CAIO VINÍCIUS LOURENÇO DEBORTOLI

ALTERAÇÕES ESTRUTURAIS E COMUNICAÇÃO INTERCELULAR NO PERIODONTO SUBMETIDO À OCLUSÃO TRAUMÁTICA.

STRUCTURAL CHANGES AND INTERCELLULAR COMMUNICATION IN THE PERIODONTIUM SUBMITTED TO TRAUMATIC OCCLUSION

ARAÇATUBA – SP

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Trabalho de conclusão de curso como parte dos requisitos para obtenção do título de Bacharel em Odontologia da Faculdade de Araçatuba, Universidade Estadual Paulista "Júlio de Mesquita Filho".

Orientador(a) : Prof. Dra. Daniela Atili Brandini de Weert

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DEDICATÓRIA

Dedico esta, bem como todas as minhas demais conquistas, primeiramente a Jesus Cristo, pois, é quem me capacita para toda e qualquer situação.

Aos meus amados pais Celso Debortoli e Meire Regina Lourenço Debortoli que me sustentaram em amor e me deram as bases e as condições necessárias pra que eu pudesse realizar este sonho, a eles meu amor e gratidão eterna.

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“Seja você quem for, seja qual for a posição social que você tenha na vida, a mais alta ou a mais baixa, tenha sempre como meta muita força, muita determinação e sempre faça tudo com muito amor e com muita fé em Deus, que um dia você chega lá. De alguma maneira você chega lá.”

Ayrton Senna

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RESUMO

Trauma oclusal primário é uma lesão no sistema de inserção periodontal resultado de uma força excessiva aplicada à superfície oclusal de um ou mais dentes com periodonto saudável. Este estudo tem por objetivo avaliar o efeito da oclusão traumática no ligamento periodontal. Para este estudo, 80 ratos Wistar de sete semanas de idade foram divididos em grupos Controle (C) e Oclusão traumática (OT), em períodos experimentais de 2, 5, 7 e 14 dias. O grupo OT teve a superfície oclusal do primeiro molar inferior direito aumentada por restauração direta de resina composta. As análises quantitativas foram feitas nos cortes histológicos corados com a reação imunohistoquímica para conexina 43 (Cx43), Runt-related transcription factor 2 (RUNX 2), e Tartrate resistant acid phosphatase (TRAcP); contracoradas com hematoxilina e eosina (HE) para verificar a área de vasos sanguíneos e matriz extracelular e número de perfis nucleares; e Tricômico de Masson para medir a área das fibras colágenas do ligamento periodontal da raiz distal do primeiro molar inferior direito. O teste de Mann-Whitney foi utilizado para comparação entre grupos. No grupo OT, no lado mesial da raiz, houve redução na porcentagem das áreas das fibras colágenas (5 e 7 dias; valor p respectivamente 0,03 e 0,05) e aumento da largura periodontal (dia 2, $p = 0,05$); no lado distal da raiz observa-se uma diminuição na área de vasos sanguíneos (2 dias, $p = 0,03$), aumento do número de perfis nucleares (dia 2, $p = 0,03$ e 7 dias, $p = 0,05$). O aumento na área de matriz celular fibrilar (Dias 5 e 7) ocorreu em ambos os lados da raiz. O número de células imunorreativas para Cx43 (Dia 5 e 7, $p = 0,03$), TRAcP (Dia 2 e 7; $p = 0,029$ e 0,016, respectivamente) e RUNX 2 (Dia 5, $p = 0,03$) aumentou do lado distal. As alterações observadas no periodonto frente a oclusão traumática estão relacionadas ao processo de reparo diante do stress mecânico, tal como um aumento na comunicação celular e suprimento sanguíneo, a degeneração das fibras de colágeno e aumento no número de fibroblastos. No entanto, estruturalmente as mudanças significativas foram observadas na largura do ligamento periodontal, fibras colágenas, vasos sanguíneos e perfil celular.

Palavras-chave: Oclusão dentária traumática, periodonto, conexina 43.

DEBORTOLI, C.V.L. **STRUCTURAL CHANGES AND INTERCELLULAR COMMUNICATION IN THE PERIODONTIUM SUBMITTED TO TRAUMATIC OCCLUSION.** 2015. TRABALHO DE CONCLUSÃO DE CURSO (BACHARELADO) - FACULDADE DE ODONTOLOGIA, UNIVERSIDADE ESTADUAL PAULISTA, ARAÇATUBA, 2015.

ABSTRACT

Primary occlusal trauma has been defined as an injury to the attachment apparatus as a result of excessive occlusal force applied to a tooth or teeth with normal and healthy support tissues. This study aims were to evaluate the structural and histochemical changes of the periodontal ligament and bone around the roots; submitted to traumatic occlusion. For this study, 80 Wistar rats of seven weeks old were divided into Control (C) and Traumatic Occlusion (TO) group, in a period of study of 2, 5, 7 and 14 days. The TO group had the inferior right first molar raised by composite direct filling. The quantitative analyses were done in the sections immunostaining with connexin 43 (Cx43) and runt-related transcription factor 2 (RUNX 2), and histochemically stain with tartrate resistant acid phosphatase (TRAcP); counterstained with hematoxylin and eosin (HE) to verify blood vessel number and area, extracellular matrix area and number of cellular profile; and Masson´s to measure collagen fibers area of the periodontal ligament of the distal root of the right lower first molar. The Mann–Whitney *U* test was used for group comparison. TO group showed on the mesial side of the root a decreased percentage of collagen fiber area (5 and 7 days; p value respectivly 0.03 and 0.05) and an increase in the periodontal ligament width (day 2 , p=0.05); and on the distal side of the root show a decrease in the blood vessels area (day 2, p=0.03), increase in the number of nuclear profiles (day 2, p=0.03 and day 7, p=0.05). The increase in the area of fibrillar cellular matrix occurs in both side of the root. The number of immuno reactive cells for Cx43 (Day 5 and 7; p = 0.03) TRAcP (Day 2 and 7; p=0.029 and 0.016 respectively) and RUNX 2 (Day 5, p=0.03) increased in the distal side. The changes observed in the periodontium in front of traumatic occlusion are related to repair process front to mechanical stress, such as an increase in cell communication and blood supply, degeneration of collagen fibers and increase in the number of fibroblasts. However, structurally the significant changes were seen in the periodontal ligament width, collagen fibers, blood vessel and cell profile.

Key words: Traumatic dental occlusion, periodontium, connexin 43.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

PDL = Periodontal ligament

RUNX 2 = Runt-related transcription factor 2

TRAcP = Tartrate resistant acid phosphatase

HE = Hematoxylin and eosin

Cx43 = Connexin 43

SUMÁRIO

1 - INTRODUCTION

Theoretically, primary occlusal trauma has been defined as an injury to the attachment apparatus as a result of excessive occlusal force applied to a tooth or teeth with normal and healthy support tissues (The glossary of prosthodontic terms, 2005; Davies et al., 2001).

Immediately after applying a force system on a tooth, initial tooth mobility occurs associated with an intra-alveolar tooth displacement, producing local stress and strain distributions in the desmodontium. At this initial phase, the deformation of the alveolar bone is reversible and of very low magnitude. Maintaining this state, the mechanical stress in the PDL triggers apposition and resorption of the alveolar bone, which is bone remodelling and results in a permanent change of the tooth position. (Papadopoulou, 2013)

At early phase (24h) of the occlusal trauma, osteogenesis in rat's alveolar bone was inhibited, and osteoclastogenesis was not significant (Wan, 2012). An increase in the pressure of the interstitial fluid of the periodontal ligament was observed after 48 hours (Palcanis, 1973). Until the 5th day the width of the periodontal ligament space decreased, there is an increase in the number (Palcanis, 1973) and a disorientation (Glickman, 1971; Palcanis, 1973; Jabôr et al., 2003) of fibroblasts, cell necrosis and venous thrombosis in the periodontal ligament (Palcanis, 1973), a decrease in collagenous fibers (Palcanis, 1973; Biancu, Ericsson, Lindhe, 1995) and elevated osteoclast activity (Palcanis, 1973; Biancu, Ericsson, Lindhe, 1995; Glickman, 1971; Sodeyama et al., 1996; Yoshinaga, 2007; Kaku, 2005). There were also a significant increasing of Ruffini endings and free nerves endings in the PDL (Sodeyama et al., 1996). The PDL space return to normality on day 7 (Kaku et al., 2005), probably owing to bone remodeling (Glickman, 1971; Sodeyama et al., 1996).

However, at clinical level it can affect tooth mobility (Paul et al., 1995; Gher, 1998; Nunn and Harrel, 2001; Harrel and Nunn, 2001), frenitus, persistent discomfort or pain on eating (Davies et al., 2001) and asseptic pulp necroses; also radiograffically, discontinuity and thickening of fasciculate alveolar bone, widening of periodontal ligament space, radiolucency and condensation of alveolar bone and/or root resorption (Davies et al., 2001) can be seen indicating that continuous destructive/patological process can occur for a longer time; creating even tooth loss.

Even though, symptoms and signs can be linked with the effect of the traumatic occlusion in the periodontium, the reviews about this subject show inconclusive results, pointing out the necessity of more randomized trials and quantitative research (Liu, Jiang, Wang, 2013; Fu and Yap, 2007; Gher, 1998; Svanberg, King and Gibbs, 1995; Carranza, 1969)

Mechanical loading is an important factor affecting the osteogenic differentiation of mesenchymal stem cells (SHI et al, 2011). It can inhibit the expression of markers of osteogenic differentiation, like alkaline phosphatase (ALP) and runt-related transcription factor 2 (RUNX2); what is enhanced in an inflammatory environment (Nokhbehsaim et al., 2010).

The RUNX2 gene provides instructions for making a protein that is involved in bone and cartilage development and maintenance. Researchers believe that the RUNX2 protein acts as a "master switch," regulating a number of other genes involved in the development of osteoblasts. Althouth RUNX2 can be also regulated by connexin 43 (Bivi et al., 2012; Niger et al. 2013) and prostaglandin E2 (PGE2)(Manokawinchoke et al., 2014).

Connexin43 (Cx43) plays a critical role in osteoblast function (Bivi et al., 2012; Niger et al. 2013) as evidenced by reducing expression of osteoblast markers and delayed ossification (Plotkin, 2011), it means that loss of Cx43 can result in both decreased bone formation and bone resorption (Loiselle et al. 2013)

2 - OBJECTIVES

This study aims were to evaluate the structural and histochemical changes of the periodontal ligament and bone around the roots; submitted to traumatic occlusion.

The specifics objectives are to make a quantitative analyses in the right first lower molar, of the:

a) number of osteoblast around the roots (immunolabeling of runt-related transcription factor 2-RUNX 2);
b) number of osteoclast around the roots (histochemically stain with tartrate resistant acid phosphatase-TRAcP);
c) number of expression of connexin 43 in limited area of periodontal ligament;
d) number of cellular profile (sections counterstained with hematoxylin and eosin -HE);
e) blood vessel area (sections counterstained with hematoxylin and eosin - HE);
f) extracellular matrix area (sections counterstained with hematoxylin and eosin - HE);
g) width of the periodontal ligament (sections counterstained with hematoxylin and eosin - HE) and
h) collagen fibers area (Masson's trichrome stained setions)

3 - MATERIALS AND METHODS

This study has been approved by the Animal Care Committee of the Dentistry School of Araçatuba (UNESP).

For this study, 80 Wistar rats (*Rattus Norvegicus, albinus*) of seven weeks old were be used. Originating from the central bioterium of the Dentistry School of Araçatuba, the animals were transferred to the bioterium of the Department of Surgery and Integrated Clinic 5 days prior to the experiment. They were kept in cages with five animals each and given granulated food and water *ad libitum*. The environment were kept at a temperature of 22 °C (±2 ºC) and 50% (±10%) humidity, and light/dark cycles of 12/12 hours.

To address the influence of mechanical loading in the alveolar bone after occlusal trauma, the animals were be divided into 2 groups: Control and Traumatic occlusion group.

The groups consist of:

a. Control group (40 rats) – rats with the same age as the rats in the experimental group that won't submitted to experimental conditions, in order to compare with normal conditions.
b. Traumatic occlusion group (40 rats) – mechanical loading at occlusal surface of the teeth by composite filling in the right inferior first molar.

The period of study were 2, 5, 7 and 14 days. Prior to the experimental section, the animals were intramuscularly anesthetized with a solution of ketamine hydrochloride (25 mg/kg, Vetanarcol, Laboratórios König, Argentina) and xylazine (10 mg/kg, Coopazine, Coopers Brasil, Brazil).

In the Traumatic occlusion group, the inferior right first molar were raised by direct filling using 37% phosphoric acid etchant for enamel and dentin (FGM, Brazil), microbrush (Microbrush® International, Grafton, USA); scotchbond multi-purpose light adhesive (3M ESPE, Sant Paul, USA), Estelite e Quick composite resin (Tokuyama Dental Corp, Japan) and photopolimerizer (Dabi Atlante, Ribeirão Preto, Brazil), as indicated by producer; creating a flat high occlusal table at the highest occlusal cusp. A piece of ligature wire 0.20mm (.008´´) (Morelli, Sorocaba, São Paulo, Brazil) were attached to the surface of the composite filling. Prior to the restoration, microretentions were made with Carbide burs FG ¼ (Beavers Dental, Canada) at high speed handpiece with water.

Perfusion and fixation

Animals were excluded in case they die of natural causes, and if they lose the occlusal composite filling.

After anesthesia, transcardial perfusion were performed. An intraventricular injection of heparin (0.1 ml / 5,000 U.I/ml) were administered. After 1 minute 100 ml saline were perfused via the aorta, followed by mixed solution of 200 ml of paraformaldehyde fixation solution at 4% (Sigma Chemical Co., St. Louis, MO, USA) and 200 ml phosphate buffered saline (PBS) at 0.1M, pH 7.4, 4ºC (Sigma Chemical Co., St. Louis, MO, USA).

After the dissection, the specimens were washed in PBS, and were kept paraformaldehyde fixation solution at 4% (Sigma Chemical Co., St. Louis, MO, USA) for 24 hours, before star the decalcification with ethylene diamine tetra acetic acid disodium (EDTA) at 10% for 20 days.

The specimens were processed with progressive dehydration in ethyl alcohol, diafanized in xylol, impregnated by paraffin at a low fusion temperature (56-58 ºC) for 3 hours and embedded according to standard protocols.

Transversal histological sections of 5 μm wide of the right lower first molar and surrounding tissue were obtained with an automated rotary microtome (Leica SMR 2000), transferred to a bain-marie (40-50 ºC) and then collected using silanised slides.

Immunohistochemistry for detection of RUNX-2 and connexin 43

The slides were deparaffinized and rehydrated. Next, they were subjected to the following washing and incubation stages: a) rinse 3 min in 3% H_2O_2; b) wash 5 min in destilled water to inactive endogenous peroxidase; c) 10 minute washings in PBS (0.1 M phosphate-buffered saline, pH 7.4, room temperature); d) incubation for 30 min in 2% BSA (albumin bovine serum) (Sigma); e) gently tap off the blocking buffer (2% BSA); f) incubation for 24 hours at room temperature, using the following primary antibodies separately for each histological slides containing samples from all of the experimental groups: mouse monoclonal anti-RUNX-2 (1:600) (Santa Cruz

Biotech); rabbit anti connexin 43 (1:150) (Santa Cruz Biotech) g) three times 10 minute washings in PBS at room temperature; h) histological sections were incubated with a labelled polymer-HRP secondary antibody for 1h and the reaction were developed using the chromogen 3,3′-diaminobenzidine tetrahydrochloride (Dako Envision + System-HRP, Dako North America, Inc – USA and i) counterstain with hematoxylin (Mayer). The negative control consisted of specimens subjected to the aforementioned procedures but without the primary antibodies.

Histochemical treatment for TRAP detection

To show TRAcP activity, sections were deparaffinized, rehydrated and rinsed in PBS. For reactivation were used a 0.1M Tris pH 9.0 solution, overnight at room temperature. After that, the section were incubated with a solution containing 30 mg Naphtol-AS-BI phosphate (Sigma D4254, Sigma Aldrich Chemie Gmbh, Taufkirchen, Germany), 0.5 ml Dimethylformamide (Sigma D4254, Sigma Aldrich Chemie Gmbh, Taufkirchen, Germany), 9 ml acetatebuffer in PVA , 0.1 ml $MgCl_2$. $6H_2O$, 0.1 ml KNa-tartrate $4H_2O$, 0.3 ml pararosanilin and 0.3 ml NaNO2; for 1h at 37°C. Subsequently, the sections were rinsed in PBS and counterstained with Mayer hematoxylin.

Histometry and Labeling analyses

The images were processed by Image J program for measurement of the area (μm^2) and to count the number of cells in the pre determined area in the periodontal ligament and/or alveolar bone, according to the case.

A number of three sections of each specimen, in a distance of 40 µm between them, were selected to evaluate the histomorphology at the time-related changes throughout the experimental period and comparation between the groups.

The sections were observed with an Aristoplan light microscope (Leica - Aristoplan, Alemanha) and micrographed with an Axiocam MRc digital camera (Carl Zeiss MicroImaging GmbH, Germany).

The visual camps of the lower right first molar and surrounding tissues of each animal were collected with 40X and 100X objectives, and also analysed using the program Axionvision Rel 4.0 (Carl Zeiss GmbH, Germany).

The number of cells immunostainned for RUNX 2 and expression of Connexin 43, and histochemically treatmented for TRAP detection, were counted in an area of 7835 μm^2 and magnification of 770x of the distal part of the all the root of the right first lower molar.

The sections counterstained with hematoxylin and eosin (HE) were used to quantitative analyzes of the number of nuclear cell profile, blood vessel area, extracellular matrix area and the width of the periodontal ligament (mesial and distal side) of the all the root of the right lower first molar. Masson's trichrome stain setions were used to quantitative analyzes of the collagen fibers area. All this variables were measured in an area of 49044 μm^2 and magnification of 620X.

The examiner were not informed to which group the images belonged, in order to avoid bias during the analysis.

Statistical Analysis

The data were analyzed using IBM SPSS 20.0 (IBM, Armonk, NY, USA) at α=0.05. The *Mann–Whitney U* test was used for group comparison. Data were expressed as mean ± SD or percentage. The Spearman correlation test was applied between number of labeled cells with Cx43, RUNX 2, TRAcP.

4 - RESULTS

Representative histological observations are shown in Figure 1. These analyses were limited to the cervical region of the periodontal ligament and alveolar bone of the right first lower molar of the mandible.

The control group showed a normal pattern of histological characteristics during the whole experimental period, periodontal ligament with the same width in the mesial and distal side, fibers and blood vessel with a regular arrangement, and irregular bone reabsorption with more activity in the distal side of the root (Table 1 and 2).

Periodontal ligament width in the TO group, in the mesial side show an significant increase ($p=0.05$) that was normalized on day 5 (Table 1).

Histological analysis between groups showed a statistically significant decrease in the percentage of collagen fiber areas in the mesial portion of the distal root in the traumatic occlusion group, on day 5 and 7 days (Table 1). However, the area of blood vessel and extracellular matrix, as well as the amount of fibroblast nuclear profiles did not show significant changes in all postoperative periods in the mesial portion of the distal root (Table 1). However, the distal portion of the distal root of the same tooth, there was a decrease in the percentage of blood vessels area ($p = 0.03$) and in the number of nuclear profiles cell ($p = 0.03$) on day 2 (Table 2).

In analyses of imuno and histochemical proteins, the data show an increase in the in the number of immuno reactive cells for Cx43 (Day 5 and 7; $p = 0.03$) TRAcP (Day 2 and 7; $p=0.029$ and 0.016 respectively) and RUNX 2 (Day 5, $p=0.03$)(Table 3 and 4).

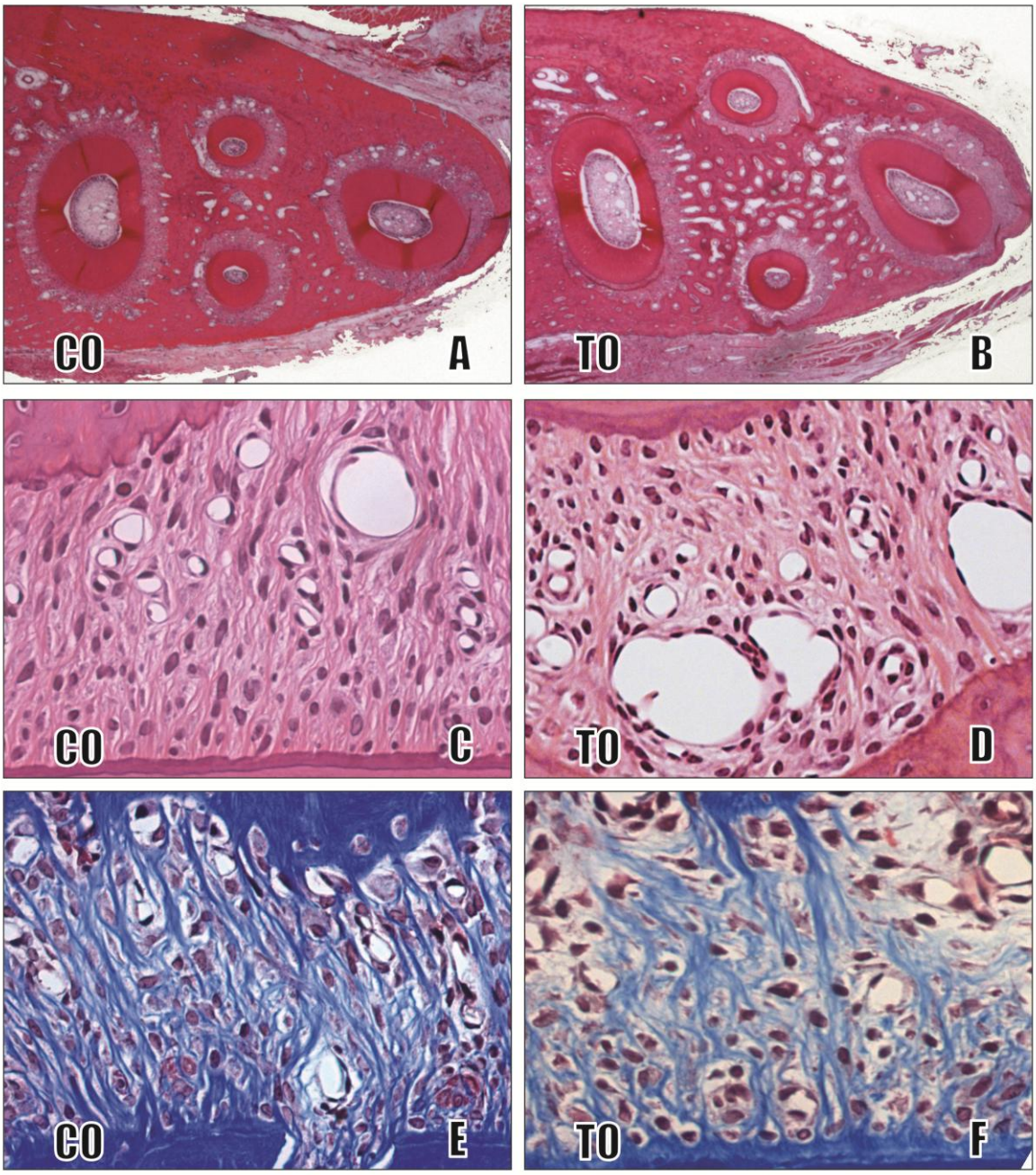

Figure 1. Transversal histological sections through the roots of the first lower right molar, HE staining in Fig1A-D (day 5) and Masson's trichrome staining Fig1 E-F (day 5). (Magnified 40x, and 620x).

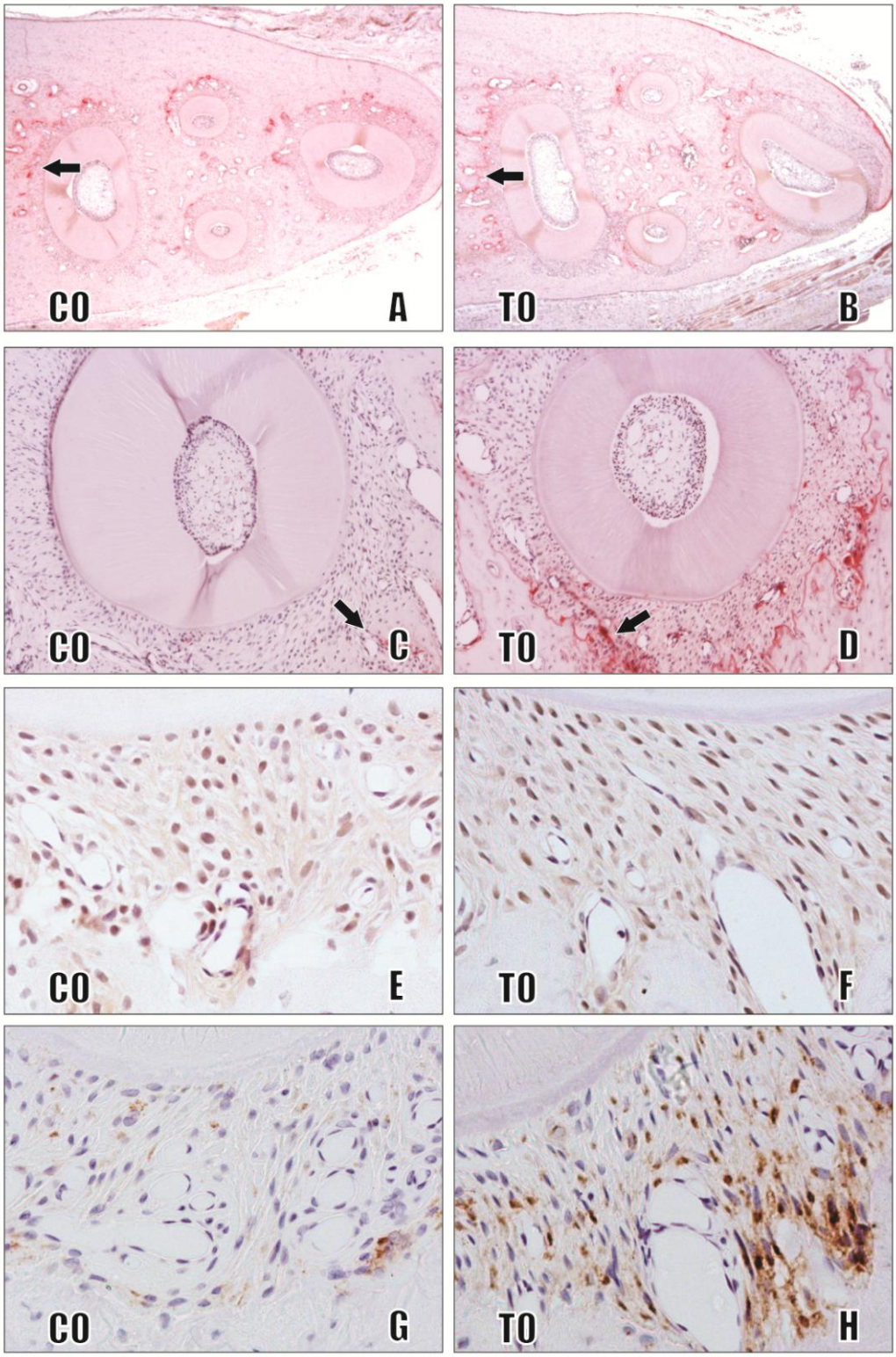

Figure 2. Transversal histological sections through the roots of the first lower right molar, staining by TRAcP Fig A-D (day 7), RUNX2 Fig E-F (day 14) and Connexin 43 Fig G-H (day 7). (Magnified 40x, 150x and 620x).

Table 1. Traumatic occlusion and morphological changes in the mesial side of the periodontal ligament.

Variables	Control Mean (SD)	Traumatic Occlusion Mean (SD)	P Value
Collagen fibers area (%)			
2 days	38.7 (12.1)	36.5 (10.7)	0.88
5 days	44.0 (5.71)	31.0 (4.24)	0.03*
7 days	43.5 (2.64)	25.0 (3.60)	0.05*
14 days	30.5 (3.31)	30.5 (2.50)	1.00
Blood vasel area (%)			
2 days	3.55 (0.80)	4.69 (1.92)	0.48
5 days	4.59 (3.07)	5.50 (2.04)	0.48
7 days	4.93 (2.41)	5.98 (2.65)	1.00
14 days	7.62 (2.00)	6.50 (0.26)	0.80
Extracellular matrix area (%)			
2 days	15.9 (12.2)	6.57 (3.48)	0.11
5 days	9.17 (4.32)	15.1 (4.16)	0.11
7 days	5.25 (2.37)	13.6 (7.96)	0.11
14 days	8.67 (5.37)	7.50 (5.65)	0.40
Nuclear cell profile (number)			
2 days	170.9 (11.2)	192.8 (23.8)	0.11
5 days	168.7 (9.64)	195.0 (34.5)	0.20
7 days	150.2 (10.5)	200.6 (34.5)	0.22
14 days	186.9 (17.8)	194.5 (35.4)	1.00
Periodontal ligament width (μm)			
2 days	138.6 (12,2)	160.2 (9,5)	0.05*
5 days	149.4 (10.5)	142.7 (21,9)	0.88
7 days	144.0 (12.2)	149.5 (8.9)	1.0
14 days	145.6 (17.2)	123.7 (10.3)	0.23

P values are for comparison between the two groups. *Mann–Whitney* U test were used for continuous variables. Data are presented as the mean ± standard deviation.*P<.05; **P<.001.

Table 2. Traumatic occlusion and morphological changes in the distal side of the periodontal ligament.

Variables	Control Mean (SD)	Traumatic Occlusion Mean (SD)	P Value
Collagen fibers area (%)			
2 days	40.7 (7.1)	39.0 (7.0)	0.89
5 days	32.5 (4.8)	35.2 (2.8)	0.34
7 days	31.9 (4.4)	30.3 (14.1)	1.00
14 days	40.0 (9.7)	28.9 (6.5)	0.23
Blood vasel area (%)			
2 days	3.2 (1.3)	1.7 (0.5)	0.03*
5 days	3.7 (3.1)	2.3 (0.7)	1.00
7 days	4.1 (2.5)	3.9 (2.9)	0.86
14 days	2.5 (2.3)	4.5 (3.5)	0.63
Extracellular matrix area (%)			
2 days	15.0 (6.4)	15.2 (3.7)	1.00
5 days	14.9 (3.4)	18.7 (5.0)	0.34
7 days	17.9 (1.5)	22.6 (4.4)	0.40
14 days	19.7 (2.2)	22.7 (4.1)	0.63
Nuclear cell profile (number)			
2 days	289.1 (18.3)	247.8 (17.5)	0.03*
5 days	250.7 (41.1)	290.6 (30.8)	0.20
7 days	304.2 (14.3)	221.2 (26.0)	0.05
14 days	215.7 (29.1)	215.5 (31.2)	1.00
Periodontal ligament width (μm)			
2 days	144.1 (10.1)	142.5 (3.5)	1.00
5 days	156.9 (11.6)	168.2 (30.6)	0.68
7 days	149.2 (30.7)	123.1 (9.8)	0.40
14 days	147.4 (52.0)	143.2 (34.7)	1.00

P values are for comparison between the two groups. *Mann–Whitney* U test were used for continuous variables. Data are presented as the mean ± standard deviation.*P<.05; **P<.001.

Table 3. Traumatic occlusion and imunohischimical reactions in the periodontal ligament.

Variables (number)	**Controle** Mean (SD)	**Oclusão Traumática** Mean (SD)	**P Value**
Connexin 43			
2 days	31.5 (2.08)	29.0 (8.40)	0.88
5 days	32.2 (4.34)	36.5 (4.20)	0.34
7 days	41.5 (8.69)	41.5 (11.2)	1.00
14 days	26.2 (3.50)	39.2 (2.50)	0.03
RUNX 2			
2 days	34.5 (2.03)	44.1 (3.59)	0.05
5 days	37.0 (2.87)	50.5 (1.52)	0.03
7 days	32.5 (4.85)	66.4 (21.2)	0.11
14 days	44.0 (8.70)	48.7 (8.63)	0.40
TRAcP			
2 days	24.1 (10.9)	98.7 (11.8)	0.03
5 days	26.6 (3.4)	109.3 (39.8)	0.05
7 days	32.2 (6.3)	76.5 (19.4)	0.01
14 days	27.0 (28.2)	95.7 (25.3)	0.05

P values are for comparison between the two groups. *Mann–Whitney* U test were used for continuous variables. Data are presented as the mean ± standard deviation.*P<.05; **P<.001.

Table 4. Nonparametric correlation between number of between Connexin 43, RUNX 2 and TRAcP expression, presented by rank correlation coefficient.

	Period	RUNX 2		TRAcP	
CX 43	2 days	-0.60	p=0,11	-0.19	p=0.68
	5 days	0.42	p=0.29	0.36	p=0.61
	7 days	-0.14	p=0.76	0.33	p=0.58
	14 days	0.58	p=0.17	0.93	p=0.02*

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

5 - DISCUSSION

This study shows the effect of traumatic occlusion in the periodontium, caused by the increase of the occlusal height by direct composite. In this model, changes in the components of the periodontal ligament were with low intensity in the most of studied variables; with some significant differences.

The reduced thickness of the periodontal ligament space in rats subjected to traumatic occlusion was observed in HE section by Sodeyama et al. 1996 and Kaku et al., 2005. On the 1st day of traumatic occlusion there was a slight narrowing of the space LP for Sodeyama et al. 1996 and greatly reduced for Kaku et al., 2005; and remained significantly lower ($p < 0.05$) up to day 5, recovering thickness of 7 days (Kaku et al., 2005). On the 2sd, 3th and 4th days, there was a PDL space narrowing at the distal side and a broadening of the mesial side (Sodeyama et al. 1996). These data show a different pattern of the periodontal ligament width changes, with an increase in the mesial side and decrease in the distal side of the roots.

This histological analysis presented that the fibers of the periodontal ligament in teeth submitted to occlusal trauma were more disorganized in the distal region of the roots. This disorganization was apparently higher in teeth restored with amalgam than with composite resin (Jabor et al., 2003). There was a continuous decrease in the collagen fibers area from day 2 to 7, getting back to the normal measure on day 14, in the traumatic occlusion group. These facts are probably because of the reduction in the mechanical force applied to the tooth due to the tooth wear and bone reabsorption.

Histological analysis in HE, were carried out by several authors. Hyalinised tissue was also common histological finding observed in the furcation region of teeth submitted of traumatic occlusion. This study did not show a regular pattern of the hyalinization area presence in none of the groups during these periods of time, in the mesial side of the root; by mean data analyses. However in the distal side of the root, the data show a constant rise in the percentage of extracellular matrix area; may caused by fibroblast death due to lower space of the periodontal ligament. The respective frequencies of these events were described for Campos et al, 2014 describe that 37.5% (day 7 and 14) of the rats of the traumatic occlusion group presented hyalinised tissue in the periodontal ligament. Jabor et al., 2003 found that

the presence of hyaline areas was more frequent in the presence of traumatic occlusion, more evident with the amalgam than with composite resin.

Nuclear cell profile slightly increases in the traumatic occlusion group during all period, probably because the mechanical force induced by this model is not enough to induce a cell death.

These findings present an increased blood vessels area on the mesial side of the root from day 2 to 5, probably because of the bone formation required by the increase in the width of the periodontal ligament in front of traumatic occlusion; and a decrease on the distal side may follow the decrease on the periodontal ligament width. This analysis was made in the roll periodontal ligament width. According to Dotto et al. (1967), the occlusal trauma caused a statistically significant increase in the number of blood vessels in the periodontal ligament experimental period of 21 days (+ 22%; p = 0.01), 60 days (a 25% increase, p = 0.01) and 120 days (24.5%; p = 0.005). Regarding the positioning and location of the blood vessels of the periodontal ligament in the control group, the blood vessels were closer to the bone than the cement. After 3 and 7 days, blood vessels had suffered a small shift, but still remain closer to the bone. On 21 days of traumatic occlusion, blood vessels occupy a position closer to the cement (p=0.005). From 60 days these blood vessels have moved to a more central position; with no statistically significant difference with the values obtained in 21 days, recovering its original position with 120 days (p = 0.02). In this finding the number of blood vessels also shows a significant difference, in some periods of the experiment, however this kind of analyses is not the best for this purpose.

The rise in the number of osteoclasts during all experimental period (2 to 14 days), in front of traumatic occlusion; was the remarkable sign in this research. The statistical analyses may lead to conclusion, that the alterations in the bone are greater than in the periodontal ligament.

The presence of osteoclasts is connected to the idea of bone reabsorption and osteoblasts bone formation. However in this research the number of RUNX 2 expression also increase in the traumatic occlusion group. It may be explained by the tooth movimentation which generates formation in one side of the tooth and reabsorption in another.

The number of osteoclasts increased dramatically from day 1 until day 14 days (Kaku et al., 2005; Yoshinaga et al., 2007; Walker et al, 2008; Nakatsu et al., 2014;

Campos et al., 2014). In the study Kaku et al., 2005, osteopontin (OPN) has been observed in a few osteoclasts from day 3 until day 14.

Although there was an increase in the number of connexin 43 expression in front of traumatic occlusion, it does not show a link with the number of RUNX 2 and TRAcP expression from day 2 to 7. Only on day 14 this data show a positive correlation between the imuno expression of connexin 43 and TRAcP; which does not support the expected results of this research.

6 - CONCLUSIONS

The changes observed in the periodontium in front of traumatic occlusion are related to repair process front to mechanical stress, such as an increase in cell communication and blood supply, degeneration of collagen fibers and increase in the number of fibroblasts. However, structurally the significant changes were seen only in the collagen fibers.

The imuno expression of connexin 43 does not show a pattern correlation with RUNX 2 expression, been so related with TRAcP expression in the periodontium in front of traumatic occlusion.

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8 - APPENDIX

APPENDIX A- Certificado de Aprovação da Comissão de Ética na Experimentação Animal.

unesp UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" Campus de Araçatuba

Comitê de Ética no Uso de Animais (CEUA)
Committee for Ethical Use of Animals (CEUA)

CERTIFICADO

Certificamos que o Projeto **"Efeito do trauma oclusal em algumas estruturas do sistema estomatognático"** sob responsabilidade da Pesquisadora **DANIELA ATILI BRANDINI DE WEERT** e colaboração de Ana Paula Farnezi Bassi e Sara Vieira Pacanaro está de acordo com os Princípios Éticos da Experimentação Animal (COBEA) e foi aprovado pelo CEUA, de acordo com o protocolo **2012-00980**.

CERTIFICATE

We certify that the research **"Effect of the occlusal trauma in some struture of the stomatognatic system"**, protocol number **2012-00980**, under responsibility of **DANIELA ATILI BRANDINI DE WEERT** and with collaboration of Ana Paula Farnezi Bassi and Sara Vieira Pacanaro agree with Ethical Principles in Animal Research (COBEA) and was approved by CEUA.

Prof. Dr. Edilson Ervolino
CEUA Vice-Coordinador

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