Single Photon Emission Computerized Tomography in the Evaluation of the Osteoblastic Activities of a New Bone Regeneration Technique

Analysis of 12 Mandibular Sites in six Experimental Pigs

C Ogunsalu[1], C Ezeokoli[1], H Daisley[1], A Adogwa[1], J Watkins[1], A Archibald[2], C Legall[1], O Khan[2]

ABSTRACT

Objectives: *To utilize single photon emission computerized tomography performed in sequence to determine the osseo-integrating capabilities and osteoblastic activities of a new bone regeneration technique called the membrane – sandwich technique (Ogunsalu sandwich bone regenerating technique) and to compare the quality and quantity of bone formed by this bone regeneration unit to bone regeneration obtained by using the same particulate bone grafting material covered with interceed® (another type of bio-resorbable membrane).*

Design and Method: *Single photon emission computerized tomography bone imaging was performed in sequence on the mandible of a total of 6 pigs on both the right and left side (total of 12 sites) at two and a half hours following the injection of technetium 99m methylene diphosphate. Imaging was performed using a Siemen Orbitar II gamma camera. The projection data was acquired in a 128 x 128 matrix over 180 arc and SPECT reconstruction was performed using a filtered back projector method with a Shepp-Logan Hanning filter and a cut-off frequency of 0.4. The surgical defect on one side of the jaw was treated with the sandwich unit with Bio-oss particulate bone within it, while the other side contained the same quantity of Bio-oss as in the left side but just covered with interceed® membrane. The osteoblastic uptake on the side with the classical sandwich was compared to the side with the particulate bone covered with interceed® membrane for dynamic physiological activities. The average activity for both sides was calculated and compared.*

Result: *For all the 12 sites, osteoblastic activities were recorded and indicated that vascularized bone was formed at all the experimental sites. Autogenous bone graft was confirmed to be superior to xenograft using this sandwich technique. Furthermore, the osteoblastic activities on the sandwich side were seen to be more when compared with the control side (Interceed® side).*

Conclusion: *The Ogunsalu sandwich bone regeneration technique has been successfully evaluated with SPECT which shows osteoblastic activity with formation of vascularized bone which integrates with the surrounding bone.*

La Tomografía Computarizada por Emisión de fotón único en la Evaluación de las Actividades Osteoblásticas de una nueva Técnica de Regeneración ósea

Análisis de 12 sitios Mandibulares en seis Cerdos Experimentales

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RESUMEN

Objetivos: *Utilizar la tomografía computarizada por emisión de fotón único (TCEFU) realizada en secuencia, a fin de determinar la capacidad óseo-integradora y las actividades osteoblásticas de una*

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nueva técnica de regeneración del hueso, denominada técnica de membrana-sándwich, y comparar la calidad y cantidad de hueso formado por esta unidad de regeneración ósea con la regeneración ósea obtenida mediante el mismo material de injerto de hueso particulado cubierto con interceed® (otro tipo de membrana bioreabsorbible).

Diseño y Método: *Mediante TCEFU, se realizó un estudio de imágenes óseas en secuencia, de la mandíbula de un total de 6 cerdos, a los lados izquierdo y derecho (un total de 12 sitios) a las dos horas y media, luego de una inyección de difosfato de metileno marcado con tecnecio 99 m. El examen de imágenes fue realizado usando una cámara gamma Siemens Orbiter tipo II. Los datos de la proyección fueron adquiridos en una matriz de 128 x 128 sobre un arco de 180 y la reconstrucción con TCEFU se realizó usando un método de retroproyección filtrada, con un filtro Shepp-Logan-Hanning y una frecuencia de corte de 0.4. El defecto quirúrgico en un lado de la mandíbula fue tratado con la unidad de sándwich con hueso particulado bio-oss dentro, mientras que el otro lado contenía la misma cantidad de Bio-oss del lado izquierdo, pero cubierto con una membrana de interceed®. La respuesta osteoblástica en el lado con el sándwich clásico fue comparada con el lado del hueso particulado cubierto con la membrana de interceed® en cuanto a actividades fisiológicas dinámicas. La actividad promedio de ambos lados fue calculada y comparada.*

Resultado: *En los 12 sitios, las actividades osteoblásticas fueron registradas de forma indicando que se formó hueso vascularizado en todos los sitios experimentales. Se confirmó que el injerto óseo autógeno es superior al xenoinjerto que usa esta técnica de sándwich. Además, se observó que las actividades osteoblásticas en el lado del sándwich eran más en comparación con el lado control (lado del interceed®).*

Conclusión: *La técnica de Ogunsalu para la regeneración ósea por "sándwich" o membrana interposicional, ha sido exitosamente evaluada mediante TCEFU, concluyéndose que la misma consiste en la actividad osteoblástica con formación de hueso vascularizado que se integra al hueso circundante.*

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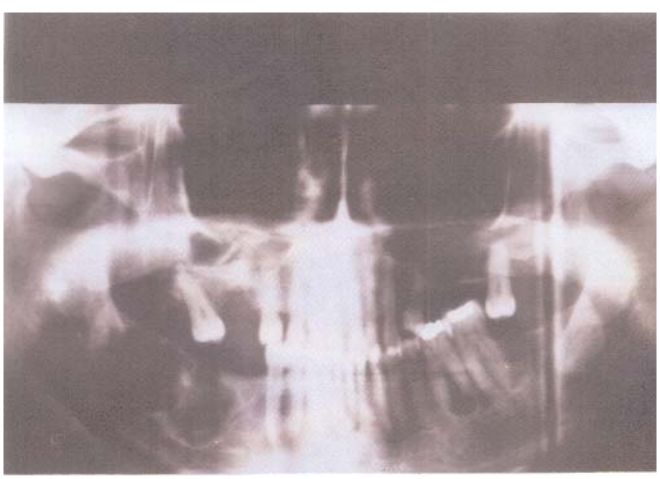
INTRODUCTION

The Ogunsalu sandwich bone regeneration unit and technique is a new addition to the various other available bone regeneration techniques (1–4). Morphologically, generous amount of bone formation has been demonstrated by 2D images (Figs. 1a and b) when it was initially used in a patient following excision of a maxillary aneurysmal bone cyst. The dynamics of osteoblastic activities in the implanted site has not yet been demonstrated; as such our current experiment stands out as being unique as it utilizes SPECT to demonstrate the osteoblastic activity around the integrating sandwich unit. Single photon emission computerized tomography (SPECT) provides an additional advancement to planar imaging and permits accurate quantitation that is common to most tomographic techniques by removing regions which are not of interest. The technique of SPECT is well



1a

1b

Fig. 1a: Dental panoramic tomogram showing the sandwich unit in place (see arrow) and figure 1b showing evidence of advanced osseogenesis a few months after the implantation of the sandwich unit.

established and has found successful clinical application for the study of many organ systems including the skeletal system (5–6). Prior to the new millennium, in their very classical study and subsequent publication in the year 2000, Khan *et al* adequately demonstrated the role of SPECT in the osseous integration process of dental implants (7). More recently three-dimensional computed tomography was utilized to document the progression of bone formation in a porcine mandibular distraction wound at various distraction rates and fixation times (8). It would have been interesting for the same documentation to be done utilizing the SPECT (a three-dimensional and dynamic imaging modality).

For the first time, the physiologic events following the implantation of the Ogunsalu sandwich technique is demonstrated using SPECT. The underlying principles of SPECT are well known and are common to most other tomographic imaging techniques. With the injection of a radiopharmaceutical agent (RPA) containing a single gamma-photon-emitting radionuclide such as technetium 99 m, it is possible to obtain a 3-dimensional representation of the distribution of radioactivity within an organ or an area of interest (AI) by using radiation detectors. This can be easily achieved with a rotating gamma camera which will detect the emitted radioactivity as the camera is rotated around the clinical area of interest. The acquired images and data are then processed by a computer which initially provides a cross-sectional (transaxial) representation of the distributed radioactivity. The transaxial data can be additionally used to reconstruct saggital and coronal images (6).

To confirm that the Ogunsalu sandwich technique integrates with surrounding bone after implantation, a preliminary experiment was done on *pig 1* in which the classical membrane/bone sandwich was implanted in the right and left mandibles, however, the bone substitute on the left sandwich was Bio-oss® and that in the right mandible was autogeneous bone. The additional reason for this preliminary experiment was to compare the quality and quantity of bone formed when xenograft is used as against when autograft is utilized.

The other five animal experiments were done similarly but rather than implanting the classical sandwich on the right jaw, the Bio-oss® particulate bone was covered *in situ* with interceed® membrane. This was to compare the quality and quantity of bone formed when the classical sandwich is used as to when the same particulate bone is just covered with interceed® membrane.

SUBJECTS AND METHODS

SPECT was performed in sequence on the mandible of six pigs at 8, 11, 13, 14, 17 and 24 weeks to determine the osteoblastic activities of the Ogunsalu sandwich bone regeneration unit after its implantation.

Experiment 1. Pig 1 had the sandwich bone regeneration unit implanted into a surgically created defect in both the right and left mandible under general anaesthesia, and SPECT was done at 14 weeks for comparative analysis and evaluation of the bone formed on the left side (the side which had xenograft as the bone substitute in the sandwich unit) with the right side (the side which had autogenous bone graft in the sandwich unit). At 14 weeks post-implantation, the pig was anaesthetized and an intravenous access was established into an ear vein, this was used to introduce 740 MBq (20mCi) technetium 99 methylene diphosphate. The pig was euthanized two and a half hours after injection and the mandible was removed from the carcass prior to SPECT imaging.

Experiment 2: Pigs 2–6 were different in that they had the classical sandwich unit with Bio-oss (xenograft) as the bone substitute implanted in the left mandible and the same amount of Bio-oss was placed in the surgical defect in the right side but was covered with interceed® resorbable membrane.

This experiment was performed in sequence and was done to compare the amount of bone formed when the side with the sandwich unit is compared with the side that utilized interceed® membrane overlay.

At 8, 11, 13, 17 and 24 weeks, the pigs were subsequently injected with technetium 99 methylene diphosphate in a similar manner as *pig 1*. Approximately two and a half hours after the injection of the radiopharmaceutical agent, all the pigs in experiments 1 and 2 were subjected to SPECT imaging. The tomographic images of the mandible with the region of interest were obtained with a Siemens Orbitar II rotating large field-off-view gamma camera (Siemens medical system Inc, Erlanger, Germany) equipped with a low energy collimeter (Fig. 2).

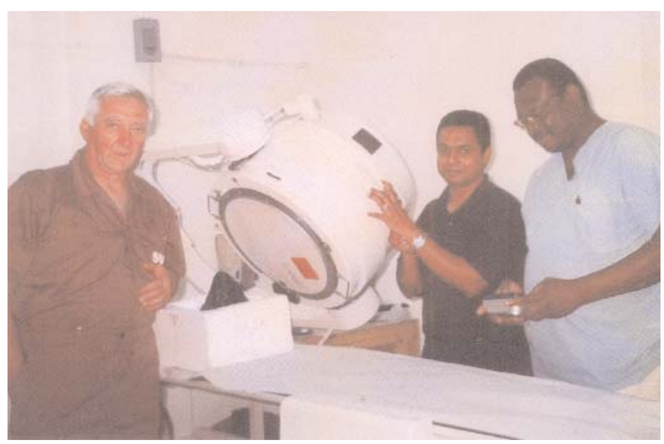


Fig. 2: Nuclear imaging team around the Siemen Orbitar II Gamma Camera.

For each mandible, 64 projection images (20s/images) were acquired at 180 degrees in a 128 by 128 matrix with a dedicated nuclear medicine computer (Siemens icon computer). The projection data were corrected for flood field nonuniformity and centre of rotation. The transverse reconstruction was then performed with a Shepp-Logan Hanning filter cut-off frequency of 0.4.

By utilizing the transverse slices, the activity in the site of implantation of the sandwich unit and the xenograft with interceed® overlay – the so called area of interest (AI) was observed, calculated and compared with a reference point within the jaw on each side. The average count (pixel) for each area of interest was compared.

RESULTS

Table 1 shows the comparative osteoblastic activity between the xenograft and autograft sandwich in *pig 1*. Table 2 compares the SPECT findings of the right side and the left side for *pigs 2–6*. Figure 3 shows the SPECT imaging for *pig 1* which had the classical sandwich implanted in both the right

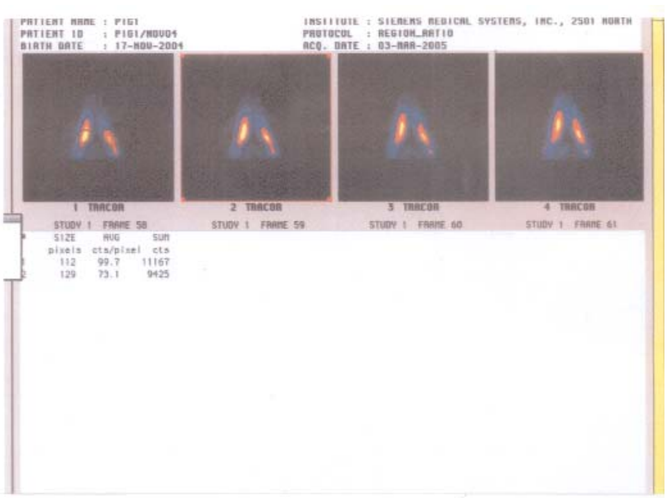
Table 1: Comparison of the osteoblastic activity between the xenograft and autograft sandwich unit

Site	Component of Sandwich Unit	Size Pixel	Avg Count	Sum
1. Right mandible	a. Membrane – Bio-Gide® b. Bone substitute – autograft	112	99.7	11 167
2. Left Mandible	a. Membrane – Bio-Gide® b. Bone substitute – xenograft (Bio-oss®	129	73.1	9425

Table 2: Comparative average count and activity ratio

Slides	Pig No. 2		Pig No. 3		Pig No. 4		Pig No. 5		Pig No. 6	
Mandibles	L	R	L	R	L	R	L	R	L	R
Time (Weeks)	8 Weeks		11 Weeks		13 Weeeks		17 Weeks		24 Weeks	
Component of Sandwich Unit Size of Pixel	SS	IS	SS	IS	SS	IS	SS	IS	SS	IS
Average Count	260	170	250	200	240	270	194	160	195	255
Activity Ratio	1.25		0.9		1.53		1.18		0.77	

SS = Sandwich Side
IS = Interceed Side



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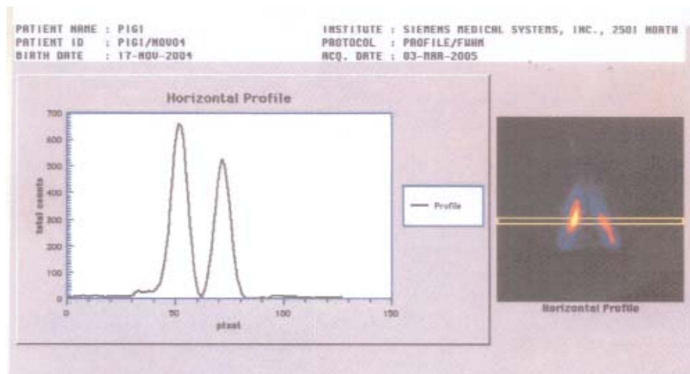
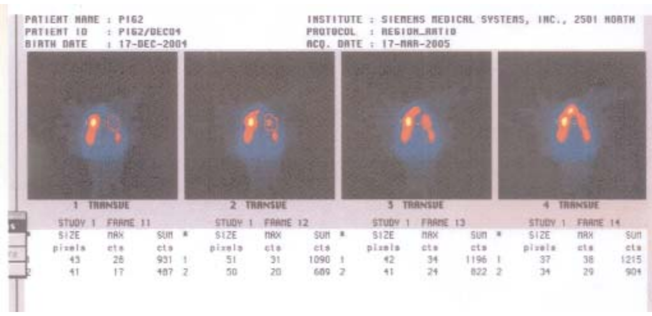
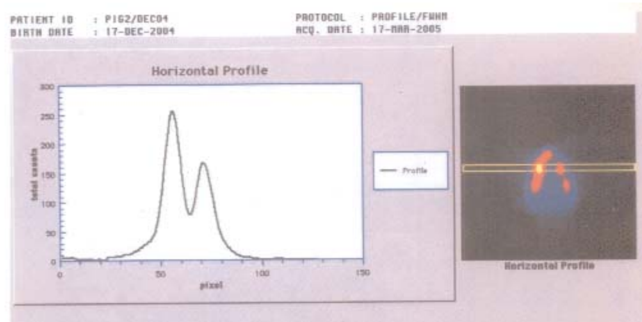


3b

Fig. 3a shows the osteoblastic activity in the mandible of pig 1at 14 weeks and Fig. 3b shows the activity curve and ratio for each side.

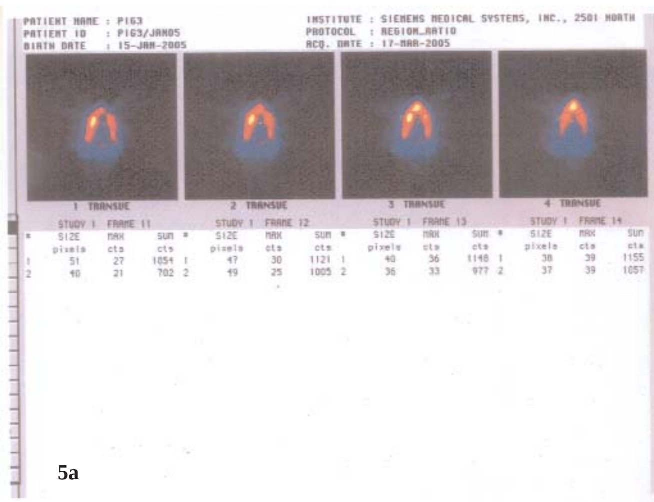


4a

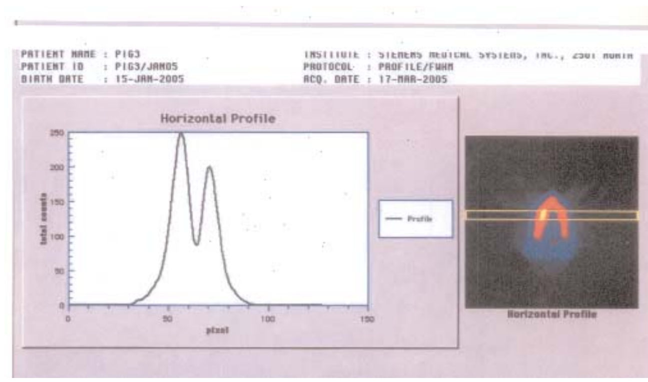


4b

Fig. 4a shows the osteoblastic activity in the mandible of pig 2 at eight weeks and Fig. 4b shows the activity curve and ratio for each side.



5a



5b

Fig. 5a shows the osteoblastic activity in the mandible of pig 3 at 11 weeks and Fig. 5b shows the activity curve and ratio for each side.

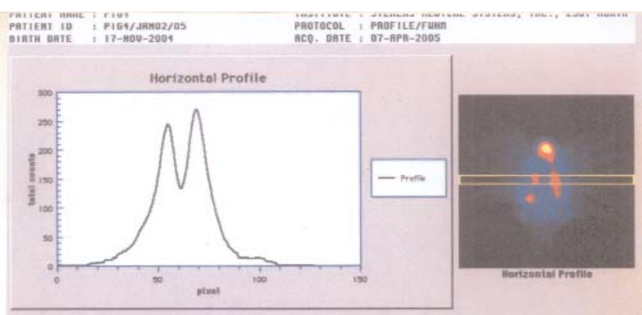


Fig. 6: Shows the osteoblastic activity and activity curve/ratio for each side of the mandible of pig 4 at 13 weeks.

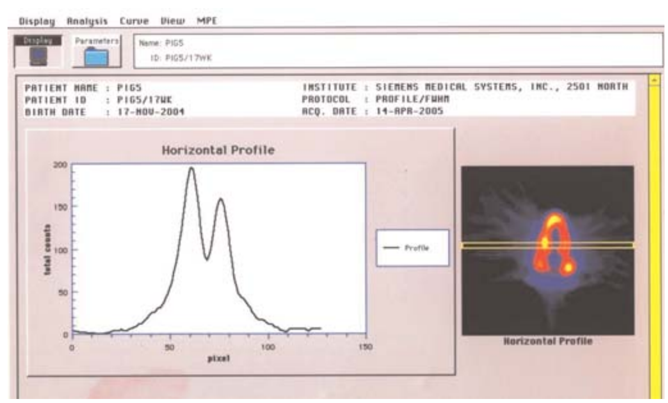


Fig. 7: Shows the activity curve and ratio for pig 5 at 17 weeks.

and left mandibular sites but differ in that the left sandwich had Bio-oss and the right one had the autogeneous bone graft.

The other five pigs had the classical sandwich with Bio-oss bone substitute implanted on the left side and Bio-oss with interceed® overlay on the right side. The SPECT images for the *pigs 2–6* are shown in Figs. 4–8 respectively.

1. Significant osteoblastic activities were observed in all the 12 mandibular sites, thus confirming that the Ogunsalu sandwich unit and the Bio-oss®/interceed® overlay side actually form bone that is vascularized from as early as 8 weeks. As such, osseo-integration with the adjacent bone will be present at this stage.
2. The total count for the sandwich unit side was more than the interceed side at the 8th and 11^{th} week, but

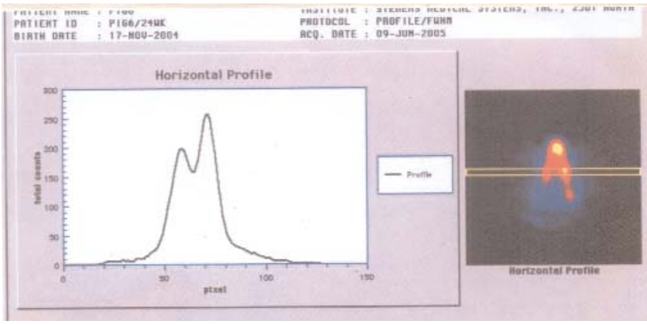


Fig. 8: Shows the activity curve and ratio for pig 6 at 24 weeks.

became less at the 13th week to overtake the interceed side at the 17th week (Fig. 9).

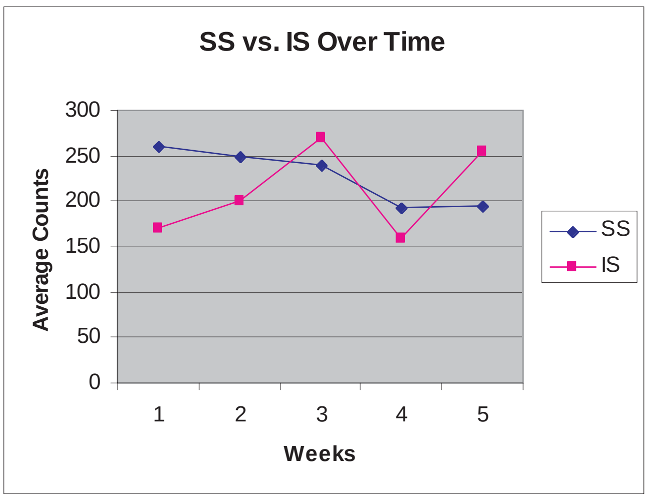


Fig. 9: Shows the total count of the interceed side against the sandwich side (demonstrating what we now call the overtaking phenomenon).

3. At the end of the 24th week, the interceed side had re-overtake the sandwich side (Fig. 9).
4. The relative activity which relates the two sites (sandwich side and the Bio-oss®/interceed® side showed a decrease from the 8th to 11th week and an increase from the 11th to 13th week with subsequent decrease from the 13th week to 15th week to 24 weeks (Fig. 10.)

   The statistical analysis for experiment II using the 2-independent group t-test revealed that the sandwich (SS) side was 16.8 greater than the interceed side(IS) on the overall (the average count for the SS was 227.8 (± 31.2) and that of the IS was 211.0 (± 49.5) with a difference of 16.8).

DISCUSSION

The Ogunsalu sandwich technique when first used in the year 2000 involved the use of Biogran® (Orthovita) – a synthetic bone substitute and Biogide® (Osteohealth) – a resorbable

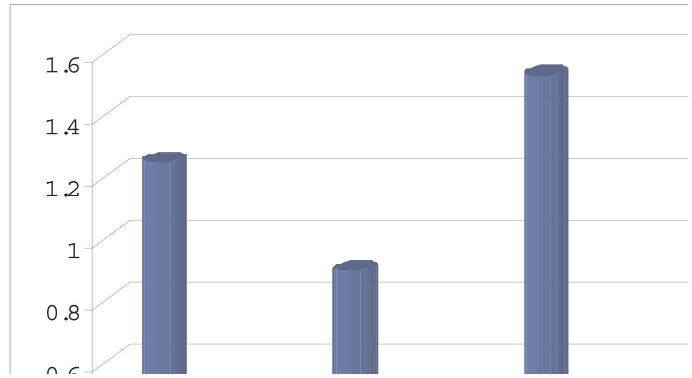


Fig. 10: Shows the relative activity curve comparing the sandwich side with the interceed side.

membrane as a unit to reconstruct the maxillary sinus floor, followed by simultaneous placement of Biogran® onlay for the reconstruction of the alveolar bone in a patient with aneurysmal bone cyst of the maxilla (1, 2). It was then suggested for use also in the reconstruction of the orbital floor, alveolar cleft and cleft palate and for reconstruction of the outer table of the frontal sinus following trauma. Subsequent to the original use, it has been successful in the management of oro-antral communications following exodontias (3) and dental implant therapy was eventually done for the same patient to replace the lost tooth in the area of the oro-antral communication (4). Recently at the otorhinolaryngology section of the national medical association conference in the United States of America where this work was presented in July 2005, it was suggested for use by a neurosurgeon for the management of CSF leaks.

From our clinical observation so far, we know that the sandwich unit integrates, however, the vascularity of the regenerated bone and its osteoblastic activity has not yet been investigated and proven until our recent animal experiment.

It is important to note that SPECT has allowed the physiological (dynamic) assessment and morphological assessment of bone regenerated by this novel technique. It demonstrated adequately that osteoblastic activity was present within the bone regenerated by this unit, and also vascularization (as osteoblast would not thrive in an avascular bone). Our experiment, which utilized SPECT, assisted in confirming that quantitatively and qualitatively the bone regenerated from autogeneous bone graft was the best (Table 1) as demonstrated by a relative activity ratio of 1:20.

Current clinical approaches to tissue replacement and reconstruction are to alleviate pain and restore mechanical stability and function by utilization of various bone substitutes. Such approaches have utilized autogenous grafts, allografts, platelet rich plasma, xenograft, synthetic material and a mixture of some of these materials. Of all these, the autogenous bone graft has been proven clinically and experimentally to be the gold standard. However, our method utilizing SPECT proved that autogenous bone is a better material when compared to xenograft in terms of quantity and quality of bone regenerated in addition to proving that the sandwich technique has osteoblastic activity, integrates and vascularizes. Biomaterials are utilized in a passive manner to facilitate the growth or regenerative capacity of existing tissue in the conductive stage of tissue regeneration. This is mostly utilized today in periodontology and implantology. Much credit goes to Nyam, Lindhe, Karring *et al* (10) who pioneered the successful use of the osteoconductive mechanism in providing a means for selective wound healing by supporting the in-growth of the periodontal supporting cells, while excluding gingival, epithelial and connective tissue cells from repopulating the sites, by using a barrier membrane.

The Ogunsalu sandwich bone-regeneration unit exclusively confines the bone graft within a resorbable barrier membrane in the desired area of bone regeneration, thus allowing osteoconductive, osteoinductive and osteogenic mechanism to take place without disturbance. SPECT as utilized in our initial evaluation of this unit and technique confirm that this bone-regeneration unit: (1) contains osteoblastic activity after implantation, (2) is vacularized and indeed it osseointegrates. Our comparison of the osteoblastic activity between xenograft and autograft utilizing SPECT confirm that autogeneous bone graft is the best substitute. Furthermore, the sequential drop and rise of osteoblastic activity in the sandwich side illustrates a new phenomenon, “the overtaking and the re-overtaking phenomenon” which is probably membrane size dependent.

With regards to the experiment II the difference of 16.8 between the sandwich side and the interceed side is not statistically significant.

Tissue engineering continues to impact on dentistry. In their review, Kaigler and Mooney (9) stressed that tissue engineering is a novel and exciting field that aims to recreate functional, healthy tissues and organs in order to replace diseased, dying or dead tissue. In their review article, they described the three major tissue engineering strategies: (a) conduction (b) induction (c) cell transplantation). They maintained that although these strategies are similar in their objectives, each maintained a unique component (9). The conductive approach makes use of a barrier membrane to exclude connective tissue cells that will interfere with the regenerative process while enabling the desired host cells to populate the regeneration site. The inductive approach uses a biodegradable polymer scaffold as a vehicle to deliver growth factors and genes to the host site. The growth factors or gene can be released at a controlled rate based on the breakdown of the polymer. The cell transportation strategy uses a similar vehicle for delivery in order to transplant cells and partial tissue to the host site.

The mechanism of bone regeneration and augmentation when the Ogunsalu sandwich technique is utilized is similar to what has been previously documented in terms of osseoinduction, osseogenesis and osseoconduction, except for the fact that such mechanism can be manipulated or varied depending on the type(s) of bone substitute that is placed within the sandwich unit. As such, the three different processes namely: osteogenesis, osteoinduction and osteoconduction (11–14) implicated in successful bone grafting can be represented at one site of bone regeneration, depending on the different combination of bone substitute placed in the sandwich unit.

The three primary types of bone graft material are autogeneous bone, allograft and alloplast, of which commercially available xenograft are generally considered a subgroup. The mechanism by which these graft materials regenerate bone normally depends on the origin and composition of the material (12, 15). Autogeneous bone, an organic material harvested from the patient, forms new bone by osteogenesis, osteoinduction and osteoconduction and whilst the allograft have osteoconductive and possibly osteoinductive properties, they are not osteogenic. Finally alloplast which consist of natural or synthetic material are typically only osteoinductive. As such, the result of this current animal experiment is valid as it shows that the side with autogeneous bone graft in the sandwich has more osteoblastic activity when compared with the side with xenograft as the bone substitute within sandwich unit.

In determining what type of graft material that one should use within the sandwich unit, the clinician must consider the characteristics of the bony defect to be restored. However, a further advantage of the sandwich technique is that a combination of graft materials can be utilized to obtain a desired quality and quantity of bone.

CONCLUSION

The Ogunsalu sandwich bone-regeneration unit has been successfully evaluated with SPECT to demonstrate osteoblastic activity. Furthermore, it regenerates vascularized bone which osseointegrates with the surrounding bone. It can be called a double GTR – technique, and in our experiment no statistically significant difference existed between this technique and the side which utilized the interceed overlay (a single GTR – technique).

Finally, we conclude, that by utilizing SPECT, autograft is a better bone-graft when compared with xenograft, however one limitation of autograft is that humans do not have sufficient store of autogeneous bone for transportation. Overall, the osteoblastic activity and bone regenerated in the sandwich side was more superior to the interceed® side probably because of our new concept of double guided tissue regeneration.

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

Site	Component of Sandwich Unit	Size Pixel	Avg Count	Sum
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Table 2: Comparative average count and activity ratio

Slides	**Pig No. 2**		**Pig No. 3**		**Pig No. 4**		**Pig No. 5**		**Pig No. 6**	
Mandibles	L	R	L	R	L	R	L	R	L	R
Time (Weeks)	8 Weeks		11 Weeks		13 Weeeks		17 Weeks		24 Weeks	
Component of Sandwich Unit Size of Pixel	SS	IS	SS	IS	SS	IS	SS	IS	SS	IS
Average Count	260	170	250	200	240	270	194	160	195	255
Activity Ratio	1.25		0.9		1.53		1.18		0.77	

SS = Sandwich Side
IS = Interceed Side