OPEN ACCESS SCIENTIFIC JOURNAL

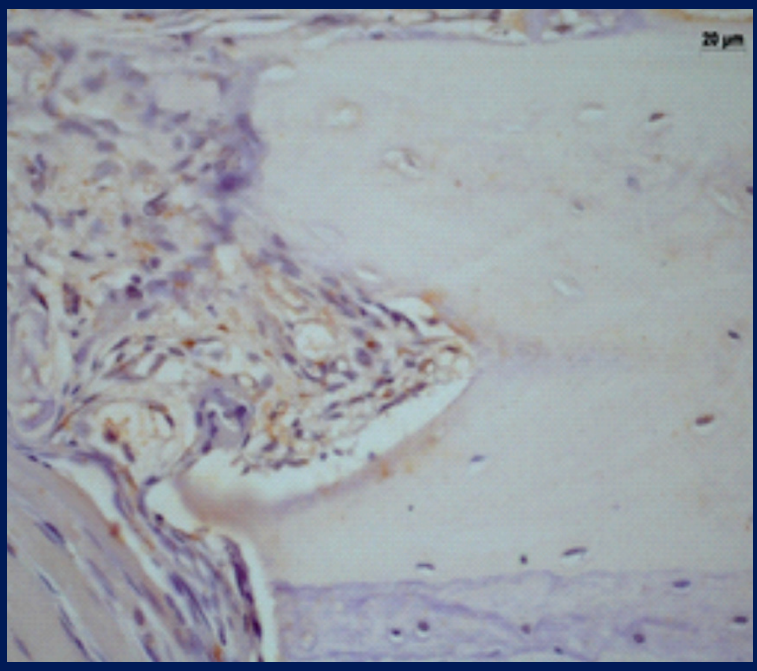
VOLUME 17 Nº 02 Supplemet - 2014

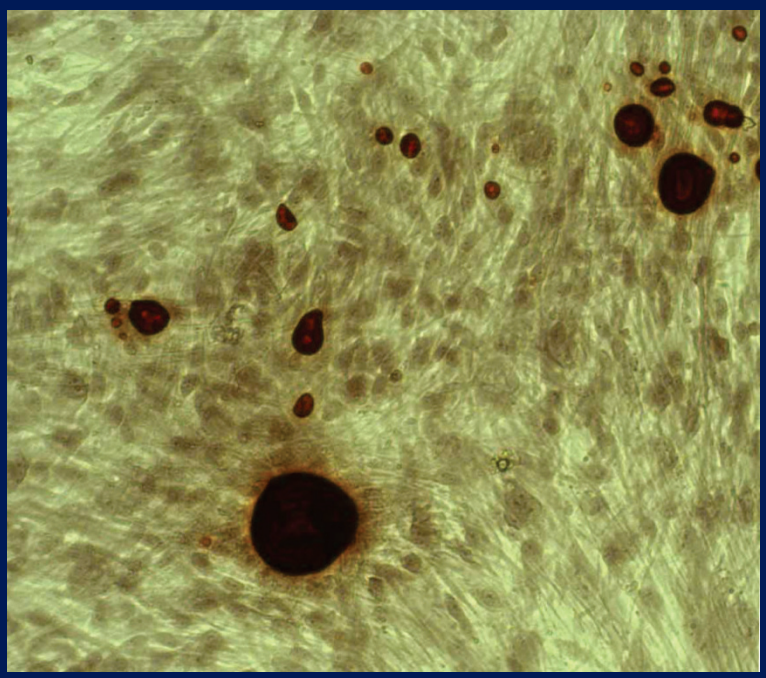
Brazilian Dental Science

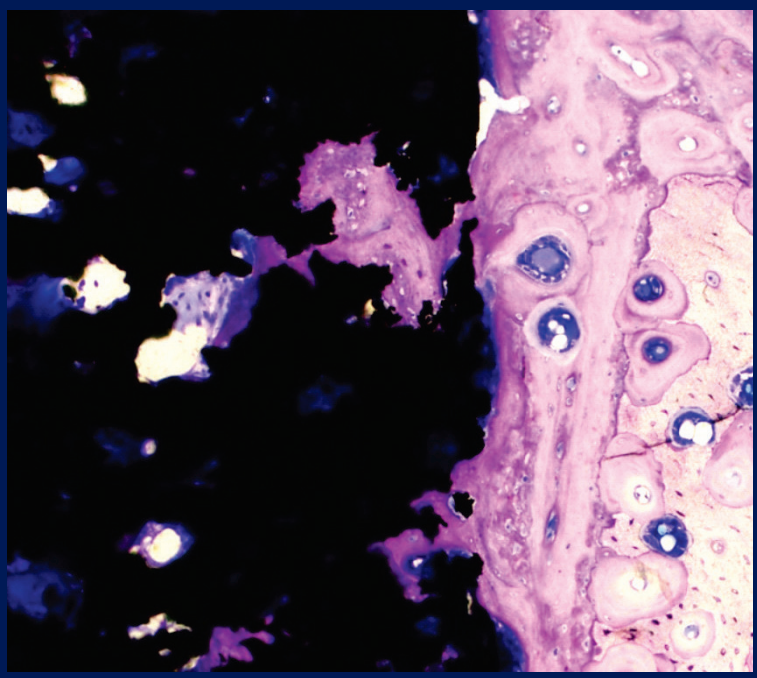
Ciência Odontológica Brasileira

1st Brazilian Workshop on Bone Biology

2 a 4 de abril de 2014







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Institute of Science and Technology

1st Brazilian Workshop on Bone Biology

2014

São José dos Campos

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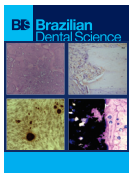

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EDITORIAL

The proceedings of the **1st Brazilian Workshop of Bone Biology,** held at ICT - Dentistry campus - UNESP, São José dos Campos, from April 02 to 04 2014, are available, with great pleasure, to all readers of the Brazilian Dental Science.

Chaired by Professor Ana Lia Anbinder, from the Department of Biosciences and Oral Diagnosis of this institution, the Congress brought together professionals in symposia and poster presentations.

Both, faculty and undergraduate and graduate students had the opportunity to discuss the results of their scientific research, exchanging relevant information to improve the quality of future work and increasing the relationship networking.

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Brazilian Dental Science: the expression of quality research.

Sincerely,

Associate Professor
Sérgio Eduardo de Paiva Gonçalves
Editor-in-Chief

*Com grande satisfação colocamos à disposição dos leitores da BDS os Anais do **I Brazilian Workshop of Bone Biology**, realizado no ICT, campus de Odontologia – UNESP- São José dos Campos, no período de 02 a 04 de abril de 2014.*

Sob a presidência da professora Dra. Ana Lia Anbinder, do departamento de Biociências e Diagnóstico Bucal da referida Instituição, o Congresso reuniu profissionais da área em simpósios e apresentações de trabalhos sob a forma de painéis.

Tanto docentes, como alunos de Graduação e de Pós-Graduação tiveram a oportunidade de discutir os resultados de suas investigações científicas, trocando informações de importância para elevar a qualidade dos futuros trabalhos e aumentar a rede de relacionamentos.

A BDS orgulha-se de ser este importante veículo de disseminação da produção científica e de construção do conhecimento.

Acesse nosso site (ojs.ict.unesp.br) e confira os trabalhos relevantes para a sua área de atuação.

Brazilian Dental Science: a expressão da pesquisa de qualidade.

Cordialmente,

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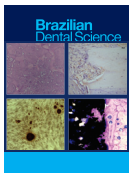

WORD OF THE PRESIDENT - PALAVRA DO PRESIDENTE

The first Brazilian Workshop on Bone Biology was an initiative of UNESP (Univ Estadual Paulista) professors aimed at bringing together researchers studying bone biology in several areas, to establish national and international cooperation, and to discuss new methodologies and innovative ideas. The workshop format allowed the discussion of various topics on bone biology by the 210 participants of the event (163 participants were present and 47 participated via online). The audience was mostly composed of undergraduate and graduate students, professors, especially from dental schools, but also veterinarians, physical trainers and physical therapists, from Vale do Paraíba and São Paulo countryside. The following institutions were represented at the event: UNESP (Campi São José dos Campos, Araraquara, Araçatuba and Botucatu); USP (Ribeirão Preto, Bauru); UNICAMP; UNIFESP; UFSCAR; UFJF; UFRN; Rio Grande University Foundation; UNITAU; UNIP; UNIVAP; FUNVIC. The financial support was provided by UNESP and by FAPESP (São Paulo Research Foundation). Based on the successful first Worskshop on Bone Biology, we are already planning the second event.

Profª Drª Ana Lia Anbinder
President of the I Brazilian Workshop of Bone Biology

O I Workshop Brasileiro sobre Biologia Óssea foi uma iniciativa de professores da UNESP (Univ Estadual Paulita) que teve como objetivo congregar os pesquisadores que estudam biologia óssea nas mais diversas áreas, para estabelecimento de redes de cooperação nacionais e internacionais, discussão de novas metodologias e ideias inovadoras. O formato de workshop permitiu a integração e discussão dos diversos assuntos entre 210 participantes do evento (163 participaram de maneira presencial, e 47 à distância, uma vez que o evento foi transmitido ao vivo, online). O público em sua maioria foi composto por alunos de graduação e pós-graduação e docentes da area da saúde, principalmente de odontologia, mas também veterinários, educadores físicos e fisioterapeutas, da região do Vale do Paraíba e interior de São Paulo. As seguintes instituições estiveram representadas no evento: UNESP (Campi de São José dos Campos, Araraquara, Araçatuba e Botucatu); USP (Ribeirão Preto, Bauru); UNICAMP; UNIFESP; UFSCar; UFJF; UFRN; Fundação Universidade Rio grandense; UNITAU; UNIP; UNIVAP; FUNVIC. O apoio financeiro foi prestado pela UNESP e pela FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo). Mediante o êxito da primeira edição do workshop, já estamos trabalhando para mais um evento de sucesso!

Profª Drª Ana Lia Anbinder
Presidente I Workshop Brasileiro sobre Biologia Óssea

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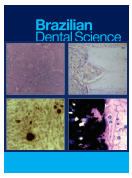

April 2nd 2014 -Wednesday

Morning

8h30 - 9h	Opening Ceremony
9h - 10h30	Bone Marrow - source of cells, cytokines and growth factors for bone regeneration.
Speaker:	Maria José Hitomi Nagata
10h45- 12h15	Bone repair and estrogen deficiency
Speaker:	Roberta Okamoto

Afternoon

14h - 15h45	Host response modulation in periodontics and its relationship with cutting edge studies of bone biology
Speaker:	Ana Lia Anbinder
16h - 18h	Influence of surface topography, composition of materials and coating of calcium phosphate in osteogenesis in vivo and in vitro
Speaker:	Luana Marotta Reis de Vasconcellos

April 3rd 2014 - Thursday

Morning

8h - 10h	Regulation of normal bone and tooth mineralization, and what goes wrong in hypophosphatasia and X-linked hypophosphatemia
Speaker:	Marc D. McKee
10h15 - 12h15	Interfaces in mineralized tissues: Implications for bone wound healing and osseointegration of implants
Speaker:	Marc D. McKee

Afternoon

14h - 14h30	Bone marrow aspirate combined with low-level laser therapy: a new therapeutic approach to enhance bone healing
Speaker:	Carolina dos Santos Santinoni
14h30 - 15h	Evaluation of bone repair in bone / implant interface in rats with induced osteoporosis treated with raloxifene or alendronate. Histological and biomechanical analysis
Speaker:	Gabriel Ramalho Ferreira
15h - 15h45	Study of the involvement of the metallopeptidase PHEX in physiological and pathophysiological processes
Speaker:	Nilana Barros
16h-18h	Discussion: Bone repair- What is the future? All speakers

April 4th 2014 –Friday

Morning

8h - 11h	Poster presentation
11h -11h30	Closing ceremony

01

Effects of chemical components of cigarette in femur of passive smoker rats

CARVALHO C, MIGUEL IM, CASTRO AP, COUTINHO JCS, SANTOS MRS
Faculdade de Pindamonhangaba – Fundação Universitária Vida Cristã (FAPI/FUNVIC) – Pindamonhangaba – SP – Brazil.

Smoking is one of the concerns of modern society due to the its deleterious effects. This study investigated the effects of chemical components present in the smoke of cigarettes in femur of 10 passive smoker male Wistar rats. The animals were kept under controlled conditions, 12-hour light/dark cycle, food and water ad libitum. For 60 days the animals were exposed for 8 minutes, three times a day, to smoke ten cigarettes in a box for this type of experiment. Ten other male rats, not exposed to smoke, were used as controls. After the treatment, the animals were subjected to euthanasia. The femur of each animal were collected, fixed in 10% buffered formaldehyde for 24 hours. After, they were cleaned and immersed into a 2% sodium hypochlorite solution to facilitate the completion of cleaning. Later, the pieces were frozen at -18oC for Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray spectrometry (EDX) and optical profilometry. Traces of toxic elements present in the cigarette smoke were detected on the femur of animals, resulting in different signs of change in bone mineral density, as an increase in the porosity, in the diameter and depth of the pores, suggestive of wearing and bone fragility.

02

Spontaneous alveolar bone loss development in diabetic rats

SOUZA DM, MEDEIROS AC, SANTOS JO, LIMA MCM, CARVALHO C
Faculdade de Pindamonhangaba – Fundação Universitária Vida Cristã (FAPI/FUNVIC) – Pindamonhangaba – SP – Brazil.

Diabetes mellitus has been associated with the occurrence and severity of alveolar bone loss. Besides this relationship presents biological plausibility, there is a need for studies in humans, animal and in vitro (cell/tissue) models to elucidate this issue. The objective of this study was to evaluate the effect of diabetes mellitus induced by Alloxan in periodontal bone support (PBS) in adult rats. Sixteen females were divided into two groups of 8 animals: Diabetes (blood glucose levels after induction greater than 200 mg/dL), Control group (not induced diabetes). Diabetes was induced by a single intraperitoneal injection of Alloxan (Sigma -Aldrich®) diluted to 2% solution of sodium citrate 0.05M, pH 4.5. Weekly the glucose level was checked using a glucosimeter, indicting initial increase and then stability of blood glucose. After 42 days of experiment, the rats were sacrificed under anesthesia and the left mandible were dissected and radiographed for measurement of PBS. A significant reduction ($p < 0.05$) in the percentage of PBS in diabetic animals compared with Control group was found. Diabetes mellitus in adult rats induced alveolar bone loss, suggesting that the same can occur spontaneously in diabetic individuals.

03

Influence of low level laser therapy on bone repair in osseointegration

FALEIROS PL[1*], BOSCO AF[1], ERVOLINO E[2], THEODORO LH[1], GARCIA VG[1,3], DE ALMEIDA JM[1]
1 – Department of Surgery and Integrated Clinic – Division of Periodontics – Univ Estadual Paulista – UNESP – Araçatuba – SP – Brazil.
2 – Department of Basic Sciences – Univ Estadual Paulista – UNESP – Araçatuba – SP – Brazil.
3 – University Center of Educational Foundation of Barretos – UNIFEB – Barretos – SP – Brazil.

The purpose of this study was to evaluate the influence of low level laser therapy (LLLT) on bone repair after placement of titanium implants in tibia of rats. Surgical alveolus were created in 60 rats (Wistar), assigned into the groups C (no local treatment) and LLLT (irradiation with low level laser prior to implant placement). The laser used was Thera Lase® (InGaAIP 660 nm, in continuous mode, in contact with the area, 35 mW, 0.14J, 4.9 J/cm^2, for 4 s). Ten animals from each group were euthanized at 15, 30 and 60 days postoperative. Histological sections were stained with hematoxylin and eosin or subjected to immunohistochemistry reaction for TRAP detection. In the region adjacent to the implant, histological analysis of bone repair process and quantitative analysis of multinucleated TRAP-positive cells were performed. Data were subjected to statistical analysis ($p \leq 0.05$). In all studied periods, alveoli of the implants were partially surrounded by a thin layer of immature trabecular bone, and quantity and thickness were comparatively higher in specimens of LLLT. Greater amount of TRAP-positive cells was observed in LLLT in comparison with C ($p \leq 0.05$). We conclude that LLLT accelerated the osseointegration via stimulation of the osteoblastic activity, since there was increased bone formation, and via stimulation of the osteoclastic activity, because such cells were presented in greater quantity either in the initial or late phases of the repair process, where bone remodeling occurs.

04

Clinical and radiographic analysis of sinus elevation using bovine bone inorganic matrix, β-tricalcium phosphate and homogenous bone graft`

NASCIMENTO RD, SANTOS JO, RALDI FV, MANHÃES JUNIOR LRC, SANTAMARIA MP, MORAES, MB.
Institute of Science and Technology – Univ Estadual Paulista – UNESP – São José dos Campos – SP – Brazil.

Due to the advances in implant reabilitation, the need for reconstructive procedures prior implant placement, as elevating the sinus membrane in the posterior maxila, becomes common. Thus, bone substitutes such as inorganic bovine bone matrix, β- tricalcium phosphate and homogenous bone graft, are employed. The objective of this study was to evaluate bone height and bone density, before and 6 months after surgery, by cone beam computed tomography, using three bone grafts options as well as to measure the final torque at the time of implant placement 6 months after bone grafting. Fifteen cases were randomly divided into 3 groups according to bone grafting selected. The comparison among groups was performed using the values of the gray scale, the alveolar ridge

height in grafted regions and the final implant torque. We observed an increase in alveolar ridge and tomographic density in all grafted groups. There was no correlation between tomographic bone density and insertion torque after statistical analysis.

Financial Support: FUNDUNESP 01377 / 12

05

Evaluation of different energy densities of the laser therapy during orthodontic movement in diabetic animals

SANTANA-MELO GF; HIROAKA CM, LIMA GMG; SALGADO MAC; VILELA-GOULART MG; GOMES, MF
Institute of Science and Technology – Univ Estadual Paulista – UNESP – São José dos Campos – SP – Brazil.

Diabetes Mellitus (DM) is increasing exponentially, acquiring epidemic characteristics in several countries, particularly in developing ones, quietly evolving and leading to various complications. At the same time the accessibility and adherence to orthodontic treatment is increasing and the bone repair process due orthodontic stimuli became a major concern, especially in diabetic patients, because of their impaired bone metabolism. The search for ways in which they can improve the process of bone remodeling during orthodontic movement is a current reality, the application of low power laser can be of great value especially in the loss of glycemic control. The aim of this study is to evaluate the influence of different energy densities of the AsGaAl laser during orthodontic movement in diabetic rats. Forty animals were used, divided into 8 groups: control (normoglycemic rats), Laser (normoglycemic rats with application of laser in densities 5 J, 10 J and 20 J), Diabetic, Diabetic-Laser (diabetic rats with application of laser in densities 5 J, 10 J and 20 J), evaluated at 21 days of orthodontic movement. Analyzes were performed by histomorphology, immunohistochemistry (PCNA) and digital radiography in the lower right first molar, subjected to orthodontic movement. The data were statistically analyzed by analysis of variance (ANOVA) and Tukey tests ($P<0.05$). Cell proliferation was higher in normoglycemic rats than in diabetic rats, but with the laser treatment led to an increase in cell proliferation in both groups. Both normoglycemic and diabetics rats irradiated with energy density of 20J / cm^2 showed better response to orthodontic movement and integrity of the periodontal tissues. According to the results and the experimental conditions in this study, it can be concluded that there was effective AsGaAl laser radiation, and the energy density of 20 J/cm^2 showed the best results, since the pathophysiology of the disease diabetes did not affect the integrity of the microarchitecture of periodontal tissues.

06

Increased bone mineral density, maximum load to failure and number of osteocytes associated with the consumption of mate in senile rats

PEREIRA CS[1], BRASILINO MS[2], TIRAPELI KG[1], PEREIRA AAF[3], GARCIA CTS[1], NAKAMUNE ACMS[2]
1 – Graduate Studies Program in Physiological Sciences – Brazilian Physiological Society – Univ Estadual Paulista – UNESP – SP – Brazil.
2 – Araçatuba Dental School – Univ Estadual Paulista – UNESP – Araçatuba – SP – Brazil.
3 – University Center Toledo – Araçatuba – SP – Brazil.

Oxidative stress has been associated with decreased osteocyte number and increased lipid peroxidation that resulted in loss of bone quality. We observed that mate tea (MT) treatment during 8 weeks increased antioxidant defense and reduced lipid damage on erythrocytes, pancreas and liver of senile female rats. We investigated the effect of MT on maximum load to failure (MLF), bone mineral density (BMD), lipid peroxidation (LP) and osteocyte number (N.Ot) in adult and senile females. Wistar rats (n=5, 4 months and n=10, 17 months) were divided into: A (adult), Senile (S) and SMT (senile treated with mate tea, daily doses 20mg/body weight, gavage for 4 weeks). We investigated LP (malondialdehyde, mmol/L/g bone) in bone homogenate (10 mmol/L phosphate buffer, pH 7.4); N.Ot (LAICA microscope, LAS V4 software); BMD (mg/cm^2) by dual energy X ray absorptiometry and MLF (Computer-controlled EMIC universal machine with a 2,000 N load cell, speed of 5 mm/min). Data (means±standard error) were compared by unpaired Student t-test. Aging resulted in reduction ($p<0.05$) on DMO (A: 0.227±0.004, S: 0.162±0.005) and N.Ot (A: 751.5 ± 39.5, S: 275.0 ± 16.1). LP was increased senile female (A: 34.70±0.8; S: 42.73±1.1). MLF was not modified by age (A: 135.0±8.3; S: 125.9±5.3). MT increased ($p<0.05$) DMO (SM: 0.183±0.006), N.Ot (546.7±38.2) and MLF e (160.2±2.8). MT reduced bone modification associated with senile female.

07

Glandular odontogenic cyst apperearing clinically as traumatic bone cyst

EL ABRAS ANKHA MV, ANBINDER AL, ALMEIDA JD, CARVALHO YR
Institute of Science and Technology – Univ Estadual Paulista – UNESP – São José dos Campos – SP – Brazil.

The glandular odontogenic cyst (GOC) is a rare developmental odontogenic cyst, most commonly found in the anterior mandible, with a potentially aggressive growth. Radiographically it presents a radiolucent unilocular or multilocular aspect, with well-defined, sclerotic margins. Resorption of the cortical bone and root resorption may also be observed. Differential diagnoses reported in the literature are botryoid cyst, lateral periodontal cyst, odontogenic keratocyst, residual cyst, central low-grade mucoepidermoid carcinoma and ameloblastoma. Although the characteristics of traumatic bone cyst (TBC) often differ from glandular odontogenic cyst, some authors put it as a differential diagnosis,

since it presents itself as a radiolucent well circumscribed lesion affecting the jaws, resembling a true cyst. GOC and TBC have different prognoses and require different treatments but sometimes they can be mistaken and treated inappropriately. While TBC is often treated by curettage and sometimes healing occurs spontaneously, GOC requires a more invasive treatment, due to its aggressive potential and high recurrence rate. The objective of this study is to report and discuss two cases with definitive diagnosis of GOC that presented, according to the surgeon, clinical, radiographic and surgical aspects of TBC. We would like to emphasize the importance of performing a biopsy for the correct diagnosis of a radiolucent lesion suggesting GOC or TBC, to establish the correct diagnosis and treatment.

08

The influence of gut serotonin inhibition in the pathogenesis of periodontal disease in rats: radiographic analysis

CORAZZA BJM, LIMA GMG, MORAES RM, CAMPOS DRC, MAZZA VCO, ANBINDER AL
Institute of Science and Technology – Univ Estadual Paulista – UNESP – São José dos Campos – SP – Brazil.

Although biofilm is considered the main etiologic agent of periodontal disease (PD), tissue damage is partially given by local host response activation. The hypothesis of gut serotonin (GS) inhibition influencing host response in PD is based on literature reports about anabolic role of LP533401 into bone. LP533401 is an inhibitor of Tryptophan hydroxylase (Tph1) enzyme, important for gut serotonin production by enterochromaffin cells. The aim of this study is evaluate the effect of GS inhibition during PD pathogenesis. Thirty male rats were divided into three groups according with PD ligature induction around lower first molars and treatment: (1) Treated Group: PD induction and LP533401 (25mg/Kg/day) by gavage; (2) Control Group without PD and (3) Control Group with PD, both treated with vehicle. All animals were treated for 28 days, when euthanasia occurred. The percentage of bone support was assessed after digital radiography of the samples. After statistical analysis (t test, $p < 0.05$), significant difference was found between Control Groups, but no difference was found between Control Group with PD and Treated Group. Based on methods used, treatment with LP533401 did not influence bone resorption during induction of PD.

Financial Support: PROPE-UNESP; FAPESP (2012/2486-0)

09

Ozone therapy in bisphosphonate-induced osteonecrosis-clinical case

MORAES MB, GONÇALVES FCP, NASCIMENTO RD, SANTOS LM, SANTAMARIA MP, RALDI FV
Institute of Science and Technology – Univ Estadual Paulista – UNESP – São José dos Campos – SP – Brazil.

Bisphosphonates (BF) have been routinely used as inhibitors of bone turnover in osteopenia, osteoporosis and as chemotherapeutic agents in cancer treatment. They have high affinity for osteoclasts, act by inhibiting the breakdown of hydroxyapatite and have therapeutic effect on suppression of bone resorption, with the estimated half-life in years. However, some complications of their use have been observed, as hypocalcemia, atrial fibrillation, and especially in Dentistry, the development of osteonecrosis of the jaw (ONJ). According the administration route, there are two types of BF, oral and intravenous. The frequency of ONJ in patients using intravenous BP is much higher than in patients using BP orally administered. The objective of this paper is to present a clinical case of a 53 year-old female patient who was receiving intravenous chemotherapy with pamidronate (90mg monthly) for 6 years, and had sites suggestive of bisphosphonate-induced osteonecrosis in the maxilla and mandible. There is no consensus in the literature on the treatment of ONJ, so we recommend the ozone therapy added to the red low power laser, which presented a satisfactory outcome, reducing the symptoms and clinical signs, since the patient needs to maintain chemotherapy for remission of her disease.

10

Establishment of critical size defect in rat femurs – model for bone regeneration analysis

CRESTE CFZ, ORSI PR, LIMA JF, FERREIRA JUNIOR RS
Center for the Study of Venomous Animals and Toxins – CEVAP – FMB – UNESP – Botucatu – SP – Brazil.

Despite high regenerative properties of bone tissue, this biological process can fail or be limited by factors such as defect size. Critical size defects are characterized by bone tissue loss and cannot be repaired spontaneously under physiological conditions. To evaluate regenerative effects of biomaterials or cell therapy alternatives it is necessary to establish critical size defects in animal models. Our objective was to evaluate the efficacy of the procedure to establish critical size defects in rat femurs. Nine Wistar male rats, aged approximately 03 months, underwent surgical procedures. Rats were weighed, identified and individualized on plastic boxes. Animals were anesthetized by intramuscular injection of a solution of 90 mg/Kg of ketamin and 5 mg/Kg of xylasin chloridrate. After bone tissue exposal, a 5mm superficial drilling was made with dental micro-motor, causing a defect on the distal epiphysis of the right femur. Post-surgical analgesia was made with 2 mg/Kg subcutaneous administration of funixin on the following three days and animals received food and water ad libitum. None of the animals showed clinical signs of infection or abnormal wound healing. All animals were

euthanized on the 42nd day after surgery and underwent X-ray imaging. Femurs were dissected and sent to histological analysis. X-ray imaging, morphological and histological analysis showed that bone regeneration on drilling site was insufficient evidenced by lack of bone tissue. Surgical technique was appropriate to determine bone critical size defects on rats and this model could be used to evaluate new biomaterials and cell therapy alternatives in bone regeneration.

Financial Support: FAPESP (2013/02004-3).

11

Florid osseous dysplasia: case report

MORAES RM, SANTANA JB, CAVALCANTI ASR, CARVALHO YR, ANBINDER AL
Institute of Science and Technology – Univ Estadual Paulista – UNESP – São José dos Campos – SP – Brazil.

Fibro-osseous lesions are a group of lesions characterized by the replacement of normal bone tissue by fibrous connective tissue that undergoes gradual mineralization. Fibrous dysplasia, osseous dysplasia and ossifying fibroma are included in this group. Osseous dysplasias can be further divided into focal, periapical and florid. The aim of this study was to report a case of fibro-osseous lesion and discuss the differential diagnosis. A 60 year-old female patient, came to us complaining of pain in the region of the right mandibular body. Clinically there was an area of bone sequestration, and radiographically multiple mixed areas were noticed. Incisional biopsy was performed and histological examination showed areas of deposition of cementoid globular material in the connective tissue. The final diagnosis was florid osseous dysplasia and the patient is under observation.

12

Expression of osteocalcin in the repair of bone graft autogenous onlay with or without membrane collagen reabsorbable in diabetics rats

BERNARDO DV, TERA TM, DE MARCO AC, KERBAUY WD, SANTAMARIA MP, JARDINI MAN.
Institute of Science and Technology – Univ Estadual Paulista – UNESP – São José dos Campos – SP – Brazil.

The increase in life expectancy is accompanied by an increased prevalence of diseases associated with older age and diabetes. With an aging population, there is an increasing demand for esthetic and functional rehabilitation procedures such as osseointegrated implants. Bone abnormalities are a major complication in diabetic patients. The aim of this study was to quantify the expression of the osteocalcin, a bone formation marker, in the repair of the onlay autogenous bone graft, with or without resorbable collagen membrane in diabetic and non-diabetic animals. We used 60 ninety

day old-rats (Wistar) and divided them into two groups: test group (D- n=30) diabetes induced and control group (C- n = 30), normoglycemic. Rats in both groups received grafts in the left hemi mandible associated with collagen membrane (LM) and in right hemi mandible, uncoated (E). Sacrifices occurred after the following periods: 0h, 7, 14, 21, 45 and 60 days. Immunohistochemical analysis was performed on the graft-layer interface. To analyse the immunohistochemical expression of osteocalcin, we used the Image J program. Not only did the results show a statistically significant difference at 5% in the Diabetic and Control intragroup with or without membrane, but they were also different when the diabetic group was compared to the control group in the presence of the membrane. Within the limits of this study, it can be concluded that the marking of osteocalcin may suffer some influence of diabetes, which showed a delay in such conditions. However, the association of the membrane graft can improve the delay as well as make the expression similar to the control group.

Financial Support: FAPESP – Process 2012/01275-0

13

Immunolocalization comparison of growth factors fgf-2 and bmp-2 in the early stages of guided bone regeneration

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Autogenous bone has been recognized as the gold standard for bone grafting, providing osteogenic cells, extracellular matrix and molecular signals for the induction of bone differentiation. The use of this kind of graft, combined with a guided bone regeneration membrane, has led to predictable and positive clinical results. With the new techniques such as immunohistochemical and in situ hybridization has allowed the study of osteogenesis and bone repair at molecular and cellular levels. The aim of this study was to evaluate and investigate the endogenous growth factors expression, FGF-2 and BMP-2, at the early stages of the guided bone regeneration. Forty eight male rats have been divided into two groups: autogenous bone graft (E) and autogenous bone graft associated with e-PTFE membrane (ME) and have been evaluated in 3,7,14,21,45 and 60 days through immunohistochemistry techniques. The highest proportion of staining has been observed in FGF-2 and BMP-2 on connective tissue around graft regardless of the evaluated group. However, the highest proportion of staining in BMP-2 occurred at 7 days in E group and 14 days to ME group. As to FGF-2, the highest proportion of staining occurred at 14 days, regardless of the group. The BMP-2 staining was detected on graft bed interface and recipient bed, especially on cells next to neo-formed bone. There was staining on bone matrix to FGF-2 and BMP-2 on the woven bone bridge between recipient bed and graft. The connective tissue around the graft was the structure that presented the highest proportion of staining to FGF-2 and BMP-2 at the 14 and 21 day- occurrence.

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Sinus lifting and later implant- case report

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The posterior maxilla presents a challenge for implant rehabilitation when compared to other areas of the mouth, due to the presence of the maxillary sinus. The literature has shown the possibility of using biomaterials, as bovine bone, for bone reconstruction. The aim of this work is to report a case of maxillary sinus lifting, using xenograft bone, for later implant placement. The patient presented at our outpatient clinic with longitudinal fractures in the first upper right molar and extensive bone loss in the second upper right molar, and the extraction of both teeth was indicated. During the surgery, there was oroantral communication, which was closed by a mucoperiosteal flap. After six months, the sinus lifting surgery and the bone grafting were performed. The bone graft (Bio Oss) was carefully placed beneath the sinus membrane. After the fulfilling, a membrane was placed in the buccal area, sealing the bone window. The flap was sutured and after a nine month-healing period, two implants were placed using the conventional technique, with perfect clamping. Randomized controlled clinical trials have shown that bone substitutes (Bio-Oss) can be used successfully.

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Immunolocalization of fgf-2 and tgf-β1 during guided bone regeneration early healing events

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Bioactive factors are demonstrated like inducers or modulators of chondrogenesis and osteogenesis, for example: TGF- β1 and BMP-2. These proteins of bone promotion stimulate chemotaxis, differentiation and matrix synthesis by diverse cell types of mesenchymal origin. The aim of this study was to evaluate immunolocalization of intracellular FGF-2 and TGF-β1 growth factors after autogenous block covered or not with an e-PTFE membrane on the early phases of bone repair. Forty-eight Wistar male rats had their mandibles augmented by either an autogenous bone block graft (B) or an autogenous bone block graft covered with an e-PTFE membrane (MB). The specimens were evaluated at baseline, 3, 7, 14, 21 and 45 days after surgery by immunohistochemistry. The receptor bed showed discreet number of stained cells after three days in both groups (B) and (MB) for both growth factors. The border of the graft demonstrated the largest number of stained cells by FGF-2 after 14 days on groups (B) and (MB). For TGF-β1 the staining was discreet after 14 days in both groups. Staining was prominent in osteoblasts and osteoprogenitor cells observed in bone remodeling areas. The connective tissue above the graft showed the greatest staining proportion after 14 and 21 days for FGF-2, groups (B) and (MB), but the staining by TGF-β1 was less for both

groups. Interestingly, stained osteoprogenitor cells were still noted at the end of the experiment (after 45 days). At the interface, the greatest number of stained cells occurred between 14 and 45 days for groups (B) and (MB) respectively, when osteoprogenitor cells were positive in woven bone edges between the receptor bed and the graft. The greatest proportions of stained structures were related to intense revascularization and osteogenesis. Osteoprogenitor cells, osteoblasts and osteocytes showed intracytoplasmatic staining, not withstanding of the period or structure analyzed. The staining by FGF-2 seemed to be more intense the than staining by TGF-β1.

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Marsupialization in an orthokeratinized odontogenic cyst: case report

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A Caucasian 15-year-old boy was referred from his orthodontist who had observed, in the pre-treatment orthopantography, a radiolucent, unilocular, well-defined lesion at the left mandibular ramus. Cone-beam computed tomography showed a hypodense image, compatible with the radiographic findings. Intraoral examination showed expansion of the cortical vestibular bone in the correspondent area, with no damage to the adjacent teeth and mucosa. Puncture aspiration was performed, collecting caseous-like material, followed by incisional biopsy with marsupialization of the lesion and instructions for irrigation with physiological saline solution. Histopathological examination revealed cystic capsule fragments, delimited by squamous orthokeratinized epithelium, showing several keratin debris in the lumen. Hence, the diagnosis was conclusive for orthokeratinized odontogenic cyst. Monthly follow-up was made and evident osseous neoformation was noticed in panoramic radiographs. After 10 months, the lesion was excised and histopathological examination of the specimen confirmed the previous diagnosis. Four months later, total bone recovery was observed.

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Unicystic ameloblastoma of the mandible

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Ameloblastoma is a benign odontogenic neoplasm of slow growth and locally invasive that predominantly affects the jaw. A 19 year old white, female patient that was in orthodontic treatment for 3 years, complained of an acute pain that started 3 months before. The patient was referred for evaluation of a lesion in the region of tooth 48. On extra oral examination, there was a facial

asymmetry on the right side. Radiographic and tomographic exams revealed a radiolucent well-demarcated and unicystic lesion associated to tooth 48, measuring approximately 2.4cm in its largest diameter. Differential diagnosis was unicystic ameloblastoma and dentigerous cyst. An incisional biopsy was performed. Microscopic examination revealed a large cystic cavity lined in most of its length by non-keratinized stratified squamous epithelium. The basal layer was formed by cubical or columnar cells arranged in palisades, and other layers showed spongiosis with intense similarity to the stellate reticulum of the enamel organ. Epithelial proliferation was both intraluminal and mural with plexiform pattern. Histopathological diagnosis was ameloblastoma with unicystic aspect with intraluminal and mural proliferation. After 6 months of marsupialization, there was bone formation. Enucleation and curettage of the remanescent area was performed.

In vitro response of human osteoblasts to porous titanium biomimetic coated

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Porous titanium implants may simulate the design of the trabecular bone, favoring cell growth and osseointegration. Biomimetic treatment is a chemical surface treatment that induces deposition of apatite and enhances titanium bioactivity. The aim of this study was to compare the in vitro response of human osteoblasts to dense and porous titanium samples submitted or not to biomimetic treatment: Cells were cultured on the surface of 7 groups: control group (1) - polystyrene plate; dense sample of titanium (2); 33% porous titanium (3); 41% porous titanium (4); and groups 2 , 3 and 4 submitted to biomimetic treatment. Biomimetic treatment corresponded to alkali treatment, followed by heat treatment and immersion in simulated body fluid. Human osteoblastic lineage cells were obtained by enzymatic digestion of bone, isolated and developed subculture. 2x104 cells were plated in 24 well plates with samples of the above groups. After 24 hours the cell adhesion test was done. After 3, 7 and 10 days determination of cell viability (MTT), total protein content (Lowry) and alkaline phosphatase activity was performed. ANOVA and Tukey tests indicated that cell adhesion and alkaline phosphatase activity were similar in all groups. The activity of the total protein and cell viability were reduced in samples submitted to biomimetic treatment. The biomimetic treatment damaged the biocompatibility of titanium. FAPESP 2011/19938-3; 2010/02778-0

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Molecular analysis of porous titanium biocompatibility submitted to biomimetic treatment

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Different topographies of titanium implants and biomimetic surfaces are studied to improve osseointegration. This study aimed to investigate the in vitro behavior of human osteoblasts cultured on porous titanium specimens with two porosities; with and without biomimetic treatment, comparing them with dense titanium. Stem cells from gnathic explants were induced to osteoblast differentiation in supplemented culture medium and plated on 24 wells plates containing different titanium surfaces. Samples were previously analyzed by metallography and X-ray diffraction. Gene expression was performed by reverse transcription by real-time polymerase chain reaction. Genes involved in osteogenesis were accessed. Data were statistically evaluated. As results, mostly gene showed statistically similar expression in all groups tested, but with smaller expressions in biomimetic treatment groups, especially those related to cell differentiation. Cell adhesion and alkaline phosphatase activity were similar in samples of titanium, regardless of porosity and biomimetic coating. The biomimetic treatment reduced the total protein content and viability of the tested cells. It is concluded that variations in porosity of titanium are unable to change osteogenesis and biomimetic treatment affect the biocompatibility of titanium, delaying cellular differentiation.

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Evaluation of the prevalence of bifid mandibular canals in cone beam computed tomography

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The mandibular canal can have different morphologies, in some cases a second canal (bifid) is present. The aim of this study was to evaluate the incidence of bifid mandibular canals (BMC) by Cone Beam Computed Tomography (CBCT). Cone beam CT scans of 300 patients, both male and female, aged 25 to 87 years old, who underwent the examination for evaluation of the mandible, were examined. Patients were divided into two groups according to sex (GM for male and GF for females), and two subgroups according to the side examined (R for right and L for left). All tomographic acquisitions were followed by the same protocol and images analyses were made on the same software. Image filters associated with cross-sectional slices, oblique and panoramic reconstruction for the analysis of BMC were used. Analyses of descriptive values and comparisons were made using ANOVA test with a significance of 95%. It was observed that there

were 80 cases of BMC (26.67%), 39 (48.75%) in male and 41 (51.25%) in females. There was no difference in measurements compared by sex (p=0.788 male; p=0.140 female) and for the affected side (p=0.365 right, p=0.412 left), although the right side was more frequent (66.67%) in both sexes. Thus, it can be concluded that the incidence of BMC is relevant and should not be overlooked by dentists.

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The influence of different implant materials on bone regeneration in vivo: histological evaluation in rabbit tibia

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The surface topography and manufacture material of surgical implant can influence the osseointegration process, due to chemical and physical changes exhibited by the implants. The aim of this study was to compare implants made from different alloys of titanium porous surface associated with dense core on bone regeneration. By means of powder metallurgy, 50 implants showing dense core integrated with porous surface, were divided into 3 groups: a) group 1: control - commercially pure Ti, b) Group 2: alloy Ti-6Al-4V (titanium -aluminum-vanadium), c) group 3: alloy Ti-35Nb-7ZR-5Ta (titanium-zirconium-niobium-tantalum). In this study, we used five rabbits to evaluate and compare the bone regeneration by means of histology. Each rabbit received an implant of each group, implants G1 and G2, were inserted into the right tibia and G3 in the left tibia. Five rabbits, randomly selected, were euthanized 2 and 4 weeks after surgery. The fragments were subjected to histological analysis to evaluate the quality of new bone formation in the bone-implant interface.

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Impairment of dental socket bone repair in non-controlled diabetic rats

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Impaired wound healing and infection are complications related to surgical procedures in diabetic patients. There is evidence that diabetes affect bone metabolism and delay bone healing. The aim of this study was to evaluate dental socket healing in diabetic rats by histometric and immunohistochemical analysis of receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG) and osteocalcin (OCN). Thirty male rats were distributed in two groups: Group C: control group (citrate buffer injection) and Group ED: experimental diabetic group, in which

diabetes was induced by Streptozotocin IV injection. After 12 days of diabetes induction, animals underwent upper right incisor extraction under general anesthesia. Animals were euthanized 7, 14 and 28 days post-operative. Specimens were processed for histometric and immunohistochemical analysis. Data were statistically analyzed. There was less new bone formation in Group ED in all periods ($p=0.002$). In the ED group compared with C was: greater immunolabeling for RANKL at day 7 ($p=0.014$); greater immunolabeling for OPG at day 7 ($p=0.050$), 14 and 28 ($p=0.037$); lower immunolabeling for OCN at day 28 ($p=0.037$). Under the limitations of this research it could be concluded that induced diabetes mellitus in rats provoked a delay bone healing of dental socket as well as different immunolabeling behavior compared to normoglycemic rat.

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Improvement of bone repair in spontaneously hypertensive rats treated with losartan: histomorphometric and immunohistochemical analysis

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Hypertension is a multifactorial clinical condition, with high rates of complications, considered one of the greatest public health problems worldwide. Hypertension alters calcium regulation pattern provoking bone loss. The aim of this research was to evaluate alveolar bone repair process in spontaneously hypertensive (SHR) and Wistar rats treated with losartan (30 mg/kg/day). A total of 60 rats were divided into 4 groups: (A) Wistar; (B) SHR; (C) Wistar treated; (D) SHR treated. Upper right dental incisors were extracted, after 7, 14, and 28 days post-operative animals were euthanized. Biological mechanisms of alveolar bone repair were histomorphometry and immunohistochemistry analyzed by OPG protein, RANKL, TRAP and PECAM expression involved in bone metabolism. Kruskal-Wallis non-parametric test was used to analyze the data. The SHR group showed alveolar bone repair delay. Losartan use improved bone repair process in Wistar and SHR, resulting in increased bone formation, as well as greater trabecular thickness. The proteins tagged participate actively in bone dynamic, it was observed losartan direct action during bone repair. It is suggested that losartan and the renin-angiotensin interfere with bone metabolism through the action of angiotensin II.

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Bone marrow stem cells from shr are vulnerable to the metabolic and osmotic stress during osteogenic differentiation

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The aim of this study was to evaluate whether the hypertensive genotype could enhance the deleterious effects of the high glucose concentration in the osteogenic differentiation in vitro. Bone marrow mesenchymal stem cells (BMSCs) were isolated from Wistar and SHR (Spontaneously Hypertensive Rats) femurs. The experimental protocols were approved by the Animal Experiments Local Ethics Committee. The BMSCs subcultures were treated with proliferation or osteogenic medium plus 25 or 35mM of glucose in the presence or absence of insulin. Mannitol was used as iso-osmolar control. Markers of osteogenic and adipogenic differentiation were analyzed by colorimetric methods, staining and gene expression by qPCR, while the MAPKs pathway was assayed by Western blotting. High glucose and mannitol concentrations induced downregulation in proliferation, ALP activity and mineralization in both strains, while the insulin reversed these effects only in the Wistar strain due the increased gene expression of Runx2, Opn, Ocn and Bsp. The lipid accumulation was positive in the SHR strain and was associated with high levels of Pparg expression. High glucose and mannitol concentration downregulated ERK1/2 and p38 phosphorylation in the both strains. The BMSCs from SHR strain are vulnerable to the metabolic/osmotic stress during osteogenic differentiation at least, in part, due to its hypertensive genotype. Financial support: FAPESP Processes No 2011/02558-3, 2011/19458-1 and 2011/06070-5.

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Immunohistochemical analysis of cytokeratins on calcifying cystic odontogenic tumor

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Calcifying cystic odontogenic tumor (CCOT) is a benign cystic neoplasm characterized by an ameloblastoma-like epithelium and the presence of ghost cells. This lesion's cytokeratin (Ck) expression pattern needs to be clarified. The aim of this study is to investigate the Ck6, 13, 14, 18, and 19 expressions in CCOT. Seven CCOT cases were classified into four types. The CK expression was evaluated by immunohistochemistry and for analysis, the epithelium lining was divided according to the following regions: basal layer, suprabasal layer and ghost cells compartment. Six cases (85.7%) were classified as type 1 and one (14.3%) as 4. All cases were Ck13 and 18 negative. The Ck14 and 19 positivity in all cases reinforced the OCCT odontogenic origin and the Ck6 restricted expression to the ghost cells may be associated with these cells' pathogenesis. Ck14 and 19 were positive in all cases and Ck6 in the ghost cells.

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Influence of ovariectomy and dietary calcium deficiency in experimental periodontitis

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Estrogen and dietary calcium deficiencies are conditions that potentially lead to alveolar bone alterations and might consequently worsen periodontal disease. The aims of this study were to evaluate estrogen deficiency associated or not to dietary calcium deficiency over bone loss and to evaluate the activity of osteoclasts on induced periodontal disease in rats. Ninety-six rats aging 90 days were used in the present study, being 64 submitted to ovariectomy and 32 to sham-ovariectomy. Thirty-two rats from the ovariectomized group received standard diet (OVZ), while the rest received calcium deficient diet (OVZ'). Periodontal disease was induced 60 days after the surgery in all rats. Euthanasia was performed 3, 7, 14 and 30 days after ligature placement. HE stained slices were analyzed by histomorphometry and tartrate-resistant acid phosphatase (TRAP) was marked by enzyme histochemistry. The results revealed that Sham group presented greater bone matrix quantity and lower TRAP+ cells in comparison to OVZ group. However, OVZ group presented lower bone matrix quantity and similar TRAP+cells as OVZ' group. It was concluded that greater bone loss, which is not accentuated by calcium deficient diet,I s a consequence of estrogen deficiency in ligature induced periodontal disease in rats. Bone loss peak occurs at 14 days regardless estrogen deficiency. However greater bone resorption occurs due to the activity of osteoclasts positive for TRAP, in absence of estrogen.

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