Single Photon Emission Computerized Tomography and Histological Evaluation in the Validation of a New Technique for Closure of Oro-antral Communication

An Experimental Study in Pigs

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ABSTRACT

Various bone regeneration techniques have evolved recently but controversies regarding vascularization and integration of such bone grafting techniques have led occasionally to animal experiment to validate such techniques. The objective of this study was to evaluate the evidence of vascularization and osseo-integration of a new bone regeneration technique utilized for the closure of oro-antral communication (OAC) by an experimental model in which Single Photon Emission computerized Tomography and histological studies were conducted in pigs. We conclude that the sandwich technique used for the closure of OAC results in a vascularized new bone formation which eventually osseo-integrate with the surrounding bone. Also, this experimental study confirmed that autogeneous bone graft was superior to xenografts when used within the sandwich unit.

Tomografía Computarizada Mediante Emisión de Fotones simples y Evaluación Histológica en la Validación de una Nueva Técnica para el Cierre de la Comunicación Oroantral

Un Estudio Experimental en Cerdos

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RESUMEN

Varias técnicas de regeneración de huesos han sido desarrolladas recientemente, pero las controversias con respecto a la vascularización e integración de tales técnicas de injerto de hueso han llevado de vez en cuando a la experimentación con animales, a fin de validar estas técnicas. El objetivo de este estudio fue evaluar la evidencia de vascularización e integración ósea de una nueva técnica de regeneración de hueso utilizada para el cierre de la comunicación oro-antral (COA), mediante un modelo experimental en el que se practicó la tomografía computarizada mediante emisión de fotones simples y se realizaron estudios histológicos en cerdos. Concluimos que la técnica de sándwich usada para el cierre de COA da lugar a una formación ósea vascularizada que acaba produciendo una oseointegración con el hueso circundante. Asimismo, este estudio experimental confirmó que el injerto óseo antólogo es superior a los xenoinjertos cuando se usa dentro de la unidad de sándwich.

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INTRODUCTION

The Ogunsalu sandwich bone regeneration unit and technique is a new addition to the various other available bone regeneration techniques (1–4). This technique has been used in the closure of an oro-antral communication (3) [Figs. 1–3], resulting in complete closure of the communication and formation of a thick bony floor for the maxillary sinus (Fig. 3). Subsequently, an endo-osseous implant was placed in the edentulous maxillary site in relation to the newly regenerated bony floor of the sinus (Fig 4). This implant showed radiographic evidence of osseo-integration. A bone grafting unit can integrate with the surrounding adjacent normal bone without necessarily being vascularized and an implant within a bone grafting site showing radiographic evidence of osseo-integration does not necessarily validate vascularization. It is for these reasons that Single Photon Emission Computed Tomography was utilized to confirm the osteoblastic activities within a bone grafting unit and to validate vascularization. It also compared such activities when autogeneous bone graft is utilized as against when xenograft (Bio-oss) is utilized using experimental animal models.

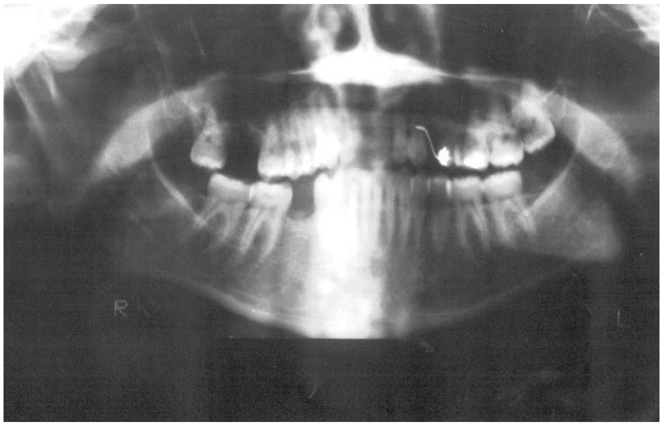

Fig. 1: Dental panoramic tomogram showing fresh oro-antral communication.

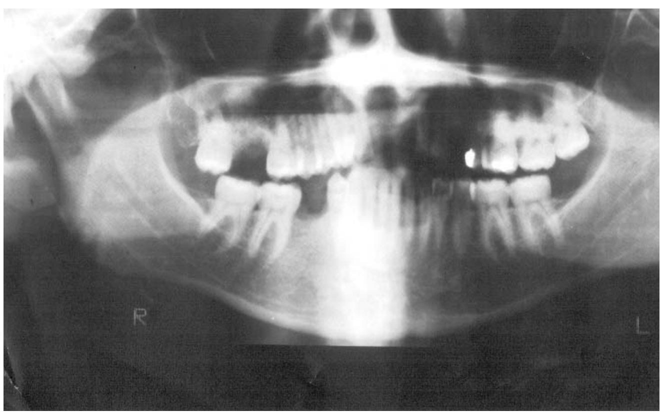

Fig. 2: Dental panoramic tomogram immediately post placement of the sandwich unit showing the sandwich unit in place within the maxillary sinus together with Bio-Oss overlay of the alveolar ridge in the area.

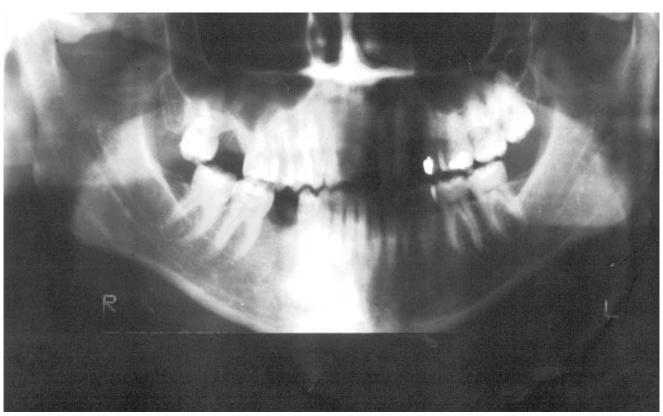

Fig. 3: Dental panoramic tomogram 8-months after the surgery showing radiographic evidence of advanced osteogenesis in the floor of the sinus and alveolar ridge.

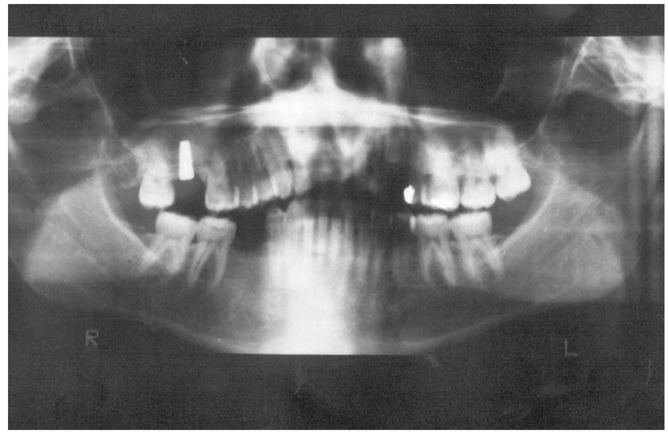

Fig. 4: Dental panoramic tomogram showing implant in place within the bone regeneration site shown in Figure 3.

MATERIALS AND METHODS

Single Photon Emission Computed Tomography (SPECT)

SPECT imaging was performed at 14 weeks after the implantation of the Ogunsalu bone regeneration sandwich unit in the right and left posterior mandible of a castrated male pig aged 22 weeks to determine its osteoblastic activities. The left side had xenograft as the bone substitute within the sandwich unit and the right side of the mandible had a sandwich with autograft bone substitute. At 14 weeks post implantation of the sandwich units, the pig was anaesthetized and an intravenous access into the ear vein was placed to allow the intravenous injection of 740 MBq (20 mci) technetium 99 m methylene diphosphate. The pig was subsequently euthanized two and a half hours after the injection and the mandible was removed. Half an hour later the tomographic images of the mandible with the region of interest (ROI) were acquired with a Siemens orbiter II rotating large field-of-view gamma camera (Siemens Medical Systems Inc, Erlangan, Germany) equipped with a low energy high resolution collimator. Sixty-four projection images (20s/ images) were acquired over 180 degrees in a 128 by 128 ma-trix with a dedicated nuclear medicine computer (Siemens ICON computer).

The projection data were corrected for flood field non-uniformity and centre of rotation. Transverse reconstruction was then performed with a shepp-Loga Hanning filter cut-off frequency of 0.4. By utilizing the transverse slices, the activity in the jaw at the site of implantation of the sandwich (area of interest) was observed and calculated and compared with a reference point within the jaw on each side. The average count (pixel) for each area of interest (xenograft site versus autograft site) was compared.

Histology

After SPECT, the overlying periostium was carefully dissected from each of the bone regeneration sites of the pigs' mandible. These periostia were preserved separately for an unrelated study. A block specimen of hard tissue was taken from a representative site in the bone regeneration area in the left (xenograft side) and right (autogeneous side) mandible.

Each of the two retrieved specimens was placed in ten per cent neutral buffered formalin. These tissues were then sent to the hard tissue research laboratory (HTRL) at the Oral Pathology department of the University of Minnesota. Upon receipt in the HTRL, specimens were dehydrated with graded series alcohol for nine days. Following dehydration, the specimens were infiltrated with a light curing embedding resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). Following 20 days of infiltration with constant shaking at normal atmospheric pressure, the specimens were embedded and polymerized by 450 nm light with the temperature of the specimen never exceeding 40°C. The specimens were then prepared by the cutting/grinding method of Donath/Rohrer Schubert (5, 6). The specimens were cut to a thickness of 150 um on an EXAKT cutting/grinding system (EXAKT Technologies, Oklahoma City, USA). Following this, core were then polished to a thickness of 45-65 um using a series of polishing sandpaper disc from 800 to 2400 grit using an EXAKT Micro-grinding system. A final polish with 0.3 micron alumna polishing paste followed this. The slides were stained with stevenals blue and Van Gieson's picro fuchsins and cover slipped for histological analysis by means of bright field and polarized microscopic evaluation.

The images of the histologic slides were then digitized by using a Nikon Coolpix 4500 digital camera and a zeis Axiolab microscope at the same magnification. After digitizing the images, they were all transferred into Adobe photoshop (Adobe systems, Inc). Subsequent to the transferring to the photoshop, all the images were assigned two pseudocolours in order to differentiate the bone and the graft material separately.

Histomorphometric measurements were completed using a combination of Adobe photoshop and the public domain NIH images programme (US National Institute of Health). At least two slides of each core were evaluated. Parameters evaluated were total area of the core, percentage of new bone formation and percentage of residual graft material by using the density slice tool. All these measurements were done in pixels and values subsequently put on an Excel spread sheet.

RESULTS

SPECT Evaluation

The SPECT images demonstrated increased osteoblastic activities on both sides of the jaw in the area of interest (A1) as shown in Fig. 5. The osteoblastic activity on the side which utilized autograft as the bone substitute for the sandwich was

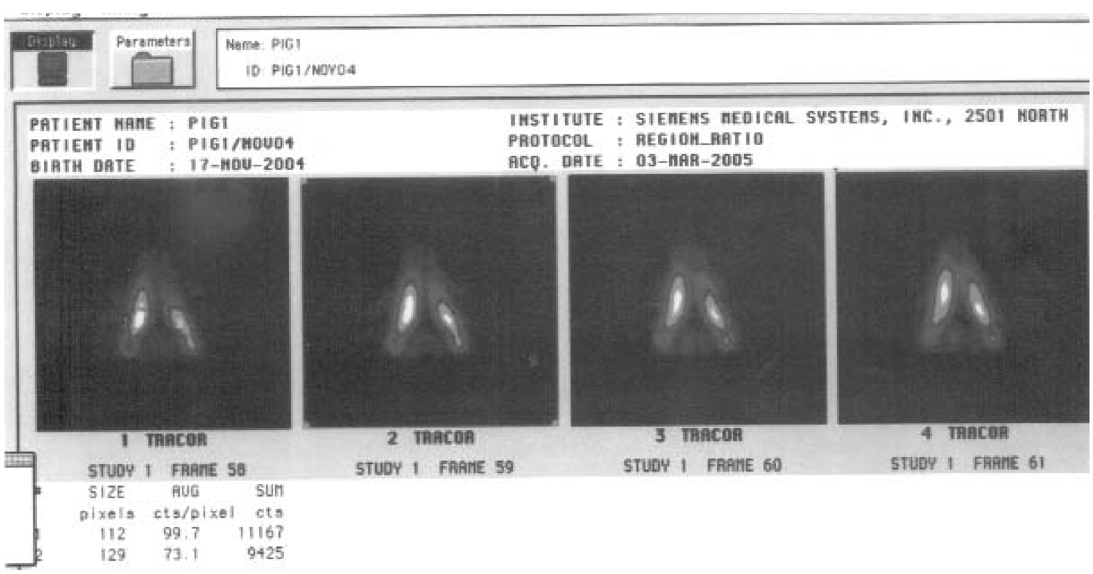

Fig. 5: Showing osteoblastic activity on both side of the jaw of the experimental pig.

more than the side in which xenograft was used as the bone substitute (Fig. 6). The average count being 99.7 pixel and 78.1 pixels respectively (Table 1) and a calculated relative activity ratio of 1:20.

Histological Evaluation

Table 2 shows the comparative histological appearance for when autogenous bone is used in the sandwich as to when Bio-Oss is used. Essentially excessive new bone formation

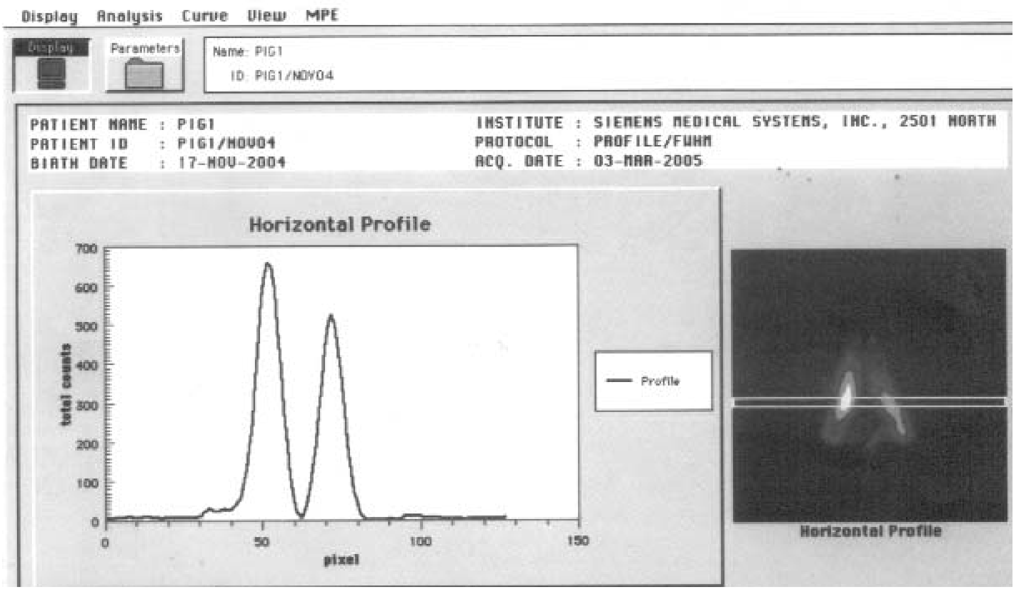

Fig. 6: Showing the activity curve and ratio for each side of the jaw. Note that the activity of the side with autogeneous sandwich is more.

Table 1: Comparison of the osteoblastic activity between the xenograft and autograft sandwich unit

Site	Component of the Sandwich unit	Size Pixel	Avg Count	Sum
1. Right mandible	a. Membrane-Bio-Gide® b. Bone substitute-autograft	112	99.7	11167
2. Left mandible	a. Membrane-Bio-Gide® b. Bone substitute xenograft (BIOSS®)	129	73.1	9425

Table 2: Histology of both sides at 14 weeks

Sandwich Side with Bio-Oss (Slide 1a/b)	Sandwich side with Autogeneous Bone (Slide 2a/b)
1. Membrane is seen, however with no support from bone because bone regeneration is not yet advanced. 2. Few Bio-Oss is seen integrating with bone 3. Both delicate and mature trabeculae is seen 4. Debris is seen (of no significance) 5. Thick osteoid with osteoblast lined around it is seen 6. Delicate trabeculae bone seen in the process of bridging with osteoblast lined up around the trabeculae bone.	1. Membrane is seen, however with no support from bone because bone regeneration is not yet advanced. 2. Excessive new bone formation is seen compared to 1a/b with more pronounced and regular bridging then seen in 1a/b.(Figs. 7a – 7d). 3. Plenty of laid down matrix is/are visible with calcification of the laid down collagen fibres. On these slides 2 pattern of bone formation are seen (Fig. 7e). i. Older pattern ii. New pattern (thus forming the network pattern of bone formation)

is seen when autogenous bone graft is used (Fig. 7a–d), which whilst showing both an older and new pattern of bone

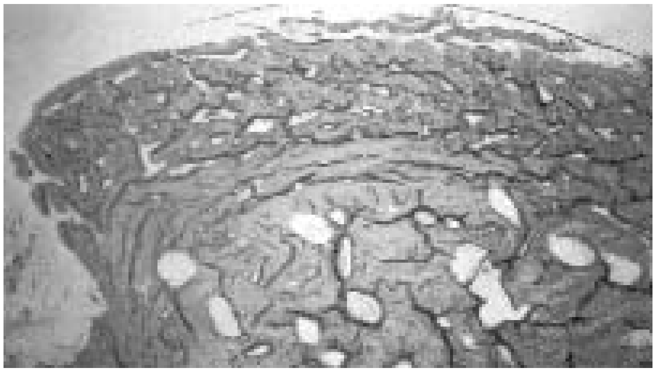

Fig. 7a: Low power showing excessive new bone formation in the autogenous side.

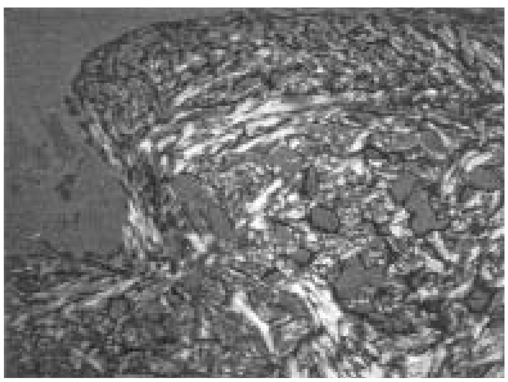

Fig. 7b: Polarized view.

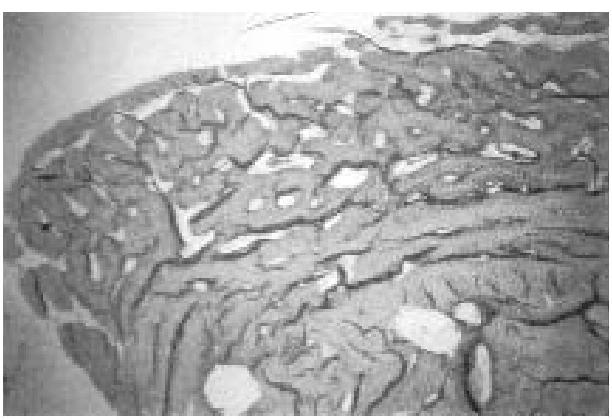

Fig. 7c: Medium power.

formation (Fig. 7e and F), the Bio-Oss side still showed delicate trabecula bone seen in the process of bridging with osteoblast lined-up around the trabecula bone (Fig. 8a – 8c).

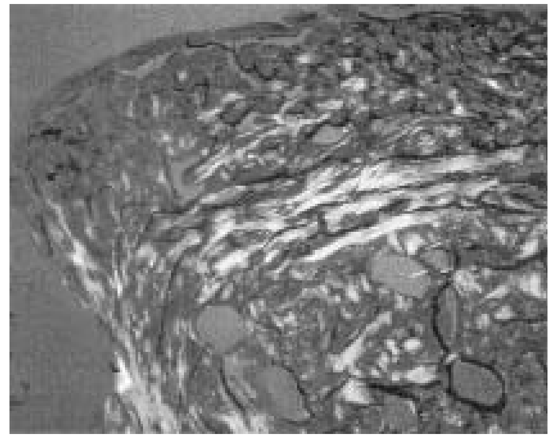

Fig. 7d: Polarized view.

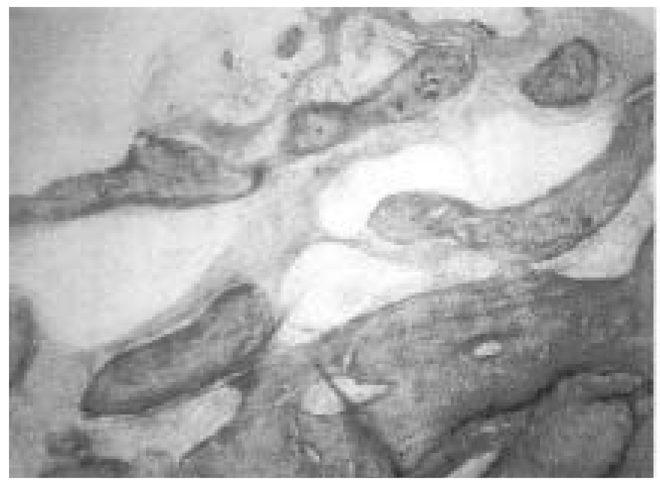

Fig. 7e: High Power showing laid down matrix with calcification at collagen fibers..

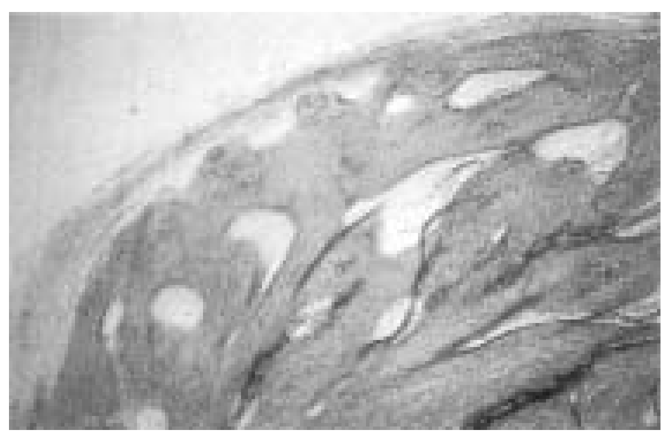

Fig. 7f: High Power showing early stage of bone regeneration. Note the overlying regenerative membrane.

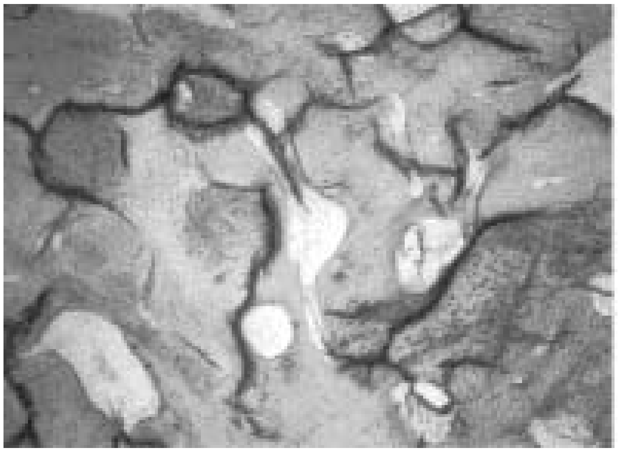

Fig 8a: Showing few Bio-Oss integrating with newly formed bone.

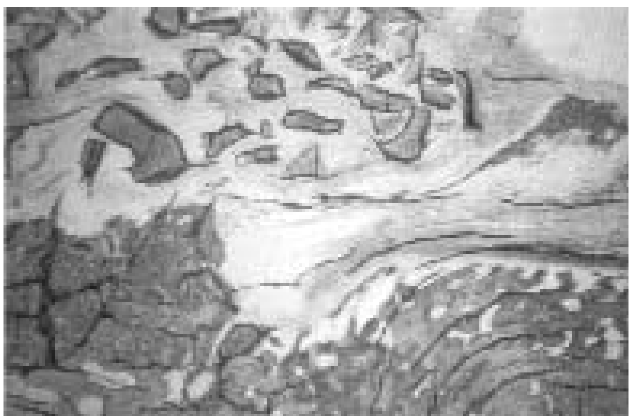

Fig 8b: Debris seen within forming new bone (of no significance).

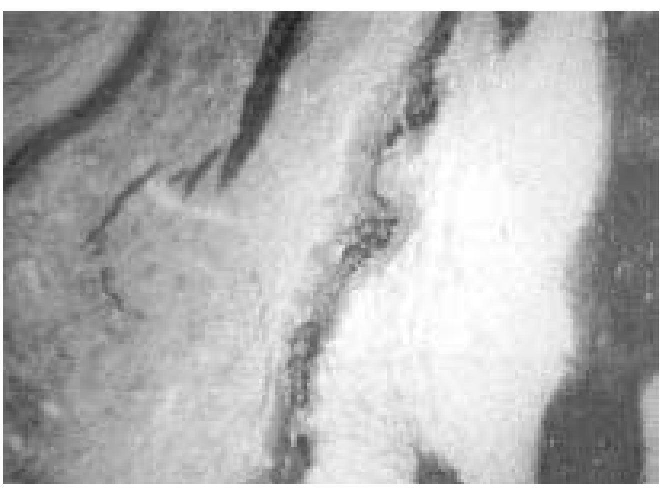

Fig. 8c: Thick osteoid with osteoblast lined around it.

Fig 9: CT-Scan showing osseointegration of newly generated bone in the left mandible of experimental pig.

DISCUSSION

The Ogunsalu sandwich technique is new (1–4). Its first use was in the year 2000 when it was utilized to reconstruct the floor of the maxillary sinus and alveolar floor after the excision of an aneurysmal bone cyst of the maxilla (1, 2). Subsequently, it was utilized for the bony closure of an oro-antral communication (3) and interestingly an implant was placed in the same site of reconstruction of the OAC (4). This implant subsequently integrated.

This unique technique of double guided tissue regeneration (D-GTR) will obviously be useful in the reconstruction of oro-antral communication and fistulae, orbital floor fracture by orthorhinolaryngologists and maxillofacial surgeons; as such, it needed to be validated scientifically in terms of its vascularization and osseointegration with the surrounding bone.

The technique of SPECT has found successful clinical application for the study of many organ systems including the skeletal system (7, 8). Khan *et al* subsequently demonstrated the role of SPECT in the osseous integration process of dental implants (9).

At 14 weeks post implantation of the sandwich unit in each of the jaw defect of the experimental animal, evidence of osteoblastic activity on SPECT (Figs. 5 and 6) and integration (osseo-integration) on CT-Scan evaluation (Fig. 9) was observed. Also such activities were noted on both jaws irrespective of the type of bone substitute (Fig. 5, 6), however the osteoblastic activity on the side which utilized autogeneous bone graft as bone substitute within the sandwich was superior to the side which utilized the xenograft as the bone substitute, thus confirming what has always been mentioned in the literature that autogeneous bone graft is more superior than all the other bone grafting materials and substitutes in the market and can be used with or without complementary therapies such as platelet–rich plasma [PRP] (10–15). Autogenous bone is the best and most reliable bone graft for achieving a predictable result and as such is referred to as the gold standard for bone grafting as it is unmatched in the amount of osteogenic cell viability it offers for bone regrowth and avoidance of histocompatability problems.

In the present study, the histological findings confirm the superiority of autogeneous bone graft when used as the bone grafting substitute for this novel technique when closure of oro-antral communication is intended (Fig. 7a–f) as compared to xenograft being used as bone substitute (Fig. 8a–c).

In summary, fresh oro-antral communication or fistulae can be closed utilizing the Ogunsalu sandwich technique. This led to bony closure and SPECT, histological and histomorphometric studies in animal model demonstrated vascularization and integration with surrounding bone. This technique can also be utilized for other reconstruction in the maxillofacial complex such as orbital floor, cleft palate and alveolus reconstruction. This study also confirmed that autogeneous bone graft is the most suitable bone substitute to be used within the sandwich.

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