

A radiological method to evaluate alveolar bone regeneration in the Chacma baboon (*Papio ursinus*)

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Introduction: The evaluation of alveolar bone healing may have a role in dental implantology, in prosthodontics in the post-extraction phase and in monitoring fracture repair. There are several radiological techniques described to evaluate alveolar bone regeneration. However, most are expensive and time consuming.

Objectives: To develop and evaluate a radiological method utilising readily available equipment to measure alveolar bone regeneration.

Materials and methods: An apparatus was designed to enable the acquisition of standardized x-ray images, consisting of a disposable impression tray, digital positioning system, aluminum step wedge, digital x-ray sensor, Rinn apparatus and laboratory putty. Bone biopsies were collected from each oral quadrant in each of five Chacma baboons (*Papio ursinus*). Accurately standardised x-ray images of the biopsy sites were taken pre-operatively, directly post-operatively and again after three and six week intervals. These images were analysed using a graded histogram provided in a computer software program.

Results: The average gray-scale value on the histogram of the selected biopsy area was determined on the series of standardised images. The average values for the three biopsied sites per quadrant were expressed as percentages of pre-operative density. The results indicated a mean increase of 6.3% ($\pm 1.4\%$) (mean ± 1 SEM) in bone density after three weeks and 12.6% ($\pm 1.7\%$) six weeks post-operatively.

Conclusion: A standardised radiological examination method was developed which, together with a computer-

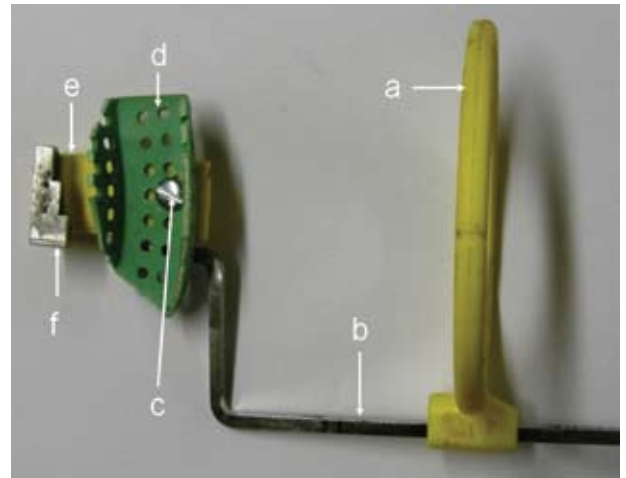


Figure 1: The apparatus assembled as a jig to ensure that all images of a quadrant were taken with tube and film alignments precisely standardised (a + b = Rinn apparatus; c = commercially available screw; d = #21 disposable mandibular impression tray; e = XPC-DS Digital Position System; f = aluminum step wedge).

ised evaluation technique, could be applied to accurately determine relative bone density. This method was shown to provide comparative bone density values during the regeneration process of alveolar bone over a six week period.

Keywords: alveolar bone; bone density; grayscale; histogram.

INTRODUCTION

Bone is a highly dynamic structure undergoing constant remodeling including repair after fracture and in response to applied forces.¹ Bone-mineral density (bone density) is a measure of the amount of minerals in the bone and usually denotes the degree to which a radiation beam is attenuated by bone, as judged from a two dimensional projection image (area bone density).² A variety of methods have been developed to study bone mineral density and the process of bone healing and regeneration, including molecular,³ cellular,⁴ humoral⁵ and mechanical⁶ approaches. Advances in the monitoring and the study of bone healing have been published extensively.^{7,8}

These methods rely principally on precision techniques in radiology⁹ and histomorphometry.¹⁰ Dual-energy x-ray absorptiometry (DEXA) and Quantitative computed tomography (QCT) are most commonly used,¹¹ and are applied primarily to identify early signs of conditions affecting bone density like osteoporosis, but are not specifically used for monitoring bone healing or bone regeneration.

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Figure 2: The XPC-DS Digital Position System (e in Figure 1) to which the XPC-DS Digital Systemsensor was fixed and the aluminum step wedge (f in Figure 1) that was used to provide a comparative standard of density, expressed as a gray scale value.

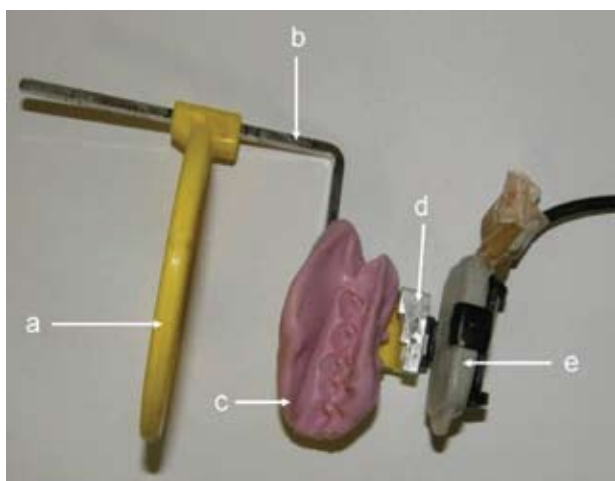


Figure 3: The impression of the teeth of the relevant quadrant enabled a precise placement over that section of the arch, and ensured that the jig was identically positioned for each radiograph (a + b = Rinn apparatus; c = #21 disposable impression tray with Lab Putty impression; d = aluminum step wedge; e = Gendex-Visualix EHD Digital Intra-Oral x-ray unit – Size 1 sensor (universal size) attached to a XPC-DS Digital Position System.

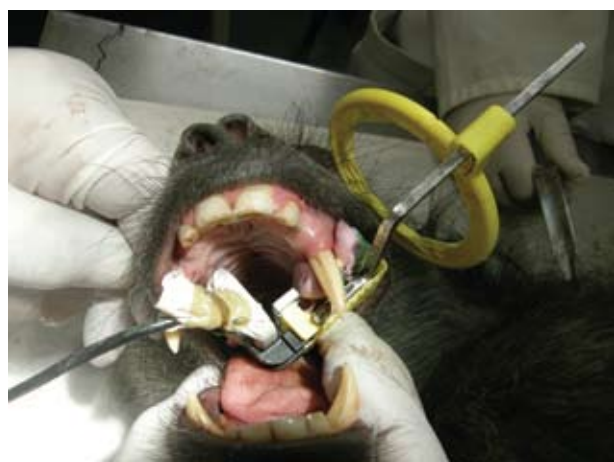


Figure 4: A photograph of the positioning of the apparatus in the mouth of a baboon before a radiographic image was acquired.

Subtraction radiography¹² is a method that can be used to visualise small differences in bone density and bone volume over time utilising the gray-scale value, a number that reflects the intensity of the radiological image at a specific location

in comparison with the rest of the image.¹³ The density of the newly formed bone, and therefore the intensity of the image, depends on the quantity of minerals deposited in the area of healing. For subtraction radiography, which analyses and compares images taken at different time points, the critical requirements are the need for standardisation and for accurate reproducibility of the films. The projection geometries of the two radiographs forming the pair of images used for the subtraction process need to be identical.^{14,15} Computer programmes may be applied to measure changes in bone density (as depicted by the gray scale values). Aluminium or hydroxyapatite are often used to standardise the radiographs for subtraction radiography, as their radiation attenuation characteristics are similar to those of bone.¹⁴

Digital intra-oral radiography has become the norm in modern dentistry. It is generally used for diagnostic purposes with a direct sensor system which includes a CCD sensor (charge-coupled device), a processor unit, a digital interface card, and a computer with specific software.¹⁶ The technique is also suitable for subtraction radiography.

The aim of this study was to develop a method to determine changes in bone density as an indicator of bone regeneration over time by using equipment present in any well-equipped dental practice. This may aid in the decision of when to place implants following the extraction of teeth, in order to restore function and aesthetics at the earliest time whilst ensuring a sound substrate for the implant.

MATERIALS AND METHODS

Five healthy Chacma baboons (*Papio ursinus*) were used. Approval for the study was granted by both the Animal Use and Care Committee (AUCC), and a subcommittee of the Committee for Research Ethics and Integrity at University of Pretoria and North West University. The animals were anaesthetised with intravenous ketamine hydrochloride (dose : 10mg/kg). In order to control haemostases and pain, 1.8 mL (9 mg) Bupivacaine with 0.5% epinephrine 1:200,000 (as bitartrate) (Novocol, Pharmaceuticals of Canada, Inc., Cambridge, Ontario, Canada) was injected intramuscularly.

Surgical intervention

Biopsies were taken in the premolar areas of the mandible and maxilla, the bone at the selected site being exposed by reflection of the overlying mucosa. Three alveolar bone biopsies, 3mm deep, were taken with a 3mm diameter trephine bur, fitted onto a straight surgical hand piece connected to a surgical drilling unit. The biopsies were positioned 2mm apart.

Radiology

Radiographs were taken pre-operatively, directly post-operatively and again after three and six weeks. Radiographs were acquired for each of the four quadrants at each time point. Acquisition of standard, reproducible radiographs for analysis was achieved as follows: for each quadrant, maxillary and mandibular in each animal, a sectional tray was prepared by cutting in half a disposable mandibular impression tray (#21, Wright Cottrell Co., Kingsway West, West Dundee, Dundee). (Figures 1 and 2). In each instance the tray was adjusted to fit properly over as many teeth as possible. A bite block (XPC-DS Digital Position System (Gendex, Lake Zurich, Illinois) was secured onto the tray with a self-tapping screw and cyanoacrylate cement. The block carried the cradle for the sensor (Gendex Visualix EHD Digital Intra-Oral

x-ray unit – Size 1 (universal size) with 25.6 line pairs/mm; KaVoDental, Gendex Imaging, Via Alessandro Manzoni, 44, 20095 CusaoMilanino, Italy) (Figure 1). A step wedge was made from a 3mm x 6mm strip of commercially available aluminium. Three steps were cut, 2mm wide and 1mm deep (Figure 2). The wedge was supported on the bite block so that it was parallel to and just touching the sensor. Removal and accurate replacement of the wedge in this position was achieved by an arrangement of location pins which fitted precisely into receptacle holes. A drill of the requisite diameter was used to make two small holes through the aluminium and into the bite block. Short straight sections of a paperclip were cut and glued into the holes in the bite block (Figure 2). These protruding pins fitted precisely into the holes in the aluminium wedge, enabling repeated removal of the wedge and its subsequent replacement in the same position. The procedure was repeated for every bite block, enabling ready transfer of the aluminium wedge for each radiograph. The bite block, sensor and tray were secured to a Dentsply-Rinn apparatus (Dentsply, Elgin, Illinois), consisting of a metal ring holder and plastic positioning ring (Figure 3).

The sectional tray (Figure 1) was loaded with laboratory putty (Coltene/Whaledent, Switzerland), a silicone base and polysiloxane activator and positioned in the quadrant to include as much of the alveolar ridge as possible. While the laboratory putty was still in the soft stage of the setting process, an x-ray was taken to enable confirmation that the sensor was correctly positioned. Once the putty had set, the impression of the teeth and the alveolar ridge provided a secure key to accurate repositioning of the set up for subsequent radiographs. The position of the ring on the holder, as well as the position in the bite block, was identical for all the radiographs (Figures 3 and 4).

A Planmeca Intra Wallmount X-ray unit (Planmeca Oy, Asentajankatu 6, 00880, Helsinki, Finland.) was used to acquire the radiographs which were taken at 8 mA with 63 kV and an exposure time of 0.08 seconds. A Toshiba D-0711 SB x-ray tube was used and the focal spot was 0.7 x 0.7 mm. The focal distance for all the images was 110 mm.

The computer software program used for the radiology was Gendex VixWin Pro (Gendex Dental Systems 901 West Oakton Street Des Plaines, IL 60018).

Evaluation methods

Four digital images were acquired per quadrant on each occasion when records were taken (Figure 5) i.e. 16 images per animal at each of the four time periods. The images were imported into Adobe Photoshop (V6.0; Adobe Systems Inc., San Jose, CA). An A4 transparent sheet was positioned on the computer screen and firmly secured. The first pre-operative image was imported on the screen and with the lasso tool of the program an area of interest was selected on the image of the step wedge (Figure 6A). The corners of the selected area were marked on the transparent sheet with a fine point permanent marker pen. Now each of the three postoperative images could sequentially be accurately positioned and oriented on the screen, using the drag and drop function, so that the marks on the transparent sheet precisely superimposed on the selected area on the image. The gray scale values for this defined area of the wedge were standardised across all four images recorded from each quadrant by using the histogram, contrast and brightness tools of the Photoshop software¹⁴ to make the required point adjustments, ensuring that the density never differed

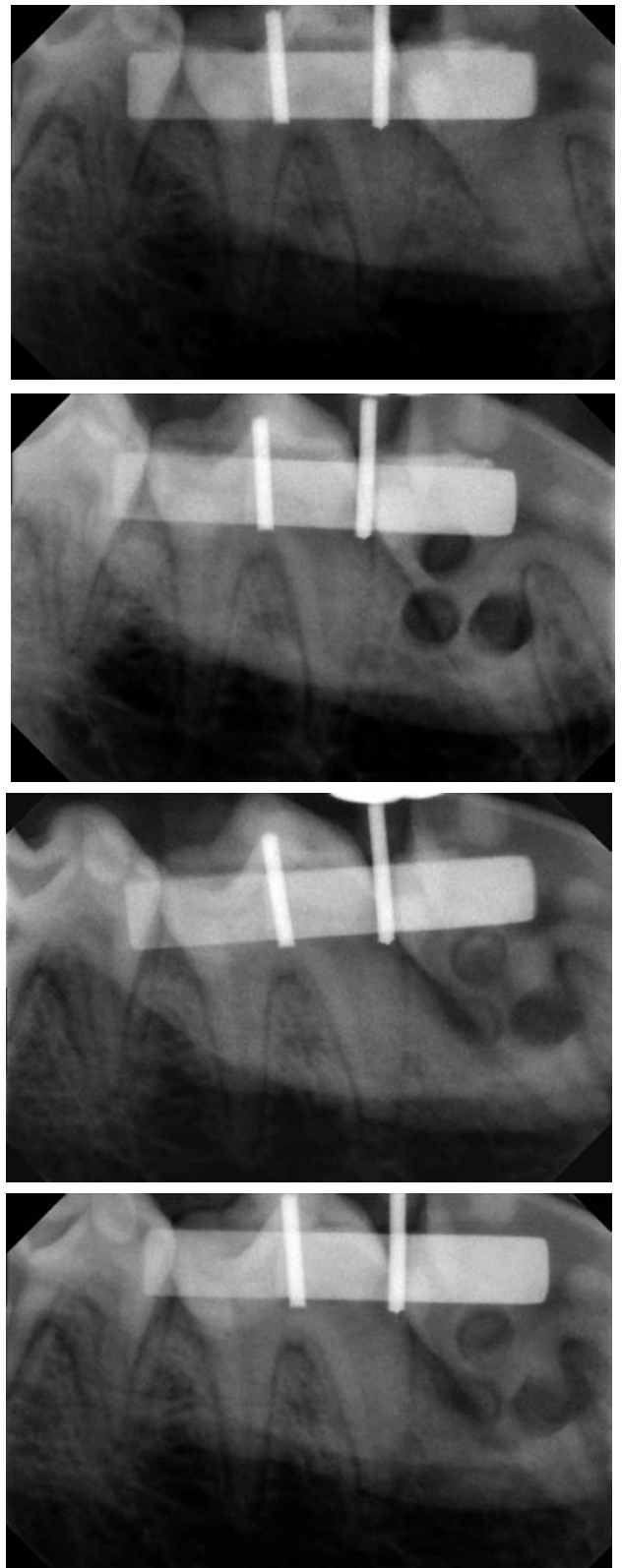


Figure 5: An example of images that were acquired pre-operatively (A), immediately post-operatively (B), 3 weeks post-operatively (C) and 6 weeks post-operatively (D).

by more than 12 data points. Hence all images now reflected comparable degrees of gray scale (Figure 6A). An area of interest was selected on each of the biopsy sites on each of the images taken immediately post-operatively (Figure 6B). The average gray-scale values for these areas on each of the three biopsy sites on each image were determined and recorded. This was done for all images of a quadrant acquired from each of the different time periods.

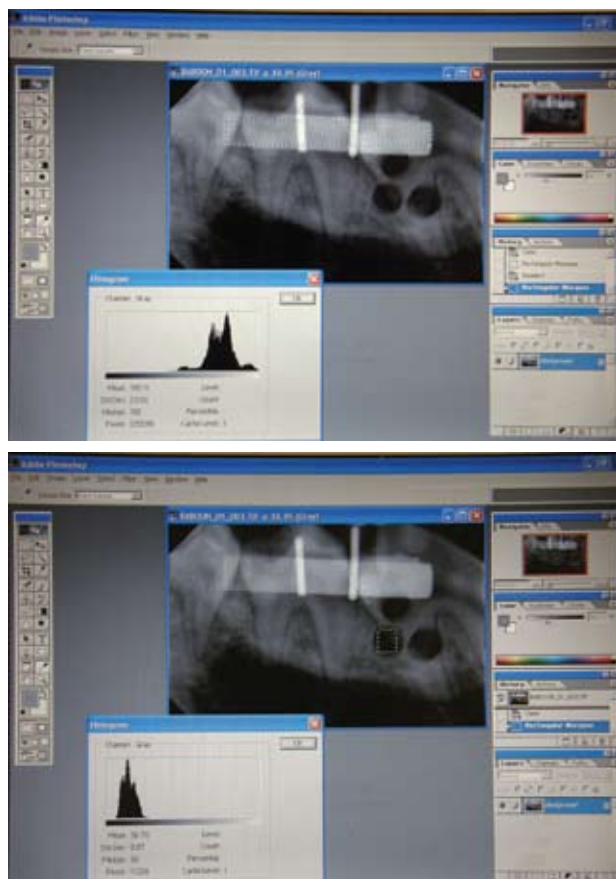


Figure 6: A histogram reflecting the radiographic intensity of the image of the selected area of the aluminum step wedge (A) and, similarly, a histogram of a selected biopsy area (B).

RESULTS

The average of the gray scale values for the three biopsy areas of each quadrant was calculated (Table 1). The average densities for the different time points were as follows: pre-operatively = 84, post-operatively = 40, post-operatively at three weeks = 45 and post-operatively at six weeks = 50. The biopsy site recorded a 53% ($\pm 18\%$) decrease in the bone density reading, measured by the gray scale value, between pre-operative and directly post-operative stages. The percentage changes were calculated using the following formulae:

$$\text{Post-operative value} = \frac{x - y}{x} \times 100$$

$$\text{Three weeks post-operative value} = \frac{a - y}{x} \times 100$$

$$\text{Six weeks post-operative value} = \frac{b - y}{x} \times 100$$

Where x = pre-operative value, y = post-operative value, a = value at three weeks post-operatively and b = value at six weeks post-operatively.

The average percentage changes per quadrant were 6.3% ($\pm 1.4\%$) at three weeks and 12.6% ($\pm 1.7\%$) at six weeks post-operatively. The average increase in bone density between post-operative three weeks to post-operative six weeks was 6.2% ($\pm 0.9\%$). The results are summarised in Table 1 and illustrated in Figure 7.

Table 1: Gray scale values expressed as a percentage relative to the pre-operative value.

Animal	Quadrant	Post-operative	Gain 3 weeks post-operative	Gain 6 weeks post-operative
B	1	53	4.9	15.0
	2	43	3.9	6.3
	3	48	4.4	10.5
	4	44	5.5	12.6
C	1	69	3.0	11.9
	2	39	3.5	15.2
	3	61	8.6	11.2
	4	37	4.0	7.2
D	1	67	5.6	9.4
	2	91	1.7	3.6
	3	78	1.3	7.3
	4	60	14.5	17.4
E	1	45	0.3	4.8
	2	60	6.1	10.4
	3	65	2.1	15.9
	4	65	16.0	26.9
F	1	52	11.1	22.7
	2	20	1.3	1.4
	3	55	25.3	31.2
	4	48	3.5	10.2
	mean	55	6.3	12.6
	SD	16	6.1	7.6
	SEM	4	1.4	1.7

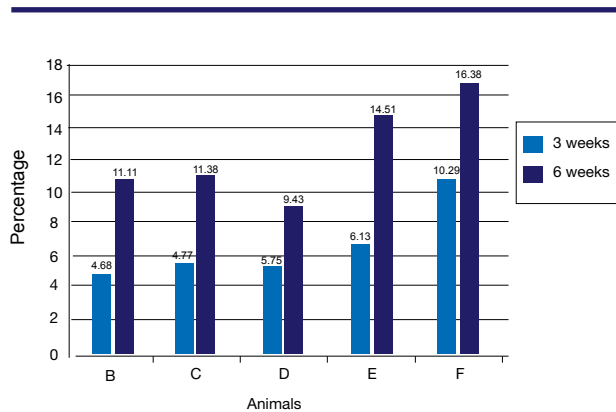


Figure 7: The percentage increase in bone density (as gray scale values) for the individual animals over three and six weeks.

DISCUSSION

The construction of the positional apparatus for the radiological sensor was a challenge due to its relatively large size occasioned by the need to accommodate the loaded impression tray, and to allow space for the aluminium step wedge. The method made it possible to reposition the apparatus accurately and repeatedly in exactly the same position and complies with all the requirements stipulated for comparable studies found in the literature survey.

A critical factor in the monitoring of bone healing is the acquisition of standardised radiological images, allowing for subtraction radiography under the direction of appropriate computer software. The process as described enabled accurate analyses of the radiographs which had been acquired of the biopsy areas at different time intervals. The results obtained from image analysis using VixWin PRO® and Photoshop 6® compared favourably with those obtained in other studies using different computer software packages.^{17,18} The linear increase in bone density correlates with the values observed in similar studies.¹⁹⁻²¹ The hypothesis was that the pre-operative value represents 100% bone density as healthy tissue, while the directly post-operative value represents 0% bone density. The mineralisation process at three weeks post-operatively is at an early phase and did not make a substantial difference to the recorded gray-scale values.²² At six weeks, higher values were recorded in the gray-scale values as mineralisation was more detectable on a radiological image. This appears to be the first study where four quadrants in the same animal were used to evaluate alveolar bone healing following similar trephine biopsies. The radiological examination method for bone density measurement is cost effective and is reasonably available in any dental practice. The accuracy essential for the standardisation of sequential image acquisition may be attained through a simple device based on a sectioned impression tray.

The method may prove useful as it is possible to determine the progress of bone healing relatively accurately, whether after extractions, surgery or fracture. The timing of subsequent interventions such as the placement of implants may be embarked upon with greater confidence.

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