UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

GILBERTO PAIVA DE CARVALHO

AVALIAÇÃO DA MECANOBIOLOGIA DO LIGAMENTO PERIODONTAL EM RATOS COM TRAUMA OCLUSAL

MECHANOBIOLOGICAL EVALUATION OF PERIODONTAL LIGAMENT IN RATS WITH OCCLUSAL TRAUMA

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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, na 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 Buco-dental Biology, in Anatomy area.

Orientador: Prof. Dr. Felippe Bevilacqua Prado

Este exemplar corresponde à versão final da tese defendida pelo aluno Gilberto Paiva de Carvalho e orientada pelo Prof. Dr. Felippe Bevilacqua Prado.

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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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“Não sabendo que era impossível, foi lá e fez”

(Jean Cocteau)

RESUMO

O comportamento das forças oclusais sobre o dente, ligamento periodontal e osso alveolar fornecem dados que permitem ao cirurgião-dentista promover um tratamento restaurador adequado. O objetivo deste estudo foi avaliar o comportamento mecanobiológico dos tecidos de suporte do primeiro molar inferior em ratos com trauma oclusal. Quinze ratos machos Wistar foram divididos aleatoriamente em dois grupos. O grupo experimental foi dividido em dois subgrupos e um fragmento de fio ortodôntico (1mm de diâmetro) foi instalado unilateralmente para simular o trauma oclusal. O grupo experimental (n=10) foi eutanasiado com 7 e 14 dias depois da instalação do fio ortodôntico. Não houve intervenção no grupo controle. As cabeças foram preparadas para o estudo histológico e a análise histomorfométrica. Foi feita uma avaliação intergrupos (controle e experimental) através da análise de variança (One-way ANOVA). Uma amostra de cabeça foi escaneada por microtomografia para obtenção do modelo de elementos finitos. Neste foi simulada a mordida normal e com trauma oclusal. A análise de elementos finitos avaliou o ligamento periodontal e osso alveolar com base na distribuição mínima de tensão (deformações de compressão), as quais apresentaram diferenças de acordo com as áreas de deformações de compressão na região de interesse bem como nos valores destas deformações de compressão. Os resultados histomorfométricos apresentaram diferenças estatísticas significativas ($P > 0,05$). Os dados histológicos mostraram nos grupos experimentais um aumento da área, alongamento e desorganização das fibras do ligamento periodontal e reabsorção óssea na região de interesse. Igualmente, os resultados da análise de elementos finitos apresentaram aumento das deformações de compressão no ligamento periodontal e osso alveolar. Nossos resultados indicaram que as forças mecânicas do trauma oclusal geram deformações de compressão no ligamento periodontal e osso alveolar, regiões onde a resposta biológica demonstrou aumento da área do ligamento periodontal e reabsorção óssea.

Palavras-chaves: mecanobiologia, ligamento periodontal, trauma oclusal

ABSTRACT

The occlusal forces behavior exerted on the tooth, periodontal ligament and alveolar bone can provide useful data allowed the dentist promotes a suitable restorative treatment. The aim of this study was to evaluate the mechanobiological behavior of the supporting tissues of the lower first molar in rats with occlusal trauma. Fifteen male Wistar rats were randomly divided in two groups. The experimental group was divided in two subgroups and a piece of orthodontic wire (1mm in diameter) was unilaterally cemented to simulated occlusal trauma. The experimental group (n=10) was euthanized on 7 and 14 days after installation of the wire/composite set. There was no intervention in control group. The heads were intended to histological evaluation and histomorphometric analyses. Intergroups data (control and experimental) were examined through analysis of variance (One-way ANOVA). One sample of head was scanned in micro-CT to obtain the finite element model to simulate a molar biting in normal occlusion (control group) and traumatic overload occlusion (experimental group). Both periodontal ligament and alveolar bone were evaluated regarding the minimum principal strain distribution (compressive strain), which presented differences according the focused compressive strain areas as well as the compressive strain quantity. The histomorphometric data presented significant statistical differences ($P < 0.05$). Histological results presented all experimental groups showed an increase area of the periodontal ligament, showing stretching, thickening and disorganization of the periodontal ligament fibers. In consideration to the region of interest, finite element analysis x histological relation showed microscopy finds periodontal ligament with an increase of collagen fiber thickness as well as increase of compressive strains in finite element models results. The alveolar bone presented resorption areas as well as areas of highest compressive strains. Our results indicate that the mechanical strength of occlusal trauma generates compressive strain in the periodontal ligament and alveolar bone, regions where the biological response demonstrated increased periodontal ligament space and bone resorption alveolar.

Key words: mechanobiology, periodontal ligament, occlusal trauma

LISTA DE ABREVIATURAS E SIGLAS

με	–	Microstrain (micro tensão)
AB	–	Alveolar Bone (osso alveolar)
AEF	–	Análise de elementos finitos
CT	–	Computed Tomography (Tomografia computadorizada)
FE	–	Finite Element (Elementos finitos)
FEA	–	Finite Element Analysis (Análise de elementos finitos)
LPD	–	Ligamento Periodontal
Micro-CT	–	Micro Computed Tomography (micro tomografia computadorizada)
N	–	Newton
PDL	–	Periodontal Ligament (Ligamento Periodontal)
RL	–	Resorption lacunaes (lacunas de reabsorção)
ROI	–	Region of interest (Região de interesse)

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1 INTRODUÇÃO

O dente tem como suporte o periodonto que é um grupo de tecidos dinâmicos que se adaptam sob o aumento das forças oclusais e de outras variações funcionais. O periodonto é composto pelo osso alveolar, o ligamento periodontal (LPD) e os tecidos gengivais (Husain, 2018). Os movimentos da mandíbula e a anatomia dental em sua relação dinâmica definem o que se denomina de oclusão. Essa relação é significante na análise da saúde bucal (Kumagai et al., 1999).

Um leve contato prematuro durante os movimentos mandibulares altera a sensibilidade do dente, sendo causa de sensação de dor. Este é considerado como um dos fatores etiológicos da hipersensibilidade da dentina, hipótese confirmada em indução experimental em humanos (Ikeda et al., 1998). A variação na forma oclusal de uma única restauração de uma coroa dentária influencia a suavidade dos movimentos, resultados que demonstram a importância clínica da restauração dos contatos oclusais e proximais. (Watamoto et al., 2008).

O trauma oclusal foi relacionado positivamente com perda de inserção periodontal ou severidade da periodontite (Branschofsky et al., 2011). As forças oclusais e seus efeitos no periodonto ainda são áreas de estudo desafiadoras para os pesquisadores (Geramy e Faghihi, 2004). Desta forma, demonstrar a relação entre o ligamento periodontal/osso alveolar e o trauma oclusal pode fornecer informações importantes sobre o problema do trauma oclusal e a doença periodontal (Ishigaki et al., 2006).

Nesta relação, o ligamento periodontal funciona como uma almofada visco-elástica entre o dente e seu osso de suporte e resiste às poderosas forças intermitentes da mastigação (Roberts et al., 2004) e o osso alveolar é um tecido rígido vivo que busca constantemente a homeostase quando é submetido à força mecânica e/ou trauma por meio de uma adaptação metabólica e estrutural que ocorre na superfície periosteal alterando sua forma, tamanho e posição (Teitelbaum, 2000; Boyle et al., 2003; Roberts et al., 2004; Kular et al., 2012). A renovação óssea constante ocorre por um processo de remodelação no qual o osso é removido das superfícies trabeculares ou corticais pelos osteoclastos e repostos na sequência por novo osso depositado pelos osteoblastos (Boyce e Xing, 2007).

Em odontologia, a relação entre osso-ligamento periodontal-dente-articulação vem sendo foco de estudo (Ho et al., 2010; Niver et al., 2011; Lin et al., 2013; Jang et al., 2014), propondo novos protocolos de estudo por alta resolução de Raio-X microscópico (Jang et al., 2014), microscopia de força atômica, microscopia eletrônica de varredura e tomografia computadorizada de raios-X de micro escala (Ho et al., 2010).

Assim, os aspectos biomecânicos da interação osso-ligamento periodontal-dente-articulações fibrosas foram analisados dentre outros fatores e os resultados de cortes transversais virtuais em 2D (obtidos de CT 3D) verificaram um aumento das áreas do ligamento periodontal e/ou compressão de tecido mineralizado. Os resultados em análise indicaram que a resposta biomecânica na articulação é devido à combinação das propriedades orgânica, inorgânica e de componentes fluidos (Lin et al., 2013).

Por outro lado, a redução da carga sobre o complexo osso-ligamento periodontal-dente demonstra respostas adaptativas dessas estruturas permitindo uma diminuição do tônus muscular mastigatório, alterações do crânio, diminuição do espaço do ligamento periodontal e alterações no osso cortical e alveolar na mandíbula (Niver et al., 2011).

As condições limítrofes determinadas por mensurações *in vivo* podem ser utilizadas em estudo *in silico* em simulações computacionais como na análise de elementos finitos para investigar estímulos biofísicos em nível tecidual (Epari et al., 2010). Os passos metodológicos descritos em uma análise de osso ao redor de um implante dental suportando uma coroa com um contato prematuro indicam que o modelo de elementos finitos é complexo, mas tem metodologia efetiva para simulação de trauma dentoalveolar, com resultados altamente precisos e compatíveis com situações clínicas reais (Chaichanasiri et al., 2009).

A Análise de Elementos Finitos (AEF) é um método que reconstrói e avalia o estresse, a tensão e a deformação das estruturas. Neste, o modelo de origem de geometria complexa é reduzido em um número finito de elementos com geometria simples, porém com as mesmas propriedades originais (Prado et al., 2014).

Um estudo buscou alertar clínicos e pesquisadores que lidam com a perda óssea alveolar, fornecendo resultados com base em um modelo de computador por meio da análise de elementos finitos. O objetivo principal deste estudo foi quantificar o estresse, a tensão e a deformação das estruturas do ligamento periodontal e relacionar com a progressão da perda óssea alveolar pela análise de elementos finitos para simular o trauma da oclusão. Apesar de não ter nenhum critério para mostrar valores máximos permitido para os tecidos vivos, este estudo tentou fornecer resultados para compará-lo com a situação normal (Geramy e Faghihi, 2004).

A análise de elementos finitos em um ensaio de pesquisa com estudo histológico e imunoistoquímico em ratos com redução experimental da carga mastigatória e também por movimento dentário revelaram por meio de análises quantitativas que as características do ligamento periodontal em desenvolvimento e maduro são influenciadas por forças físicas (Huang et al., 2016). Quando expostos em graus de variações de magnitude, frequência e duração da força mecânica, o osso alveolar e tecido periodontal adjacentes demonstram grandes alterações macroscópicas e microscópicas (Apuzzo et al., 2013).

Assim, entender como o movimento do dente ocorre no alvéolo na presença do ligamento periodontal e como acontece o processo de adaptação por meios das constantes forças funcionais pode permitir obter informações sobre as adaptações presentes nos tecidos que promovem a regeneração/remodelação para manter a função biomecânica de uma articulação (Lin et al., 2017). Neste contexto, a análise de elementos finitos é um método gerador de hipóteses que pode ser aplicada para investigar o comportamento biomecânico no ligamento periodontal de ratos com trauma oclusal.

2 ARTIGO: Evaluation of periodontal ligament mechanobiological of rats with occlusal trauma

Artigo submetido ao periódico: *Periódico* (Anexo 1).

ABSTRACT

The occlusal forces behavior exerted on the tooth, periodontal ligament and alveolar bone can provide useful data allowed the dentist promotes a suitable restorative treatment. The aim of this study was to evaluate the mechanobiological behavior of the supporting tissues of the lower first molar in rats with occlusal trauma. Fifteen male Wistar rats were randomly divided in two groups. The experimental group was divided in two subgroups and a piece of orthodontic wire (1mm in diameter) was unilaterally cemented to simulated occlusal trauma. The experimental group (n=10) was euthanized on 7 and 14 days after installation of the wire/composite set. There was no intervention in control group. The heads were intended to histological evaluation and histomorphometric analyses. Intergroups data (control and experimental) were examined through analysis of variance (One-way ANOVA). One sample of head was scanned in micro-CT to obtain the finite element model to simulate a molar biting in normal occlusion (control group) and traumatic overload occlusion (experimental group). Both periodontal ligament and alveolar bone were evaluated regarding the minimum principal strain distribution (compressive strain), which presented differences according the focused compressive strain areas as well as the compressive strain quantity. The histomorphometric data presented significant statistical differences ($P < 0.05$). Histological results presented all experimental groups showed an increase area of the periodontal ligament, showing stretching, thickening and disorganization of the periodontal ligament fibers. In consideration to the region of interest, finite element analysis x histological relation showed microscopy finds periodontal ligament with an increase of collagen fiber thickness as well as increase of compressive strains in finite element models results. The alveolar bone presented resorption areas as well as areas of highest compressive strains. Our results indicate that the mechanical strength of occlusal trauma generates compressive strain in the periodontal ligament and alveolar bone, regions where the biological response demonstrated increased periodontal ligament space and bone resorption alveolar.

1. INTRODUCTION

Mastication, orthodontic tooth movement, and functional occlusal habits continuously reverberate on the periodontal tissues and these biomechanical forces interact by means of inflammatory signals in periodontal ligament cells (Nogueira et al., 2014). The periodontal ligament is a complex, vascular, and highly cellular soft connective tissue between the tooth and its supporting bone (McCulloch et al., 2000; Roberts et al., 2004).

The dynamic bone-periodontal ligament-tooth fibrous joint consists of two adaptive functionally graded interfaces, the PDL-bone and PDL-cementum (Lin et al., 2017) that resists the powerful intermittent masticatory forces (Roberts et al., 2004; Lin et al., 2017). Functional loads on periodontal ligament induce tissue adaptations by converting mechanical energy into chemical energy at a cell level (Leong et al., 2012). Thus, in normal bone remodeling, osteoblastic bone formation always occurs as a programmed manner accurately and quantitatively just after osteoclastic bone resorption (Monnouchi et al., 2014; Udagawa et al., 2017).

Whether excessive loading exerts on the tooth, occlusal trauma occurs. Thus, this kind of trauma results in tissue changes within the attachment apparatus (Hallmon, 1999; Hallmon, Harrel, 2004). Thereby, occlusal trauma on periodontal ligament results in disappearance of alveolar hard line, enlargement of the periodontal ligament space, stretching and disorganization of periodontal ligament fibers, and destruction of alveolar bone (Kanzaki et al., 2002; Goto et al., 2011; Tsutsumi et al., 2017). Under homeostasis conditions, osteoclasts function in remodeling turnover. However, in cases of occlusal trauma, imbalance happens, causing process of pathological resorption, mainly influenced by inflammatory process (Li et al., 2017; Kaval et al., 2018).

Recent researches have been investigating occlusal trauma, periodontal ligament and bone loss using morphological, histological, immunohistochemical, micro-CT (Walker et al., 2008) and finite element method (Chaichanasiri et al., 2009; Qian et al., 2009). The finite element analysis (FEA) is an upcoming and relevant research tool for biomechanical analyses in biological research (Trivedi, 2014). Periodontal ligament was generated as a contact model between the tooth and alveolar bone instead of a solid meshed FE model with poor geometric morphology or very dense mesh. It was stated that this model saves time and pre/post processing workforce, increases the accuracy and adds to the smoothness of interface stress distributions as well (Tuna et al., 2014).

In this way, the Finite Element Analysis (FEA) is an useful method to evaluate the stress, tension and deformation of the tooth, periodontal ligament and alveolar bone, mimeting the complex form of these structures and transferring the respective physical properties to the model (Rees, 2001; Poppe et al., 2002; Prado et al., 2014), being an effective method for simulating occlusal trauma, with highly accurate results and compatible with real clinical situations (Silva et al., 2011).

The aim of this study was to evaluate the mechanobiological behavior of the supporting tissues of the lower first molar in rats with occlusal trauma.

2 MATERIALS AND METHODS

This study was approved by the Committee for Ethics in Animal Experimentation of the Institute of Biology/State University of Campinas (Protocol number CEUA-IB-UNICAMP-3661-1).

2.1 Sample

Fifteen male Wistar rats (*Rattus norvegicus albinus*), weighing among 250–350 g, obtained from Multidisciplinary Center for Biological Research (CEMIB) of the State University of Campinas (Campinas, São Paulo, Brazil), were kept in group of five individuals per cage, with controlled temperature (22 ± 2°C), light-dark cycle (12/12h), 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 in two groups.

The experimental group was designed according Rossi et al. (2017) called group 1 composed of ten rats (n=10) and group 2, control group (n=5). When completed the age of 2 months, a piece of orthodontic wire (1mm in diameter) was unilaterally cemented with composite (Fill Magic®–Vigodent, Brazil), in upper right first molar establishing a condition premature contact and, therefore, occlusal trauma. The group 1 was divided in two subgroups (n=5) and were euthanized on 7 and 14 days after installation of the wire/composite set. The control group, called group 2, composed of five rats. When completed the age of 2 months, the teeth were kept without the experimental occlusal alteration (wire/composite sets were not installed) and were euthanized on 14 day after completing 2 months old.

2.2 Procedures

The procedure was carried out under general anesthesia using injection of ketamine hydrochloride (40-87 mg/kg) (Dopalen®, Agribrands Brazil Ltda., Paulínia, São Paulo, Brazil), by intraperitoneal injection, and muscle relaxant xylazine hydrochloride (5-13 mg/kg) (Anasedan®, Sespo Ind. Co. Ltda, Paulínia, São Paulo, Brazil), by intramuscular injection. The condition of traumatic occlusion was performed by installing wire/composite set on oclusal surface of upper right first molar in the subjects of group 1 (adapted from Kumazawa et al., 1995, according Rossi et al., 2017).

2.2.1 Sample preparation and histological evaluation

At 7 and 14 days after installing wire/composite sets, the rats of group 1 (experimental group) were euthanized by excessive anesthesia. The five animals of control group were also euthanized at 14 days. The heads were disarticulated, dissected to separate mandibles, then, fixed in 10% neutral buffered formalin solution, during 24h at 4ºC. The 15 mandibles were demineralized in 5% EDTA (Life Science Research Products. AMRESCO Products), dehydrated in increasing series of alcohol and embedded in paraffin. Buccolingual serial sections of 6 µm thickness were prepared (rotary microtome Leica RM2235, Leica Systems, USA) and stained in hematoxylin-eosin (H&E).

2.2.2 Histomorphometric analyses

The fifteen heads (control, 7 and 14 days groups) were intended to histomorphometric analyses. The mandibles were removed after euthanasia, fixed in 10% formalin for 24 hours and decalcified in EDTA (Life Science Research Products. AMRESCO Products) 5%. After dehydration and diaphanization, the pieces were included in Paraplast® (Embedding Media, McCormick Scientific, OH, USA) and submitted to microtomy (Leica RM2235 rotary microtome, Leica Systems, EUA) in serial sections 6 µm thick, in the buccolingual direction of the periodontal attachment of the first lower right molar.

The analyses were performed under a microscope coupled to Nikon camera (Eclipse 80i, Shinagawa, Tokyo, Japan), an objective with the magnification power of at least 10x and using ImageJ program (National Institutes of Health, Bethesda, Maryland, USA), area of the periodontal ligament was measured in square millimeters (mm^2). The measurements were recorded and organized into a table for statistical analysis of the control groups, 7 and 14 days.

2.2.3 Statistical analyses

Statistical analyses were performed using statistical by GraphPad Prism 7 software. Intergroups data (control and experimental) were examined through analysis of variance (One-way ANOVA). Data were examined for normality by the Brown-Forsythe and Bartlett's tests. The significance level for all tests was 0.05.

2.3 Finite element analysis

2.3.1 Geometry acquisition

One sample of each group was scanned in micro-CT using the microtomography SkyScan 1174 (SkyScan, Belgium) with 50 Kv and 800 mA. To obtain a high accurate geometry in a microscopic level, the structures were reduced to the upper and lower first molars and their support structures (alveolar bone, and periodontal ligament). From micro-CT images, the 3D surfaces were constructed using the software Materialise MIMICS Research v18 (Materialise, Leuven, Belgium). The surfaces were obtained from segmentation based in the pixel marking by gray values threshold. The final structure was composed by alveolar bone (supporting the upper and lower first molars), filled periodontal and pulpal spaces (representing the periodontal ligament and pulp) and the upper and inferior molar teeth. To construct the geometry of the experimental group, the 3D surfaces of resin and orthodontic wire fragment cemented on the upper right first molar were created using the CAD software Rhinoceros 3D 5.0 (McNeel & Associates, USA). Thus, two groups of 3D surfaces were designed featuring the control and experimental groups. To conclude the geometry acquisition, all 3D surfaces were exported as stereolithographic format (STL).

2.3.2 FE mesh construction and FE analysis configuration

The two groups of 3D surfaces were imported in the software Materialise 3-Matic Research v10 (Materialise, Leuven, Belgium), where the surfaces were optimized and converted to volumetric meshes (FE meshes), composed by tetrahedral elements (Figure 1). All FE models presented an average mesh quality $q = 0.81$, in a scale from 0 to 1.

The models were imported in the software Ansys v17 Structural Mechanics (Ansys, Inc., USA) and the structures were assigned according to their mechanical properties (table 1), which all models were considered as linear elastic and isotropic.

Table 1. Mechanical properties of the structures. Elastic modulus is expressed as megapascal (MPa) unit.

Structures	**Mechanical Properties**	
	Elastic modulus (E)	**Poisson's ratio (*v*)**
Tooth*	30000	0.3
Periodontal ligament**	50	0.4
Pulp**	2	0.4
Alveolar bone*	19920	0.3
Resin^{+}	16600	0.24
Stainless steel (wire fragment)$^{\perp}$	19500	0.3

*Cox et al., 2012
**Rayfield, 2017
+ Willems et al., 1992
⊥ Ansys v17 database

The analysis was set to simulate a molar biting in normal occlusion (control group) and traumatic overload occlusion (experimental group) (Figure 1). From *in vivo* conditions it is suggested that the first lower molar is directed to occlusal contact to upper molar and, then the biting force is dissipated. Then, the reaction force is dissipated to the lower first molar (Weijs, 1975). Furthermore, the molar bite force was set as 20 N magnitude directed to the upper right first molar, which features the normal occlusal contact, i.e. without significant bone remodeling changes (Nozaki et al., 2010). In the occlusal overload condition, the lower right first molar was directed to upper molar on the premature contact (cemented orthodontic wire) with a bite force set as 40 N, featuring the dental traumatic occlusion, whose effects were described (Nozaki et al., 2010).

Restraints were applied on the cutting planes of alveolar bone to keep the stability of models during biting force action. The x, y and z axis were fixed on the upper alveolar bone. For the lower alveolar bone, the x and y axis were fixed, and the z axis was kept free to allow the superior direction during the action of biting force.

The results were directed to evaluate the minimum principal strain in the region of interest (ROI): the inter-radicular septum, at cervical level according to dental root reference (alveolar bone crest), as well as the periodontal ligament between the alveolar bone and the furcation site of the lower molar (Figure 2). Qualitatively, the minimum principal strain represents the compressive strain, which are expressed by negative values of microstrain (μƐ). This calculation was selected once the evidence of periodontal ligament compression was observed in previous studies by histological analysis (Kaku et al., 2005; Goto et al., 2011). However, the compressive strain configuration is unknown, as well as its association to the histological characterization. Thus, the results from FEA were compared to the histomorphometric analysis.

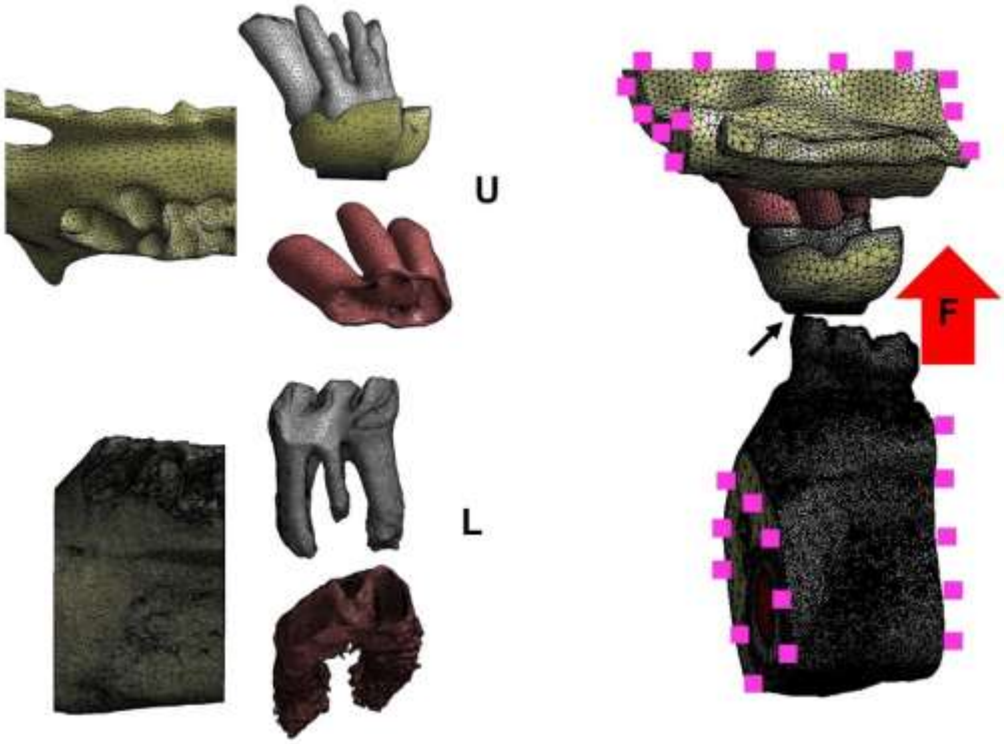


Figure 1. Finite element models (alveolar bone, molar tooth and periodontal ligament) obtained from geometry and the biting force configuration (experimental group). (U) Upper structures – note the presence of orthodontic wire fragment and the resin on the occlusal surface; (L) Lower structures. The load and boundary conditions are indicated by red arrow (F – Force) and pink squares, respectively. The black arrow indicates the premature contact.

3 RESULTS

3.1 Finite element analysis

Both periodontal ligament and alveolar bone were evaluated regarding the minimum principal strain distribution (compressive strain), which presented differences according the focused compressive strain areas as well as the compressive strain quantity.

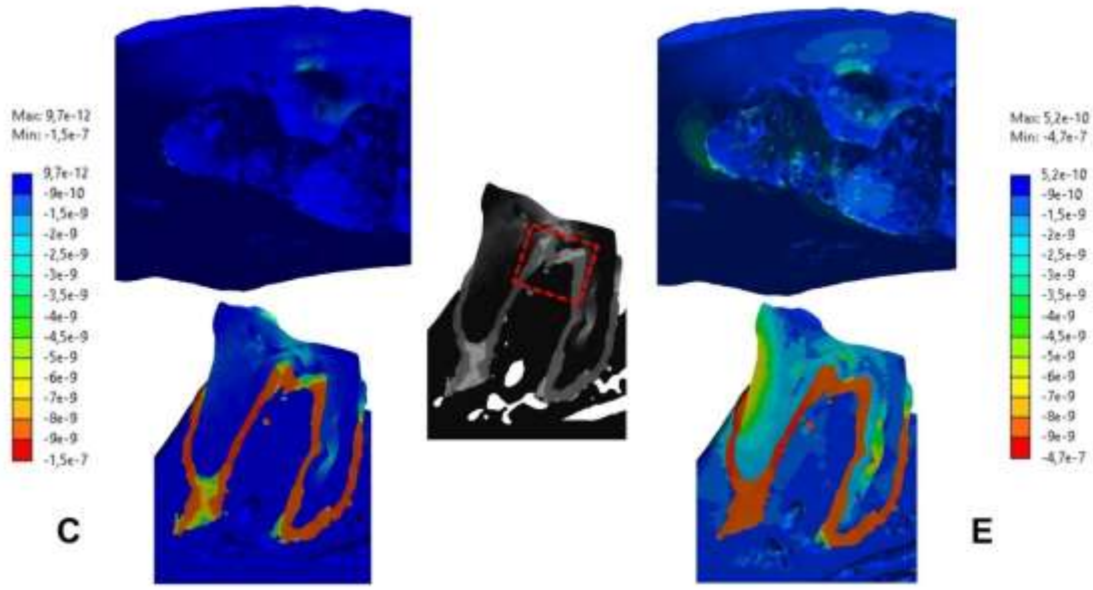


Figure 2. Minimum principal strain distribution in the control group (C) after normal occlusion and experimental group (E) after traumatic overload. The color scales indicate the negative values of strains (compressive strain). At the center of image, the red dashed square indicates the ROI, where the results were observed.

The periodontal ligament showed variations of compressive strain distribution in the control group and a uniform distribution in the experimental group (Figure 2). But, the experimental group presented higher compressive strain than the control group, ranging from -7 x 10^{-3} µƐ to -9 x 10^{-3} µƐ and from -3 x 10^{-3} µƐ to -9 x 10^{-3} µƐ. The interval values of compressive strain show this variation.

Regarding the alveolar bone in the ROI, the first observation was according the bite force configuration, where the control group presented a uniform compressive strain distribution on the bone surface (Figure 2) from a correct occlusal contact. The experimental group presented a non-uniform compressive strain distribution on the bone surface, composed with some areas with high compressive strains (Figure 2) from a premature contact. The same aspect was observed in a longitudinal cut view, where the alveolar bone crest presented a uniform compressive strain distribution in the control group and specific areas of high compressive strain in the experimental group

The compressive strain quantity in the control group on the bone surface and internal bone tissue of alveolar bone crest presented an interval ranging from -1 x 10^{-5}µƐ to -9 x 10^{-4} µƐ. The experimental group presented specific areas with highest compressive strain ranging from -9 x 10^{-4} µƐ to -2.5 x 10^{-3} µƐ.

3.2 Statistical analysis

The histomorphometric evaluation of the area of the periodontal ligament space are present in figure 3. The data present significant statistical differences (P <0.05). The data presented normal distribution without significant deviations (P <0.05). All experimental groups showed an increase area of the periodontal ligament. Larger areas are present in the group of 14 days when compared to the group of 7 days and control.

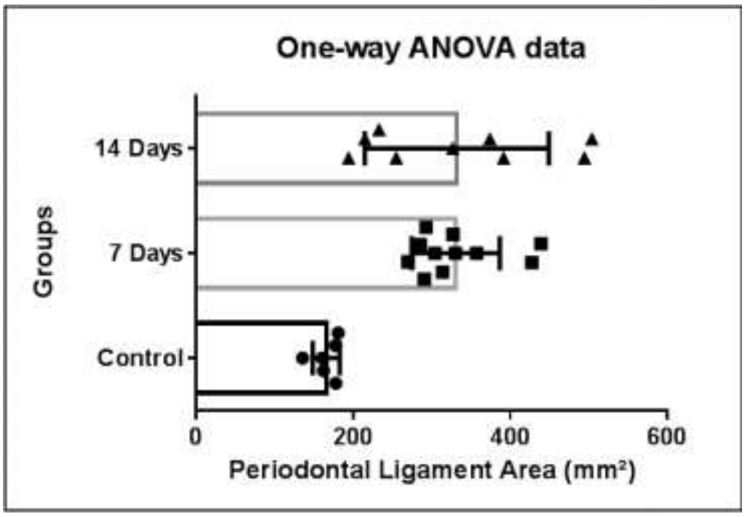


Figure 3 - Statistical analysis of the periodontal ligament areas in the control (Control), 7 days (7 Days) and 14 days (14 Days) groups presenting statistically significant differences (P <0.05)

3.3 Histomorphometric Results

Representative histomorphometric observations are present in figure 4 where the periodontal ligament blood vessels are present in and close to the alveolar bone. A comparative board control, 7 e 14 days groups are present in figure 5. The periodontal ligament maintains constant width, arrangement of the main fibers without changes until the cement and alveolar bone ordered in the region of interest in control group (figure 6).

The 7-day group had a larger periodontal ligament area when compared to the control group showing stretching, thickening and disorganization of the periodontal ligament fibers. Some regions of the cement boundary are disorganized. Gaps with presence of multinucleated cells (osteoclasts) and proliferation of inflammatory cells are observed. Hard tissue reabsorption gaps are present in the alveolar bone and cement (figure 7).

The 14-day group demonstrates a significant increase in the periodontal ligament area, higher than the control group and 7 days. There is widespread disorganization in periodontal ligament space with periodontal ligament fibers, loss of cement boundary, presence of multinucleated cells (osteoclasts), expansion of blood vessels. Osteoclasts are more intense in the areas of alveolar bone resorption (figure 8).

In consideration to the ROI, the relation FEA x histological showed microscopy finds periodontal ligament with an increase of collagen fiber thickness as well as increase of compressive strains in finite element models results. The alveolar bone presented resorption areas as well as areas of highest compressive strains.

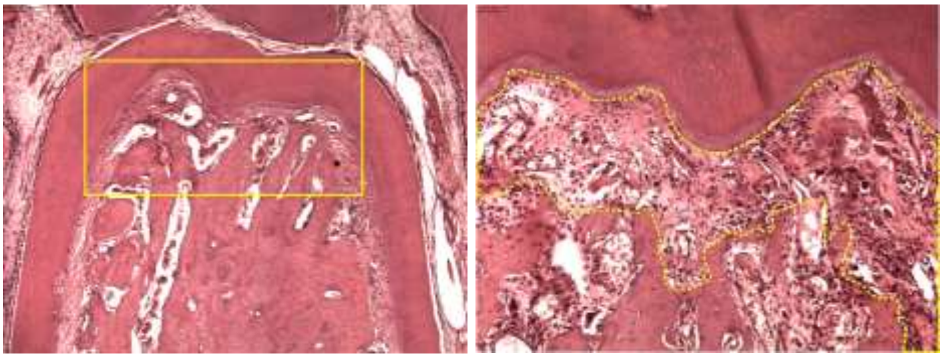
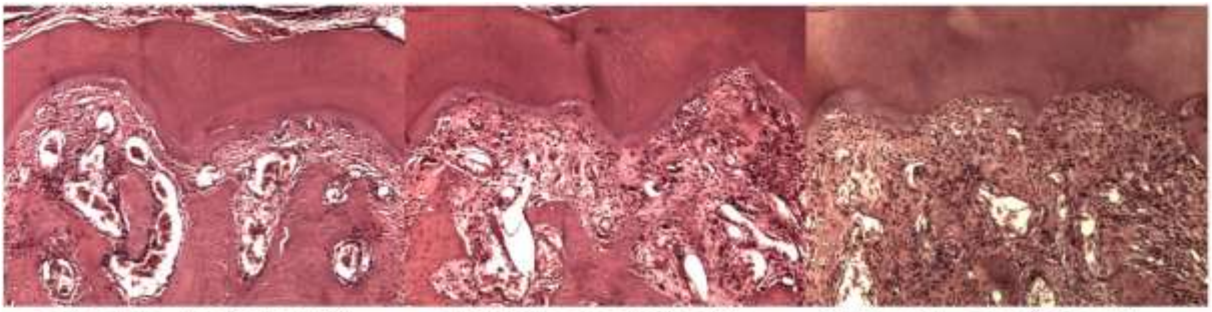


Figure 4 – Region of interest (magnification de ×5; H&E) and measurement of periodontal ligament area.



Control group – magnification ×10; H&E; Bar~49.50µm. 7-Day group – magnification ×10; H&E; Bar~49.50µm. 14-Day group – magnification ×10; H&E; Bar~49.50µm.

Figure 5 – Comparative board control, 7 e 14 days groups (magnification ×10; H&E; Bar~49.50µm).

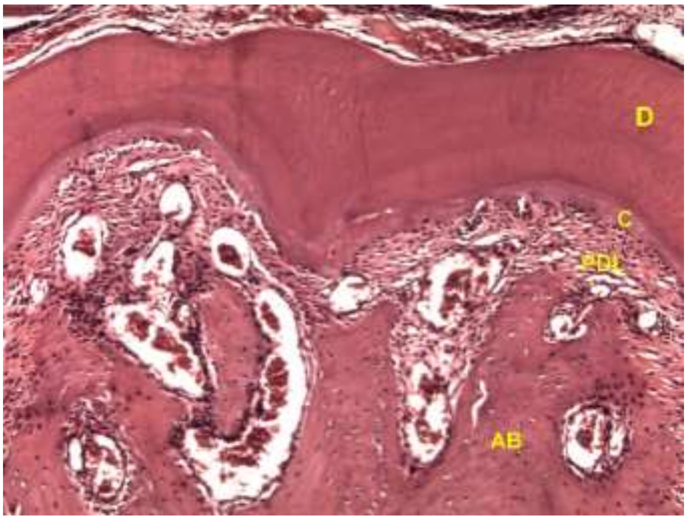


Figure 6 – Control Group; Dentin (D), Cementum (C), Periodontal Ligament (PDL) and alveolar bone (AB)

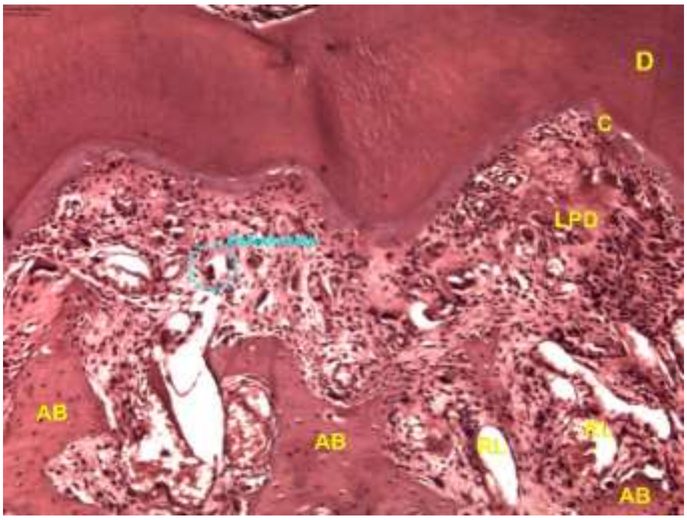


Figure 7 – 7-Day group, Dentin (D), Cementum (C), Periodontal Ligament (PDL), alveolar bone (AB), resorption lacunaes (RL) and osteoclast (blue).

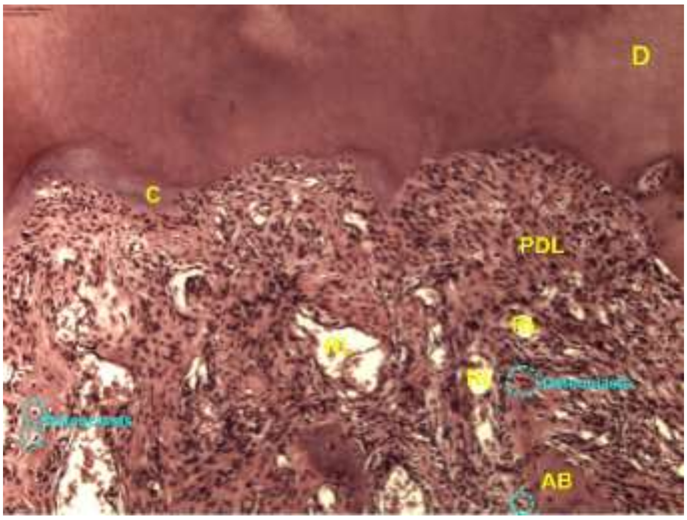


Figure 8 – 14-Day group, Dentin (D), Cementum (C), Periodontal Ligament (PDL), alveolar bone (AB), resorption lacunaes (RL) and osteoclast (blue).

4 DISCUSSION

Our investigation carried out a finite element analysis to evaluate the tooth-ligament-periodontal-alveolar bone under occlusal trauma. The presence of periodontal ligament fibers in the finite element model had an influence on the magnitude and distribution of deformations produced in the bone (McCormack et al., 2014). Results showed variations of compressive strain (-3×10^{-3} με to -9×10^{-3} με.) distribution in control group, because periodontal ligament is a dynamic soft tissue that support masticatory occlusal loads (McCulloch et al., 2000; Husain, 2018). The uniform compressive strain distribution in the experimental group indicated occlusal overloading (-7×10^{-3} με to -9×10^{-3} με) exceeding the periodontal ligament ability of supporting mechanical stress. Based on fundamental biomechanics, the tooth is subjected to a variety of loads of which compressive loads dominate the occlusal surface. Considering bone-periodontal ligament-cementum distortion, under small displacements the compressive occlusal loads result in pure shear stress state across the periodontal ligament space (Ho et al., 2010).

Thus, in this way, we suppose that in the experimental group mechanical force will be transferred directly to the alveolar bone. The findings observed in the alveolar bone in the experimental group with some areas with high compressive strains in the ROI confirmed our hypothesis. The alveolar bone in the control group with uniform compressive strain distribution on the bone surface from a correct occlusal contact pointed to physiological features. In this way, the compressive strain quantity on the bone surface and internal ranged $-1 \times 10^{-5} \mu\varepsilon$ to $-9 \times 10^{-4} \mu\varepsilon$ in the control group and highest compressive strain ranging -9×10^{-4} $\mu\varepsilon$ to -2.5×10^{-3} $\mu\varepsilon$ repeat features with specific areas with highest compressive strains.

Occlusal trauma in rats with a device installed in the first lower molar associated to periodontitis confirmed increase in the area of the periodontal ligament in the day 5 of study when compared to the group without occlusal trauma (Nakatsu et al., 2014). Other test found resorption of alveolar bone in furcation region and observed a few inflammatory cells and many osteoclasts on day 5. Many dilated blood vessels were observed around the bone crest. Osteoclasts disappeared and there were few dilated blood vessels at day 10 (Yoshinaga et al., 2007). In this sense, intragroup analyses demonstrated bone loss at 14, 21 and 28 days (Lopes et al., 2016).

Occlusal trauma caused the degeneration of collagen fibers and probably increased the permeability of antigens. The immune complex then spread widely, and the infiltration of inflammatory cells, which is related to tissue destruction, became stronger (Nakatsu et al., 2014). The results of our study demonstrated that in the group of 7 days the area of the periodontal ligament is larger when compared to the control group. Stretching, thickening and disorganization of the fibers of the periodontal ligament were observed, as well as disorganization in some regions of the cement boundary. Gaps of hard tissue resorption were observed in the alveolar bone and cementum. Our results allow us to state that occlusal trauma causes alterations in the periodontal tissues. Our hypothesis was confirmed by showing that occlusal trauma stimulates disorganization of the periodontal ligament tissue, increasing the space and consequently stimulating a process of bone resorption, corroborating to previous studies (Kaku et al., 2005; Yoshinaga et al., 2007; Walker et al., 2008; Fujii et al., 2014; Nakatsu et al., 2014; Takaya et al., 2015; Lopes et al., 2016; Tsutsumi et al., 2017).

The microscopy finds in histological periodontal ligament with an increase of collagen fiber thickness were compare with FEA results that exposed increase of compressive strain. The alveolar bone presented resorption areas as well as areas of highest compressive strains. This fact allows to affirm that the periodontal ligament features are influenced by physical forces (Ho et al., 2010; Huang et al., 2016). Occlusal trauma facilitates the recruitment of osteoclasts in order to maintain a certain periodontal ligament width, and periodontal ligament cells have an essential role in the mechanotransduction of force signals upon the periodontal ligament (Tsuzuki et al., 2016).

The FEA showed that the mechanical force in occlusal trauma was transmitted from the tooth directly into the alveolar bone and the periodontal ligament was unable to withstand this force on the tooth. Histological features indicate that the increase in the periodontal ligament space and bone resorption are the response of high-intensity mechanical action imposed on the first molar. The computational and histological results allow to identify the mechanical influence in the tissue in presence of occlusal trauma.

5 CONCLUSION

The mechanobiological behavior of the supporting tissues of the lower first molar in rats with occlusal trauma was demonstrated. Our results indicate that the mechanical strength of occlusal trauma generates compressive strain in the periodontal ligament and alveolar bone, regions where the biological response demonstrated increased periodontal ligament space and bone resorption alveolar.

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3 CONCLUSÃO

O comportamento mecanobiológico dos tecidos de suporte do primeiro molar inferior em ratos com trauma oclusal foi demonstrado. Nossos resultados indicaram que a resistência mecânica do trauma oclusal gera uma deformação de compressão no ligamento periodontal e osso alveolar, regiões onde a resposta biológica demonstrou aumento do espaço do ligamento periodontal e reabsorção óssea alveolar.

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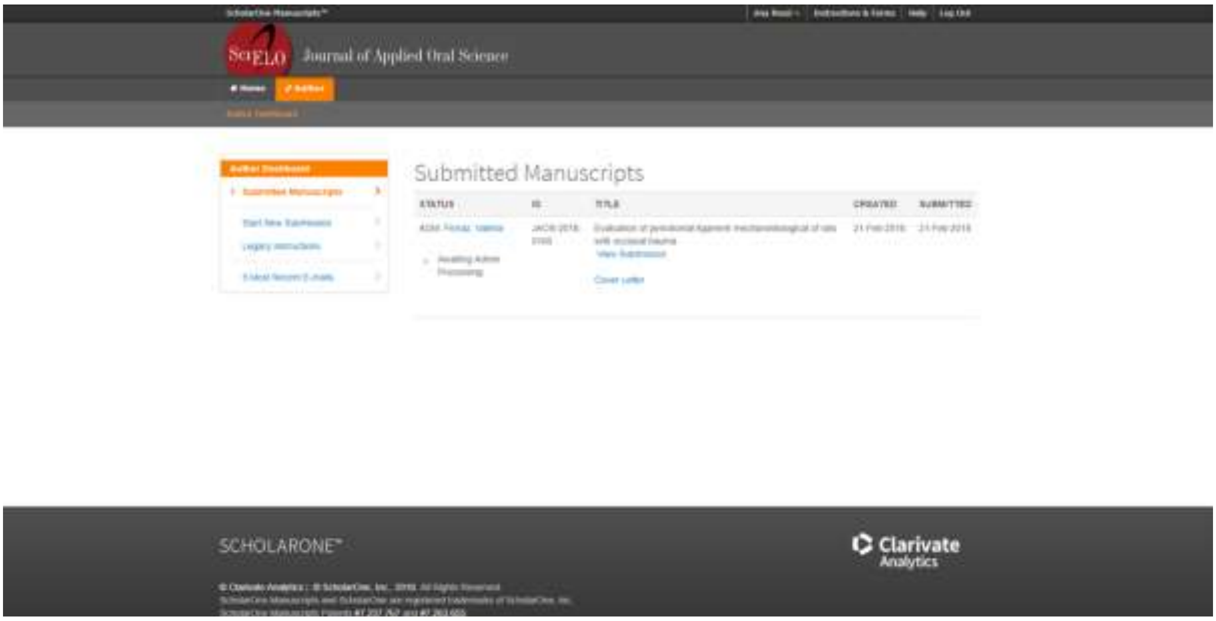
ANEXOS

ANEXO 1 - Comprovante de submissão



ANEXO 2 - Certificação do Comitê de Ética

UNICAMP

CEUA/UNICAMP

INFORMAÇÃO

A Comissão de Ética no Uso de Animais da UNICAMP - CEUA/UNICAMP - esclarece que não há necessidade de submeter para análise desta Comissão o projeto ANÁLISE DE ELEMTNOS FINITOS E ANÁLISES HISTOLÓGICAS NO OSSO ALVEOLAR E LIGAMENTO PERIODODNTAL EM RATOS ADULTOS SUBMETIDOS A CONDIÇÃO DE CONTATO PREMATURA, de responsabilidade do Prof. Dr. Felippe Bevilacqua Prado e dos executores Gilberto Paiva de Carvalho e Rodrigo Ivo Matoso.

Justifica-se por se tratar do uso de imagens microtomagróficos provenientes do protocolo CEUA 3661-1 - REMODELAÇÃO ÓSSEA E ANÁLISE BIOMECÂNICA DO OSSO ALVEOLAR MAXILAR EM RATOS COM SOBRECARGA MASTIGATÓRIA, não havendo manipulação *in vivo* De animais para desenvolvimento do projeto.

Campinas, 23 de outubro de 2017.

Prof. Dr. WAGNER JOSÉ FÁVARO
Presidente da CEUA/UNICAMP

ANEXO 2 - Certificação do Comitê de Ética (continuação)

UNICAMP

CEUA/Unicamp

CERTIFICADO

Certificamos que o projeto intitulado "**REMODELAÇÃO ÓSSEA E ANÁLISE BIOMECÂNICA DO OSSO ALVEOLAR MAXILAR EM RATOS COM SOBRECARGA MASTIGATÓRIA**", protocolo nº **3661-1**, sob a responsabilidade de **Prof. Dr. Felippe Bevilacqua Prado / Alexandre Rodrigues Freire**, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo *Chordata*, subfilo *Vertebrata* (exceto o homem) para fins de pesquisa científica ou ensino, encontra-se de acordo com os preceitos da **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais e do **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**, e com as normas editadas pelo **Conselho Nacional de Controle da Experimentação Animal - CONCEA**, e foi aprovado pela **Comissão de Ética no Uso de Animais** da **Universidade Estadual de Campinas - CEUA/UNICAMP**, em **09 de fevereiro de 2015**.

Vigência do projeto: 01/2015 - 03/2015
Espécie/Linhagem: Rato heterogênico Wistar
No. de animais: 25
Peso/Idade: 200g / 02 meses
Sexo: machos
Origem: CEMIB/UNICAMP

A aprovação pela CEUA/UNICAMP não dispensa autorização prévia junto ao **IBAMA**, **SISBIO** ou **CIBio**.

Campinas, 02 de setembro de 2015.

2ª. VIA

Profa. Dra. Liana Maria Cardoso Verinaud
Presidente

Fátima Alonso
Secretária Executiva

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