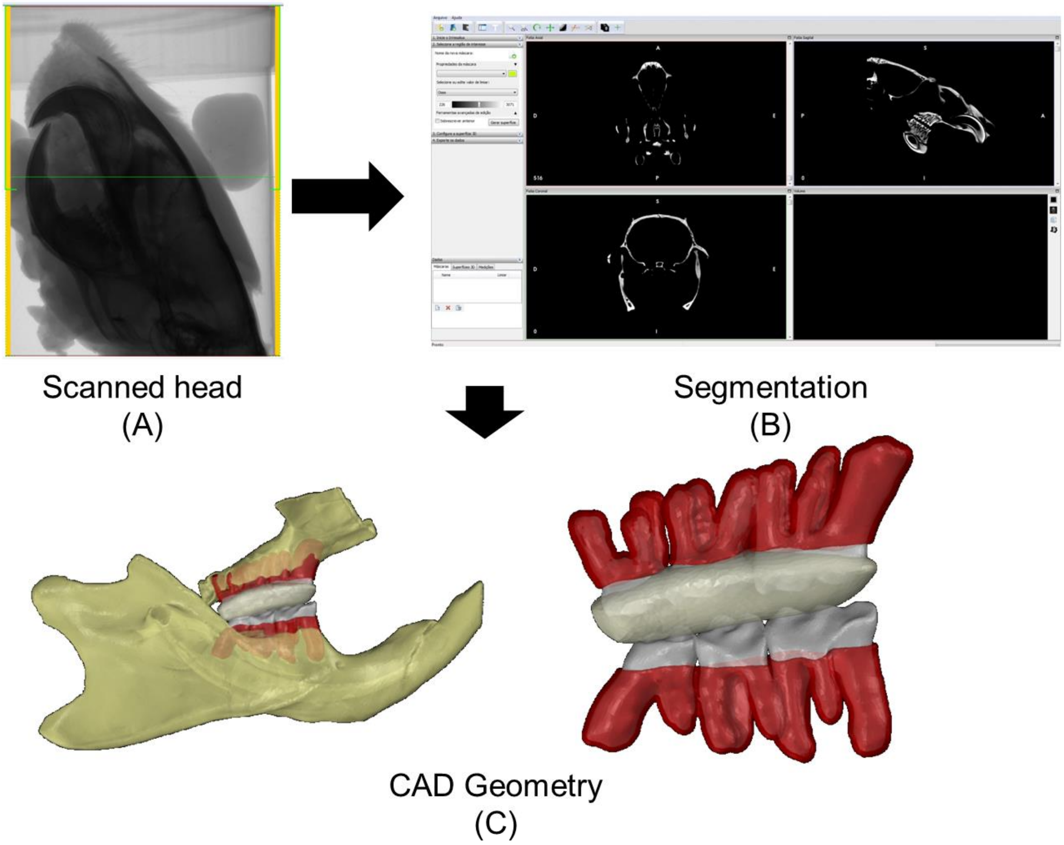

Figure 3. Construction of CAD geometry (C) using Rhinoceros software from scanned head in micro-CT (A). Three-dimensional surfaces were obtained through segmentation (B) using InVesalius software, in which the structures were marked by pixel selection.

Construction of finite element mesh

ANSYS v14 Structural Mechanics software (ANSYS, Inc., USA) was used to construction of finite element mesh. Geometries were converted to a volumetric three-dimensional finite element mesh with tetrahedral shape (Figure 4A). The elements were assigned according to the stiffness mechanical properties, involving the modulus of elasticity (E) and Poisson's ratio (*v*), featuring as isotropic. A single value was applied to the bone structure, since it was determined that the rat skull has no significant amount of trabecular bone (Cox et al., 2011). The mechanical properties (values) applied in our study were determined experimentally by Cox et al, 2012. Thus, the structures were assigned with the values: E = 19920 MPa and v = 0.3 for bone structure; E =

30000 MPa and v = 0.3 for the molars; E = 50 MPa and v = 0.4 for the periodontal ligament; E = 16600 MPa and v = 0.24 for resin (Willems et al., 1992).

Simulation parameters

The analysis was conducted to simulate the posterior occlusion and the action of the masticatory muscles in rats (Cox et al., 2011). The following parameters to simulation were applied:

- Boundary condition (Figure 4C): To obtain higher fidelity in simulation and better results interpretation, the mandible and the maxilla were positioned to establish the occlusal contact between the teeth and resin, resulting in occlusal stress. The boundary of the upper block were restricted to characterize the static position of the head during the occlusion. In the articular surface of the condyle, a restriction was also applied, featuring the bite on maximum muscle action.
- Loading conditions (simulation of the forces) (Figure 4B): Cox et al. (2011, 2012) defined the action of muscle forces. The force values of each muscle were used in maximum magnitude (Table 1), simulating a posterior occlusion. Each muscle was marked on the mandible surfaces according the insertion (Cox & Jeffery, 2011)

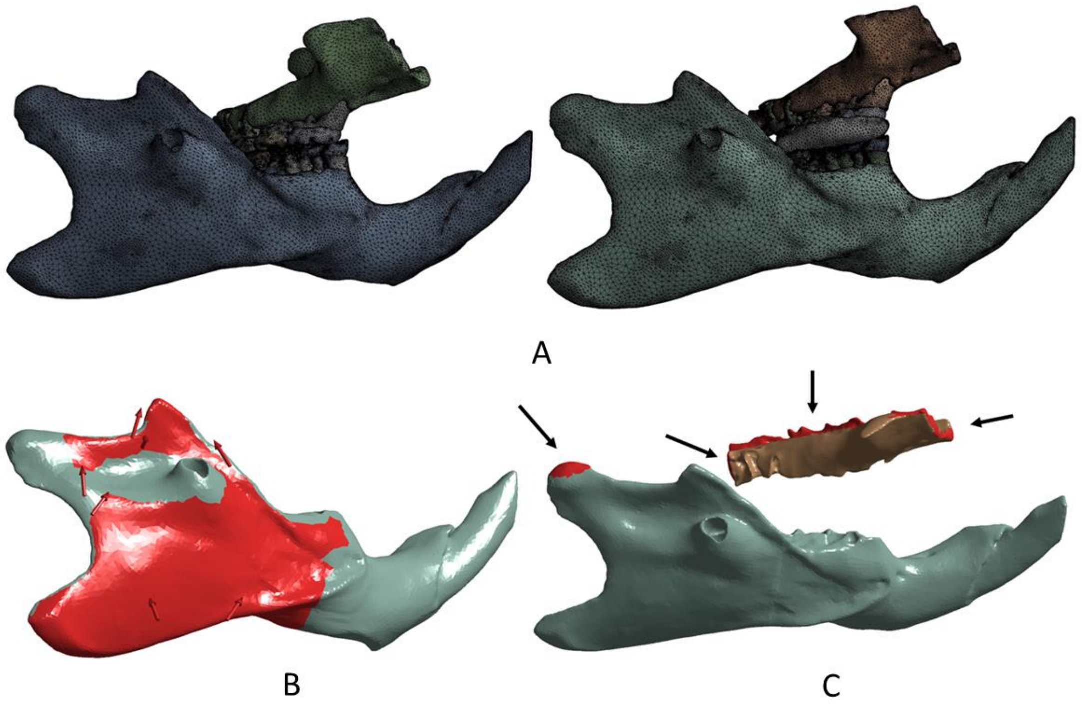

Figure 4. Finite element analysis configuration using Ansys v14 software. A) Finite element mesh of C and E group. B) Masticatory muscles insertion and force direction (marked by red). C) Boundary conditions showing restrained areas.

Table 1. Rat masticatory muscle forces* in Newtons (N).

Muscles	Forces (N)
Masseter – superficial	5,95
Masseter – deep anterior	6,01
Masseter – deep posterior	11,49
Anterior zigomaticomandibularis	1,16
Posterior zigomaticomandibularis	1,03
Temporal	9,56
Internal pterigoid	7,44
External pterigoid	2,36

***** Cox et al., 2011

Result analysis in FEA

The region of interest in FEA results was the alveolar bone crest (cervical third of interradicular septum) of the maxillary first molar (upper block) and mandibular first molar (lower block), as the region of interest assessed in the morphometric analysis. The stress and strain were evaluated according to the following theories:

- Equivalent von Mises stress (VM): the stress was evaluated according to energy flow transferred from the muscular action on the teeth. The stresses were calculated in megapascals (MPa).
- Principal strain: the strains were evaluated with positive and negative values, which are interpreted as tension and compression, respectively. To more accurately results, the maximum principal strain (MAP) (positive values) and minimum principal strain (MIP) (negative values) were evaluated.

RESULTS

Bone Volume 3D of Alveolar Crest

From volume morphometry was constructed three-dimensional geometries, which allowed observing the visible volume reduction in upper and lower alveolar bone crest of first molars (figure 5).

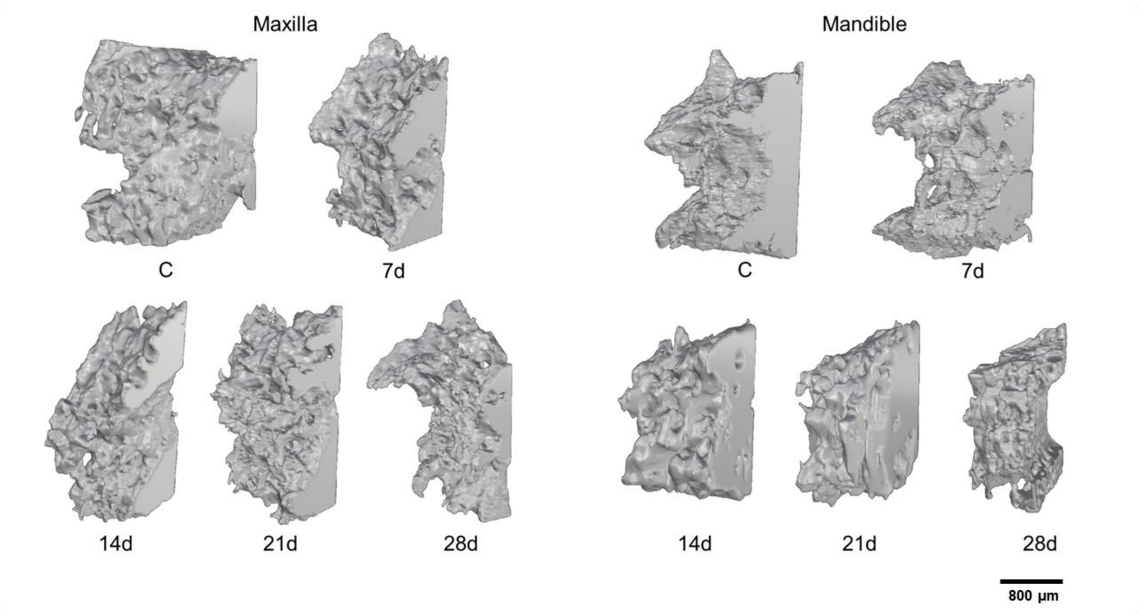

Figure 5. Three-dimensional aspect of upper and lower alveolar bone crest according the periods: control (C), 7 days (7d), 14 days (14d), 21 days (21d) and 28 days (28d).

In both tests in the upper and lower first molar occurred reduction of volume in the alveolar bone crest. The t-test showed that the reduction in bone volume was significant in the first week (7 days) with the occlusal stress ($P<0.05$) (figure 6). Along the periods, volume was reduced with minor intensity (Table 2). Although bone volume reduction has occurred in the upper and lower alveolar bone crest, its comparison showed, in general, that the upper region was major ($P<0.001$) (figure 6). Initially (group C), the upper alveolar bone crest presents major bone volume. At 7 and 14 days, volume reduction was major in the upper region. At 21 days the volume remained virtually unchanged, and a small increase occurred in the upper region. At 28 days the volume also decreased, being also major in the upper alveolar bone crest.

Table 2. Mean, Standard deviance and Standard error of volume morphometry of upper and lower alveolar bone crest according the periods.

GRUPOS	**Control**	**7 days**	**14 days**	**21 days**	**28 days**
Superior					
Mean	*0.8060	0.5240	0.4440	0.4720	0.2740
Std. Deviation	0.03435	0.03362	0.02702	0.008367	0.01517
Std. Error	0.01536	0.01503	0.01208	0.003742	0.006782
Inferior					
Mean	0.4720	0.3760	0.3360	0.3380	0.2940
Std. Deviation	0.02588	0.01342	0.005477	0.004472	0.01140
Std. Error	0.01158	0.006000	0.002449	0.002000	0.005099

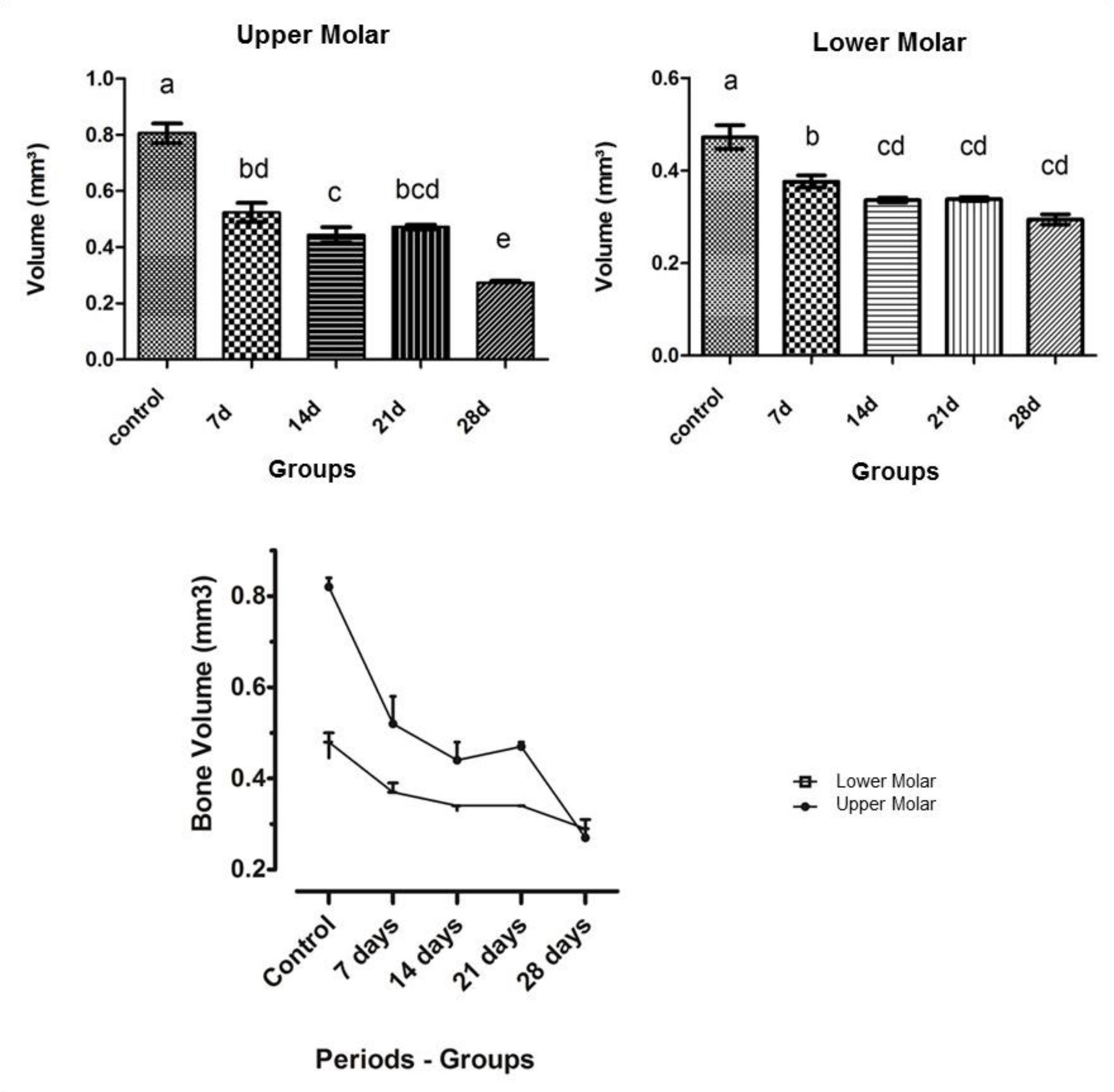

Figure 6. Graphics showing bone volume progression according the periods. Superior graphics shows volume reduction based from t test, in which the data represents the mean of control and the four periods (mean ± SEM). Upper molar graphic presented significant difference between control and all experimental periods (a) ($P<0.05$), 7d and 14d and 28d (b) ($P<0.05$), 14d and 28d (c) ($P<0.05$) and between 21d and 28d (d) ($P<0.05$). Lower molar graphic presented significant difference between control and all periods (a) ($P<0.05$), 7d and 14d, 21d and 28d (b) ($P<0.05$), 14d and 28d (c) ($P<0.05$) and between 21d and 28d (d) ($P<0.05$). The inferior graphic shows the alveolar bone crest volume reduction comparing the upper and lower (Two-way ANOVA), where the volume reduction was significantly major in upper region ($P<0.001$). The data represents the mean in four periods (mean ± SEM).

FEA

The calculation of VM stress in the C group showed minor intensity and uniform stress in the upper alveolar bone crest. There was major intensity and stress concentration in the E group (figure 7). In the lower alveolar bone crest, in both groups, stress had similar characteristics in terms of location and increase in intensity. Comparing the upper and the lower regions, the E groups showed major stress concentration and the intensity was major in the upper alveolar bone crest.

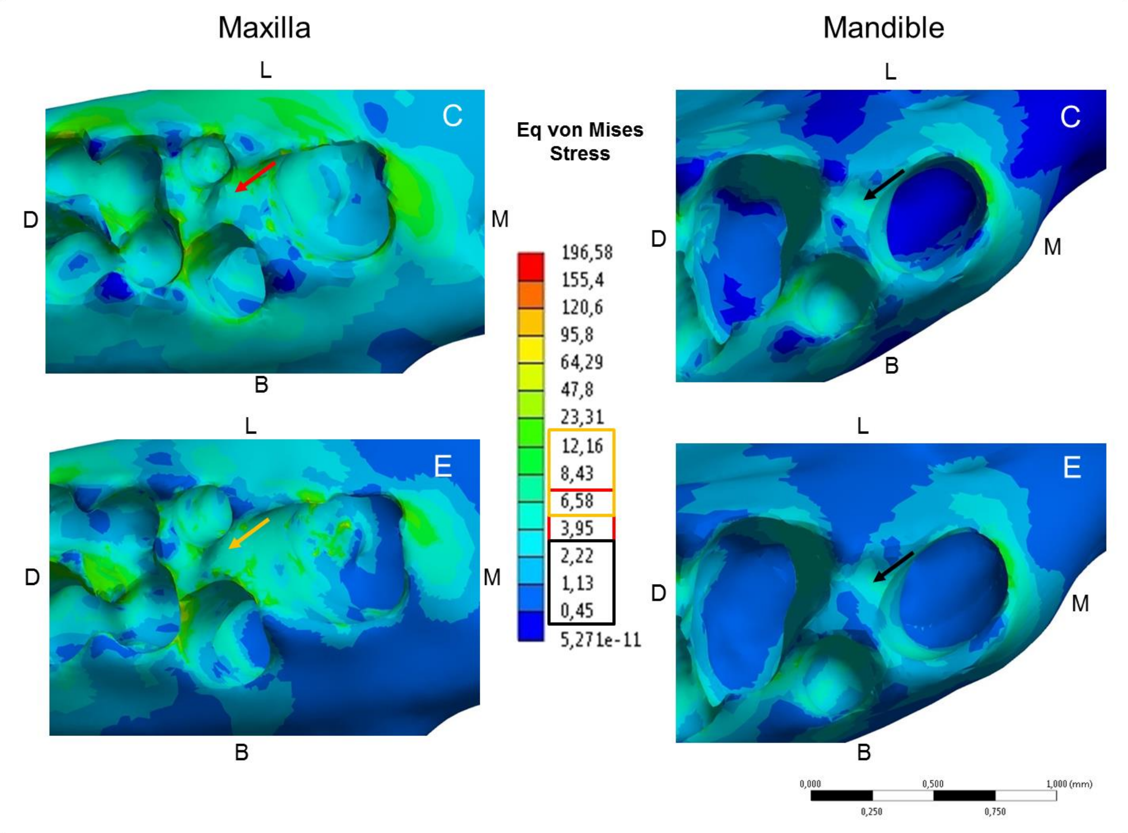

Figure 7. The VM stress evaluation using Ansys v14 software in upper and lower alveolar bone crest, maxilla and mandible, respectively. Orange arrow and square show stress areas and interval values of E group in upper region (major intensities). Red arrow and square show stress areas and interval values of C group in upper region (intermediary intensities). Black arrow and square show stress areas and interval values of C and E group in lower region (minor

intensities) (C: Control; E: Experimental; M: Mesial; D: Distal; B: Buccal; L: Lingual).

In MAP, all analyzes presented low intensity across the alveolar bone crest and was not considered for comparison between the groups. In MIP, the strains showed compression in the E group compared to C group in both the upper and lower alveolar bone crest. Between the upper and lower regions, major compression occurred in the upper alveolar bone crest (figure 8).

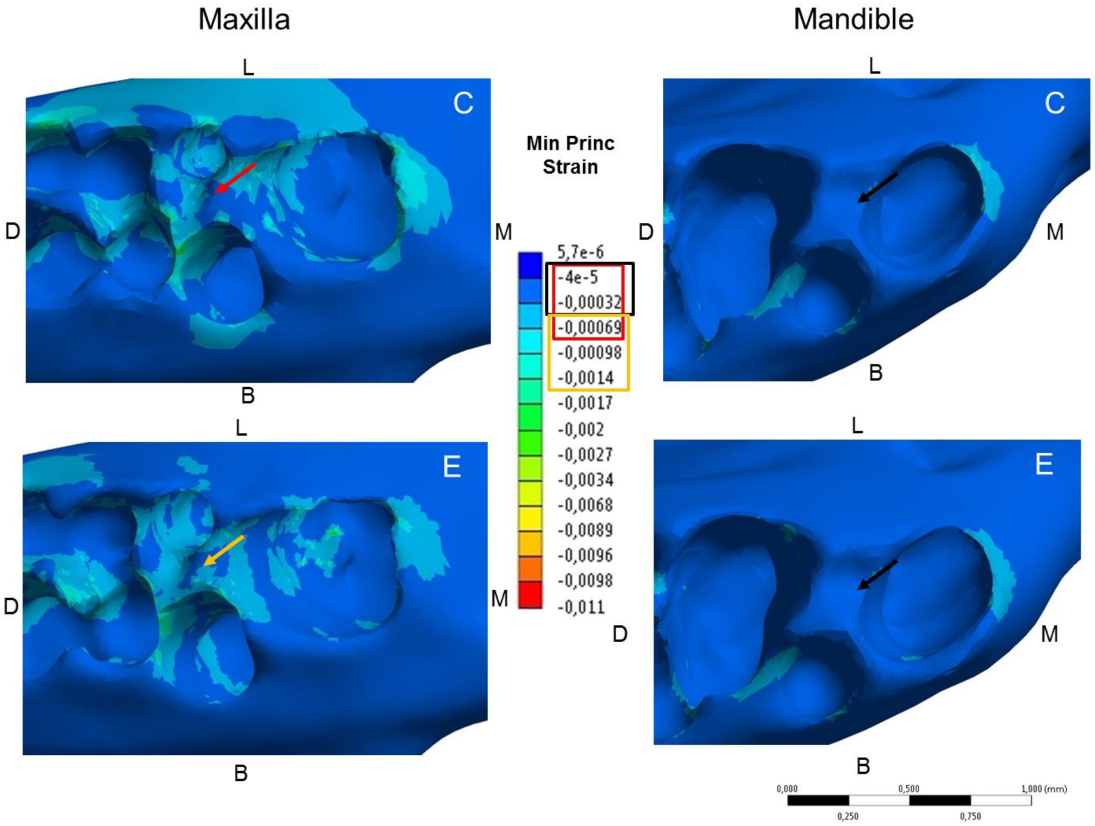

Figure 8. The MIP strain evaluation using Ansys v14 software in upper and lower alveolar bone crest, maxilla and mandible, respectively. Orange arrow and square show strain areas and interval values of E group in upper region (major intensities). Red arrow and square show strain areas and interval values of C group in upper region (intermediary intensities). Black arrow and square show strain areas and interval values of C and E group in lower region (minor intensities) (C: Control; E: Experimental; M: Mesial; D: Distal; B: Buccal; L: Lingual).

DISCUSSION

3D morphometric analysis showed a reduction in bone volume in the progressive periods. From the initial period (control) in both alveolar bone crest of the upper and lower molars was observed that bone loss was significant at 7 days post occlusion alterations. In subsequent periods, bone loss was not significant from 14 to 21 days. After 21 days, the upper block showed new bone morphology, and a significant reduction in bone volume. Kaku et al. (2005) studied an experimental condition of occlusal trauma (steel wires on the occlusal surface) in rats and found periodontal inflammation of the upper first molar. Goto et al. (2011) revealed in a similar experimental condition, the activation of genes related to osteoclastogenesis. Both studies confirmed and justified the biological response of bone loss under occlusal stress observed in our study.

Few studies related about the bone remodeling changes mediated by mechanical stimulation as the mechanostat theory (Nilsson and Westlin, 1971; Jacobson et al, 1984), where physical strains reach a threshold tissue responds to bone formation or resorption. To assess these changes, in our study, the simulation by FEA was important, because, qualitatively, we verify energy dissipation difference from the occlusal load on the presence of the resin. The minimum strains were more intense in the presence of resin resulted in increased compression on the alveolar crest. The compressive stimulation in bone is widely associated to bone resorption (Frost, 2000; Nozaki et al., 2010).

In the alveolar bone crest of both the upper and lower first molar, the bone volume reduction was mainly at 7 days after induction of occlusal stress. This fact is explained by experimental models of occlusal stress in rats have shown that as a response the supporting tissue, inflammation of the PDL, bone resorption and, hence, increased periodontal space for 7 days (Waddington and Embery 2001; Kaku et al, 2005). These studies evaluated by histological analysis the alveolar bone crest in rats characterized by the presence of osteoclasts and resorption-related proteins such as RANK-L (receptor activator of nuclear factor κB - ligant). Furthermore, there are no reports in the literature of the tissue characterization after the first two weeks (14 days) of occlusal stress situation in which suggests an adaptation and maintenance of bone tissue after morphological changes. Moreover, in our study, we found significant reduction in bone volume at 28 days. These findings generate questions about how the bone tissue responds after higher dose of mechanical stimulation. Cairns (2010) reported that repetitive strain effort from mechanical stimuli causes injury to the masticatory system, which could result from activities such as teeth clenching or grinding may be an initiating factor for the development of tenderness and pain in masticatory muscles. In an animal model, repetitive lengthening contractions of the masseter muscle resulted in an increased number of localized inflammatory sites, which would appear to bolster this concept (Hutchins and Skjonsby, 1990).

Thus, we observed that the occlusal stress resulted in a greater effect of bone volume reduction on the upper molar. Although studies have showed changes in the alveolar bone crest of upper first molar (Kaku et al., 2005) and lower first molar (Goto et al., 2011), the comparison between them in the same experimental model had not been evaluated. Both situations are characterized by the premature contact occlusal stress, which has in response inflammation of the PDL (Kumazawa et al., 1995), bone resorption and, hence, increased of periodontal space (Mavropoulos et al., 2004; Kaku et al, 2005). Such events explain the reduction in bone volume in our study. However, the FEA has shown that compressive strains were more evident in the alveolar crest of the upper first molar, and in the alveolar crest of the lower first molar with a similar result to the control group.

We suggested that in the alveolar bone crest of upper first molar the occlusal stress generates a mechanical stimulation above of the tolerance threshold of the bone tissue for maintenance of their normal morphology and therefore resulted in a bone volume reduction when compared to the lower first molar. Furthermore, it can suggest that the presence of resin results in increased energy dissipation to the alveolar bone crest of upper first molar, as seen in VM.

CONCLUSIONS

In summary and considering the FEA limitations for biological structures, the computational mechanics and morphometric results presented important association between mechanical stress/strain stimulation and biological response by structural alterations. According the occlusal stress model of our study, it was demonstrated that:

i) Occurred progressive reduction in volume by occlusal stress in upper and lower alveolar bone crest of first molars in rats, according three-dimensional morphometry;

ii) The period of major morphological changes in bone volume occurred in the first weeks (14 days) after induction of occlusal stress.

iii) The occlusal stress/strain caused major morphological changes in alveolar bone crest of the upper first molar, as shown by the major volume reduction and higher intensity of the stresses and strains in this region.

iv) Our experimental and computational approach will provide new insights into the alveolar bone tissue adaptation after a period of bone loss by altered mechanical stimuli in masticatory system, as well in experimental model of occlusal stress.

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CONCLUSÃO

Os estudos contribuíram para determinar uma associação entre resultados obtidos por simulações computacionais e os dados provenientes de efeitos biológicos. Assim, tais resultados possibilitaram avaliar mais detalhadamente as características dos estímulos mecânicos que ocorrem na presença de um trauma oclusal. A partir dos estudos realizados pode-se concluir que:

- O trauma oclusal, sendo por perda parcial da dentição ou contato prematuro, provocou aumento na compressão mecânica do osso alveolar de suporte dos primeiros molares, superior e inferior.
- Os efeitos do aumento da compressão sobre o osso alveolar pôde ser associado à perda óssea nesta região, a qual foi verificada pelas alterações estruturais.
- Devido à variabilidade biológica, associação entre as avaliações dos estímulos mecânicos por simulação computacional e os efeitos biológicos por morfometria 3D nestes estudos não determinam o limiar numérico de estimulação para que ocorra o recrutamento de osteoclastos. Porém, pode-se determinar a localização das regiões com alteração de estímulo, as quais podem ser estudas conforme as atividades celulares e moleculares.

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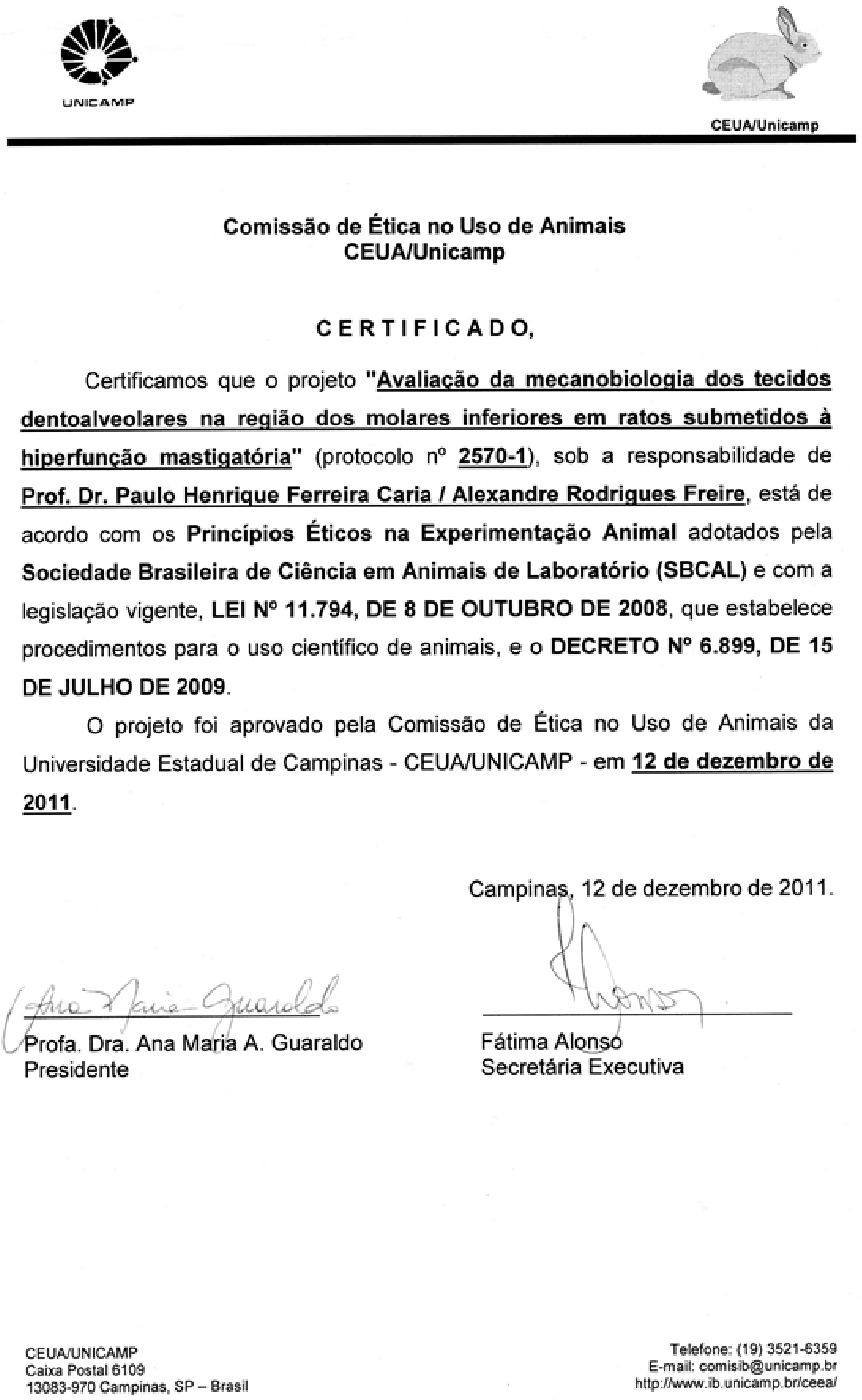

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CERTIFICADO,

Certificamos que o projeto "**Avaliação da mecanobiologia dos tecidos dentoalveolares na região dos molares inferiores em ratos submetidos à hiperfunção mastigatória**" (protocolo nº **2570-1**), sob a responsabilidade de **Prof. Dr. Paulo Henrique Ferreira Caria / Alexandre Rodrigues Freire**, está de acordo com os **Princípios Éticos na Experimentação Animal** adotados pela **Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL)** e com a legislação vigente, **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais, e o **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**.

O projeto foi aprovado pela Comissão de Ética no Uso de Animais da Universidade Estadual de Campinas - CEUA/UNICAMP - em **12 de dezembro de 2011**.

Campinas, 12 de dezembro de 2011.

Ana Maria Guaraldo

Profa. Dra. Ana Maria A. Guaraldo
Presidente

Fátima Alonso
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ANEXO 3

From: Journal of Periodontology < julie@perio.org >
Date: 2015-02-02
Subject: Submission Confirmation
To: alerfreire@gmail.com

Dear Mr. Freire:

Thank you for submitting your manuscript, "FINITE ELEMENT ANALYSIS AND BONE VOLUME 3D OF THE ALVEOLAR BONE CREST OF RATS UNDER OCCLUSAL STRESS" (JOP-15-0073), to the Journal of Periodontology. Your manuscript will be forwarded to Dr. Kornman or an Associate Editor prior to peer review.

As a reminder, the Journal of Periodontology requires all authors to complete a conflict of interest and financial disclosure form via the submission site. You will receive a separate e-mail with information regarding the form.

You may track the status of your submission on the web at https://mc.manuscriptcentral.com/jperio. To access the system, you will need to login with your User ID and password. The manuscript status can be tracked in your Author Center.

If you have any questions, or if I can provide further information, please do not hesitate to contact me at julie@perio.org. It will be most helpful if you would include both your manuscript title and reference number in any correspondence.

Sincerely,

Julie Daw
Managing Editor
Journal of Periodontology
E-mail: julie@perio.org
https://mc.manuscriptcentral.com/jperio