



Detection of Simulated Periodontal Bone Defects Using Digital Images: An *in vitro* Study

Rafael Scaf de Molon¹, Mario Henrique Arruda Verzola¹, Talita Mesquita Paquier¹, Juliana Aparecida Najarro Dearo Morais-Camillo¹, Ivy Kiemle Trindade-Suedam², Leonor de Castro Monteiro Loffredo³, Gulnara Scaf¹

¹ Department of
Diagnosis and Surgery
Araraquara School of Dentistry
Univ Estadual Paulista - UNESP
Araraquara, São Paulo, Brazil

² Department of
Biological Sciences
Bauru School of Dentistry
University of São Paulo
Bauru, Sao Paulo, Brazil

³ Department of Social Dentistry
Araraquara School of Dentistry
Univ Estadual Paulista - UNESP
Araraquara, São Paulo, Brazil

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Corresponding author:
Dr. Rafael Scaf de Molon
Department of Diagnosis and Surgery
Araraquara School of Dentistry
Univ Estadual Paulista - UNESP
Rua Humaitá, 1680, 14801-903
Araraquara-SP, Brazil
molon.foar@yahoo.com.br

Abstract

Aim: The aim of this study was to evaluate the periodontal bone defects created only mechanically and by combined mechanical and chemical techniques.

Materials and Methods: The samples comprised 24 hemi-mandibles from pigs, which were allocated into 3 groups; G1 (before acid application), G2 (after acid application and G3 (without bone defect and acid treatment). Periodontal bone defects were created with round burs between the second and third pre-molar. The radiographs were taken using the Visualix eHD sensor. The central ray was perpendicular to the sensor and to the hemi-mandible at a 40 cm focal-spot to sensor distance (settings 70 kVp, 10 mA and 15 impulses). After the defects were created in groups G1 and G2, they were treated with 100% perchloric acid for 48 hours. Images were zoomed to the level of 125% and interpreted by three examiners. Sensitivity and specificity were computed for the detection of periodontal bone defects with acid application and created using only round burs. The examiner's radiographic interpretation produced a diagnosis based on a five-point confidence scale. If the interpretation received the scores 1 or 2, it was concluded that no bone defect was present, whereas the scores 3, 4, or 5 were considered to reflect evidence of a bone defect.

Results: There was no difference between groups G1 (Sen -95%CI=0.9167; Spec -95%CI=0.9167) and G2 (Sen -95%CI=0.8333; Spec -95%CI=0.9167).

Conclusions: There is no difference in the detection of periodontal bone defects created using round burs and defects created using round burs followed by acid treatment.

Key words: Periodontal disease, dental radiography, digital radiography, alveolar bone loss

Introduction

Periodontal disease is a chronic infectious disease characterized by periodontal tissue inflammation in response to biofilm accumulation on the tooth surface, which results in the destruction of the connective tissue and alveolar bone, leading to tooth

loss [1,2]. Periodontitis is considered the most prevalent form of bone disease in humans [3].

Radiographic imaging and *in vitro* radiographic studies are a useful aid and provide valuable information in the diagnostic accuracy in dental research [4], and in the

detection of periodontal bone lesions. However, there are limitations related to two-dimensional (2D) imaging, mainly related to the superposition of the cortical plates into the trabecular bone, leading to a reduced radiographic diagnostic efficacy during the interpretation of small lesions [5,6].

In vitro studies need to be as accurate as possible when reproducing the effects of human periodontal disease. There are two possibilities when creating periodontal bone loss in vitro: using only round burs and using round burs followed by acid application at the margins of the defect. The use of burs gives a radiographic image of the defect with a well-defined margin [7,8]. The chemically treated periodontal lesions can produce an ill-defined margin, creating a more realistic periodontal bone loss for the radiographic imaging interpretation [6].

The use of digital radiography is increasing and has been an alternative to film-based imaging. One advantage of digital radiography is the ability to manipulate the image using brightness, contrast, zooming tools [8], embossed [9], inversion [10], subtraction [4] and colorization. Additional advantages of the digital imaging compared to film based-radiography are: no need for darkroom or chemical processing, lower radiation dose, faster, image acquisition and the possibility of manipulating radiographic images that may improve the interpretation of images [9]. The zooming function related to the detection of periodontal bone defect using round burs was evaluated by Morais et al. [8]. The authors found similar results in the detection of periodontal bone defects using digital images zoomed to 100%, 200% and 400%.

The literature shows few in vitro studies that simulated periodontal bone defects. It is possible to mechanically create periodontal bone defects using only round burs [8-11] and also using acid to treat the margins of periodontal defects [6,12]. Some authors used only burs [12] and others acid to chemically create the periapical lesions [7,13-19]. However, none of them compared radiographic images of the defects created using burs and treating the defect walls with acid.

To the best of the authors' knowledge, this is the first in vitro study that compared periodontal bone defect created using round burs or using round burs followed

by acid application. Thus, the aim of this study was to evaluate the periodontal bone defects created only mechanically and associated to two techniques, mechanical and chemical, aiming to improve the diagnostic accuracy of periodontal bone defects in in vitro studies.

Material and Methods

Sample

This study was carried out using 24 dry hemi-mandibles from domestic pigs (*Sus scrofa*). The mean age of the pigs was 160 days. The protocol was approved by the local Institutional Experimentation Committee for Animal Care and Use (Protocol # 30/2007). This condition allowed for standardization of the sample in regards to size and bone mineral content. A total of 12 defects were created using a 1.5 mm round bur that was also treated with acid applications. 12 dry hemi-mandibles without bone defect and acid treatment were the reference in the comparison between the experimental groups. Thus, we established three groups, namely: G1 (hemi-mandibles with 1.5 mm bone defect before acid application), G2 (hemi-mandibles with 1.5 mm bone defect after acid application) and G3 (hemi-mandibles without bone defect and acid treatment).

Periodontal Bone Defects

Periodontal two-wall bone defects were created with high rotation round diamond burs (KG Sorensen, Sao Paulo, Brazil). The burs were #1.5mm in diameter and the entire diameter was used to determine the bone defect width under constant irrigation and the defect depth was random. The defect was created on the alveolar ridge between second and third pre-molar, in the buccal-lingual direction to give the best access to create the periodontal bone defect (Figure 1a).

Image Acquisition

Digital images were acquired using the Visualix eHD sensor (Gendex Dental Systems). The X-ray unit (GE 1000; General Electric, Milwaukee, WI) was operated at 70 kVp, 10 mA and 15 impulses.

The hemi-mandible and the sensor were stabilized using a fixing device. The radiographs were taken with the long hemi-mandible axis perpendicular to the central ray and the sensor parallel to the hemi-mandible at a 40 cm focal-spot to sensor distance. A wooden block, 2 cm in width, was placed between the x-ray source and the mandible to simulate soft tissues.

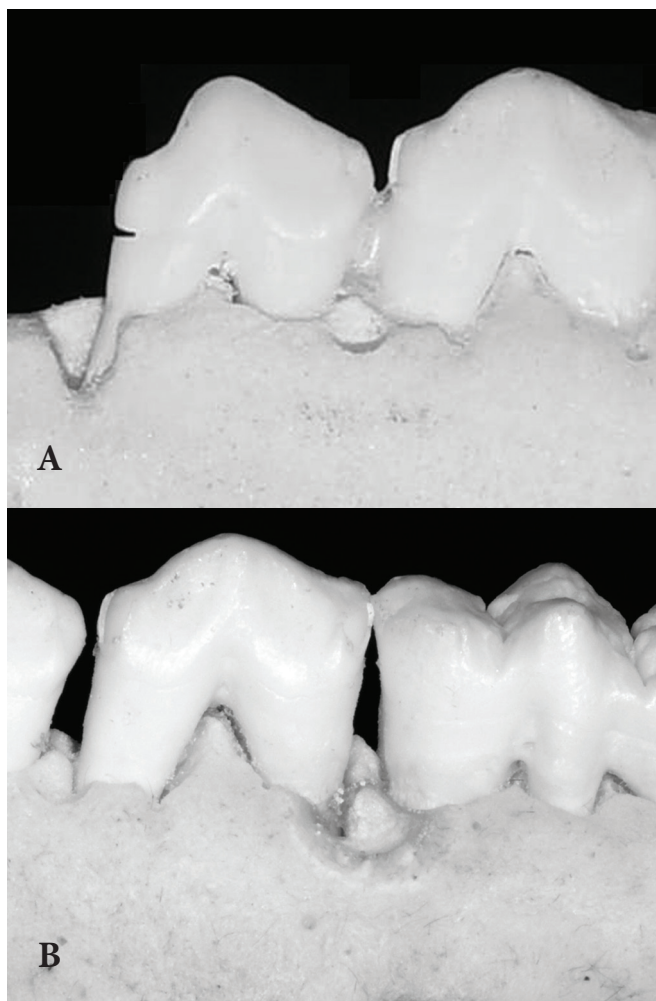


Figure 1. Bone defect created with 1.5mm round bur in a dry pig hemi-mandible (a) and treated with 100% perchloric acid application (b).

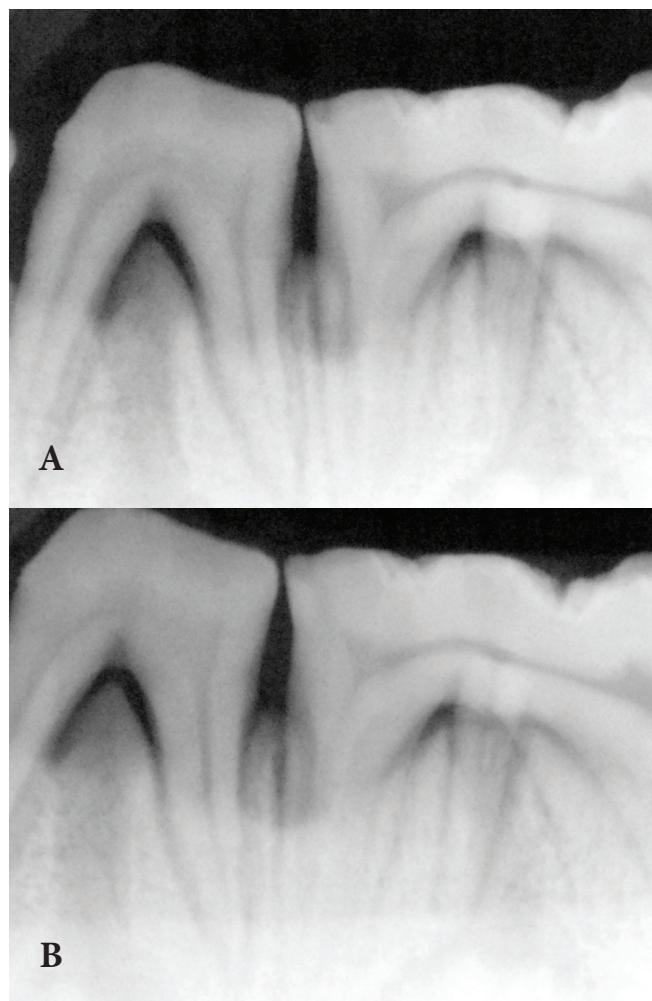


Figure 2. Digital image of bone defect with 1.5 mm before acid application (a) and after acid application (b).

Acid Treatment

A pilot study was performed using formic acid at different concentrations, but since the margins of the defects did not change, perchloric acid at 100%, which proved to change the margins of the defects, was used. The perchloric acid was applied to the hemi-mandibles and left in contact with the defect walls for 48 hours (Figure 1b). The hemi-mandibles were then washed under running water. The radiographic images were acquired as previously described. The images were saved in TIFF (Tagged Image File Format), at a resolution of 1,593 x 1,024 dpi.

Radiographic Interpretation

Images were zoomed to the level of 125% using the Image J software (<http://rsb.info.nih.gov/ij/index.html>), running on a portable computer (Toshiba Satellite P25-S507 -Taiwan) on the Windows XP operating system and a 17" flat screen WXGA, with a resolution of 1,440 x 900 pixels.

Three calibrated examiners randomly interpreted a total of 36 direct digital radiographic images of periodontal bone defects, before and after acid application, and of the reference group, i.e. without bone defect and acid treatment. The examiners did not perform the bone defects neither treated them with acid. No information was provided to the examiners about the real diagnosis of the periodontal bone defects. The screen brightness and contrast adjustments were automatically adjusted. The radiographic interpretation was carried out in a room with minimum light at a distance of 50 cm to 100 cm from the computer screen. Inter-examiner agreement was obtained by consensus during the evaluation of the defects. The examiners applied a five-point confidence scale during the radiographic interpretation of the periodontal bone defects: (1) definitely absent; (2) probably absent; (3) uncertain; (4) probably present; (5) definitely present (Figure 2a and 2b).

Statistical Analysis

In order to evaluate diagnostic procedures, sensitivity and specificity were computed for the detection of periodontal bone defects with acid application and periodontal bone defects created using only round burs. The examiner’s radiographic interpretation produced a diagnosis based on a five-point confidence scale. If the interpretation received the scores 1 or 2, it was concluded that no bone defect was present, whereas the scores 3, 4, or 5 were considered to reflect evidence of a bone defect. The diagnostic matrix was done according to validation criteria.

Results

Table 1 and table 2 summarize the results of this study. The results of table 1 show the diagnostic matrix at 125% magnification for defect size of 1.5 mm created mechanically with burs, without acid application in the detection of the periodontal bone defects (Table 1). Table 2 shows the diagnostic matrix at 125% magnification for defect size of 1.5mm with acid treatment in the detection of the periodontal bone defects (Table 2).

The findings from tables 1 and 2 provide significant evidence that there is no difference in the detection between the radiographic interpretations of periodontal bone defects created using round burs and defects created using round burs followed by acid treatment.

Table 1. Diagnostic matrix at 125% magnification for defect size of 1.5 mm created mechanically (burs). Sensitivity- Sen (95%CI) and specificity- Spec (95% CI).

Burs	Bone defect	No bone defect	Total
Positive (score ≥ 3)	11	1	12
Negative (score ≤2)	1	11	12
Total	12	12	24

Sen (95%CI) = 0.9167 (0.8022-1.0000)
 Spec (95%CI) = 0.9167 (0.8022-1.0000)

Table 2. Diagnostic matrix at 125% magnification for defect size of 1.5 mm chemically treated (acid treatment). Sensitivity- Sen (95%CI) and specificity- Spec (95% CI).

Acid treatment	Bone defect	No bone defect	Total
Positive (score ≥ 3)	10	1	11
Negative (score ≤2)	2	11	13
Total	12	12	24

Sen (95%CI) = 0.8333 (0.6842-0.9824)
 Spec (95%CI) = 0.9167 (0.8022-1.0000)

Discussion

In the present study, it was decided to apply acid to the margins of the mechanically created periodontal bone defects due to the criticism that mechanically created defects produce a sharp margin and do not imitate the nuances of natural periodontal disease [6,11]. The intention of this work was improve the diagnostic accuracy and to mimic what occurs in vivo in studies of periodontal bone defects.

Experimental models in the field of research can be defined as the materialization of part of reality [2]. They need have an appropriate accuracy and should demonstrate the limitations on the representation of the reality. In vitro radiographic studies to access and evaluate the periodontal bone loss are useful tools for the detection and measurements of periodontal bone defects. Thus, this study evaluated the detection of mechanically created and chemically treated 1.5 mm periodontal defects in pig mandibles using direct digital imaging zoomed to 125% of magnification. There was a non-significant difference in detection of the defects before and after acid application. Although the acid treatment demineralizes the alveolar bone in the border of the defect and should generate a radiographic imaging with less contrast, resulting in a more difficult radiographic interpretation, the observers did not find any difference between the two types of images related to the detection of the periodontal defects.

Dry pig mandibles were used to create the periodontal defects because it is very difficult to find human mandibles that have remaining teeth and natural periodontal bone loss. Two papers [6,12] have used 40% formic acid to demineralize the border of the defect in human cadaver segments of maxillae and mandibles. The results of these articles showed that defects of different size modify the diagnostic accuracy of bone lesions. It is possible that in our study the different defect size and margin modification were not effective in modifying the diagnostic accuracy, probably due to the type of defects and the nature of the pig mandibles.

The comparison among our results and others found in the literature is impaired because the majority of published papers show only the acid application in the in vitro periapical lesions [7,14-19]. We were able to find only two published papers [14,15] that deal

with periodontal bone defect using burs and acid treatment in the defect walls, although the authors did not compare the effect of acid treatment, as we did in this study. Besides these differences in the study design, it is important to discuss the nature of the periapical and periodontal of in vitro lesions. Periapical lesions were created by removing the tooth and placing a cotton pellet with acid at the bottom of socket [7,14-19] and periodontal lesions were created initially using a bur in the interproximal areas of alveolar crest and after the acid treatment was done [7,12]. Basic differences related to anatomical aspects can be considered between periapical and periodontal in vitro lesions.

Another aspect that should be addressed is the use of a control group. A set of radiographic imaging without defect was included in this study, which was an important reference when the examiners classified the defects related to the presence or not in a five-point reference scale. This statement is the agreement with Tirrell et al. [19], who have compared conventional radiography to digital imaging in the detection periapical lesions created chemically. On the other hand, the control group decreases the limitation related to radiographic interpretation of the anatomical landmarks in pigs, whose foramen and canals have shapes similar to the periodontal bone defect [8].

Although we have tried to create periodontal bone defects using burs and treated the margins with perchloric acid, resembling a true periodontal defect, this system has limitations as a model of the natural bone loss, which is in agreement with a previous study [12]. Within the limitations of this study and considering the methodology adopted, there is no difference in the detection of periodontal bone defects created using round burs and defects created using round burs followed by acid treatment.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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