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Effects of ovariectomy on periodontal tissues following tooth replantation

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ABSTRACT

Objectives: The aim of the study was to analyze the effects of ovariectomy on periodontal tissues following immediate tooth replantation by histomorphometric, immunohistochemistry, and μCT analysis.
Materials and methods: Eighty wistar rats (*Rattus norvegicus albinos*) with normal estrous cycles were randomly divided into two groups: ovariectomized (OVX) and Sham. Two months after surgery, the rats' upper right incisor was extracted followed by immediate reimplantation. The animals were sacrificed after 28, 45, and 60 days healing time. Histomorphometric and immunohistochemical analysis were performed by evaluation of PCNA and TRAP straining.
Results: The periodontal ligament was reinserted into the bone and cementum in the both groups. The immunohistochemical analysis revealed PCNA positive cells on the periodontal ligament in both groups at 28 days. Root resorption was noted at 45 days with immunoreactive cells for TRAP present in bone and tooth surface however no statistical differences between the groups were noticed. Histomorphometric analysis showed significant difference between groups in the periodontal ligament and root resorption parameters for the sub-items: intensity of chronic inflammatory infiltrate at 60 days ($p < 0.01$), the organization of the periodontal ligament at 28 days ($p < 0.05$), depth of root resorption at 45 days ($p < 0.05$) and at 60 days ($p < 0.001$). The μCT analysis showed multiple areas of bone resorption in association with OVX at 28 and 60 days with no significant differences between times in vivo.
Conclusion: The ovariectomy did not have significant influence in periodontal tissue parameters following tooth reimplantation.

1. Introduction

Avulsion of permanent teeth, while not very frequent, is one of the main sequelae of dental trauma due to its esthetic and functional impact on the patient (Panzarini et al., 2013). Identifying the periodontal healing pattern remains a challenge, especially after reimplantation procedures.

The success of tooth reimplantation depends on the maintenance of vitality of the periodontal ligament, which allows the avulsed tooth to recover their functions (Andreasen, Borun, Jacobsen, & Adreasen, 1995). Resorption is the main cause of failure of reimplantation and the prognosis is related to the type of resorption that may lead to complete destruction of the root (Panzarini et al., 2013).

Root resorption is basically classified as follows: surface resorption which may occur even in the absence of a significant

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inflammatory process; inflammatory resorption, which occurs in the presence of inflammatory connective tissue; and replacement resorption, in which the periodontal ligament is resorbed or removed and replaced by bone tissue (Andreasen et al., 1995).

Degeneration of the periodontal ligament depends on several factors, such as trauma, management of the root, extra-alveolar period and storage medium and pulp necrosis (Panzarini et al., 2013; Marão et al., 2012). In addition, in certain systemic diseases, such deficiency of estrogen could interfere in the periodontal tissue phenotypes.

Estrogen is known to play an important role in regulating bone homeostasis and preventing postmenopausal bone loss (Girasole, Passeri, Pedrazzoni, Giuliani, & Passeri, 1995). The lack of estrogen causes an imbalance in bone remodeling, causing the rate of bone resorption to exceed the bone formation (Riggs, Khosla, & Melton, 2002; Luvizuto, Dias, Queiroz, Okamoto, & Okamoto, 2010).

The regulation of bone homeostasis occurs by the balance of protein expression (Jabbar et al., 2011) and pro-inflammatory cytokines (Zhou, Fu, Li, & Qi, 2009) such as interleukin (IL)-1, IL-6, tumor necrosis factor-alpha (TNF-α), and protein receptor activator of nuclear factor-k B ligand (RANKL), which have been identified suggesting the impact on bone resorption in periodontal tissues (Pfeilschifter, Köditz, Pfohl, & Schatz, 2002).

Estrogen may play an important role in hold antiresorptive effects on alveolar bone, at least in part by increasing the expression level of osteoprotegerin (OPG) versus that of RANKL via estrogen receptor β in human periodontal ligament cells (Liang, Yu, Wang, & Ding, 2008). The interaction between RANKL and the receptor activator of nuclear factor-k B (RANK) has been shown to be required for osteoclast differentiation (Theoleyre et al., 2004). The imbalance between RANK/RANKL/OPG leads to activity of osteoclasts assessed by an increase in tartrate-resistant acid-phosphatase (TRAP) staining (Said et al., 2012). Because multinuclear TRAP-positive cells resorb bone, TRAP is a specific osteoclast differentiation marker (Kwak et al., 2010). On the other hand, 17β-estradiol could promote the proliferation and viability of osteoblastic MC3T3-E1 cells, associated with proliferation cell nuclear antigen (PCNA) mRNA expression, and also could stimulate osteoblastic differentiation and bone formation as assessed by alkaline phosphatase (Song, Zhang, & Zhou, 2011).

Understanding the circumstances that lead to the regeneration of oral tissues has been a major challenge for dental research. It is known that a great variety of signals are released when an injury occurs, inducing neighboring cell populations to respond with proliferation, migration, or differentiation (Andreasen and Andreasen, 2007). Despite abundant work on the effects of estrogen deficiency in appendicular bone, the method by which multiple tissue phenotypes in the periodontium respond to ovariectomy and tooth reimplantation is poorly understood. In this study we established an animal model to analyze how estrogen deficiency affects multiple periodontal tissue phenotypes following tooth reimplantation.

2. Materials and methods

2.1. Animal care and ethics

The research protocol was approved by the Animal Care and Use Committee of the Aracatuba Dental School, UNESP—Universidade Estadual Paulista, Aracatuba, Sao Paulo, Brazil (protocol # 2008-003266).

Eighty adult female Wistar rats (*Rattus norvegicus, albinus*) weighing between 150 and 250 g were selected for the study. The animals were housed in plastic cages under climate-controlled conditions (12 h light/12 h dark; thermostatically regulated room temperature) and were fed a standard solid chow (Racao Ativada Produtor; Anderson & Clayton S.A. Industria e Comercio, Sao Paulo, SP, Brazil) and water *ad libitum*, except for the 12 preoperative hours. The rats were selected for the experiment after the regular estrous cycle confirmation.

2.2. Study design and surgical procedure

The animals were randomly divided into two groups ($N = 10$ per group) and submitted to either fictitious surgery (Sham) or ovariectomy (OVX) surgery under general anesthesia with xylazine hydrochloride (Dopaser, Laboratorio Calier do Brasil Ltda., Osasco, SP, Brazil; 0.03 mL *per* 100 g body weight) and ketamine hydrochloride (Dopalen; AgriBrands do Brasil Ltda.; 0.07 mL *per* 100 g body weight). Fifteen days after ovariectomy, all animals were subjected to the estrous cycle during seven consecutive days to confirm estrogen depletion.

2.3. Tooth extraction

Two months after the surgical procedure, the animals were anesthetized and the anterior maxilla asepsis was performed with 1% iodine polyvinylpyrrolidone (Riodeine; Industria Farmaceutica Rioquimica Ltda., Sao Jose do Rio Preto, SP, Brazil), followed by extraction of the right maxillary incisor and immediate tooth reimplantation.

2.4. Tissue processing

The rats were sacrificed by anesthetic overdose after 28, 45 and 60 days and the perfusion (Cole-Parmer Instrument Company, Vernon Hills, IL, USA) was performed with the infusion of 4% formaldehyde (Acros Organics, NJ, USA) to remove the right maxilla. The anatomic pieces obtained were fixed in 4% formaldehyde, demineralized in a 10% EDTA solution, pH 7% (Sigma–Aldrich) and embedded in paraffin. Semi-serial longitudinal 5 μm-thick sections were obtained and divided interchangeably for histological (hematoxylin and eosin staining) and immunohistochemical analysis.

2.5. μCT and 3D reconstruction

For the μCT analysis, the early postoperative time point of 28 days ($n = 5$) and the final postoperative time point of 60 days ($n = 5$) were concerned. The harvested maxillae were removed from the formaldehyde, washed and stored at 70% ethanol and separated from the anatomic pieces. A vertical cut was performed on the distal third molar, exposing both the area of the jaw containing the reimplanted tooth and the native tooth (control).

These samples were examined using μCT (μCT 40, Scanco Medical, Basserdorf, Germany) at 50 kV/160 μA, with an integration time of 1×380 ms and a high resolution of 18 μm. Volumetric data was converted to DICOM format and imported in Amira program (Visage Imaging GmbH, Berlin, Germany), which allowed the quantification of the root resorption extension, the periodontal ligament space, the bone resorption and the depth of root resorption. All the μCT measurements were performed by blind examiners and by one investigator.

The data were submitted to statistic analysis using analysis of variance (ANOVA) followed by a post hoc Mann–Whitney test. The significance level was set up at 5%.

2.6. Histomorphometric analysis

The analysis of the outcomes was performed by one of the authors in a blind fashion, according to 16 histomorphometric parameters listed by Holland et al. (1999), Panzarini et al. (2007),