# FINAL REPORT

# IN VIVO STUDY OF A LOCALLY-MANUFACTURED HYDROXYAPATITE-BASED MATERIAL AS BONE REPLACEMENT MATERIAL

Dr. Noor Hayati Abdul Razak
Prof. Ab. Rani Samsudin
Nor Shamsuria Omar

Account No.: 304/PPSG/6131208
USM Short Term

UNIVERSITI SAINS MALAYSIA

**PEJABAT PENGURUSAN & KREATIVITI PENYELIDIKAN**
*RESEARCH CREATIVITY AND MANAGEMENT OFFICE [RCMO]*

# LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA *PENDEK*
## *FINAL REPORT OF SHORT TERM RESEARCH PROJECTS*

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1) **Nama Ketua Penyelidik** :
*Name of Research Leader :*

| Ketua Penyelidik<br>*Research Leader* | PTJ<br>*School/Centre* |
|---|---|
| Dr. Noor Hayati Abdul Razak | School of Dental Sciences, USM |

**Nama Penyelidik Bersama**
*(Jika berkaitan)* :
*Name/s of Co-Researcher/s*
*(if applicable)*

| Penyelidik Bersama<br>*Co-Researcher* | PTJ<br>*School/Centre* |
|---|---|
| Prof. Ab. Rani Samsudin | School of Dental Sciences, USM |
| Pn. Nor Shamsuria Omar | School of Dental Sciences, USM |
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2) **Tajuk Projek** : .....
*Title of Project:*
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In Vivo Study of Locally Manufactured Hydroxyapatite Based Material As Bone Replacement Material

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3) **Abstrak untuk penyelidikan anda**

(Perlu disediakan di antara 100 – 200 perkataan di dalam **Bahasa Malaysia dan Bahasa Inggeris**. Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & luar).

*Abstract of Research*

*(Must be prepared in 100 – 200 words in Bahasa Malaysia as well as in English. This abstract will later be included in the Annual Report of the Research and Innovation Section as a means of presenting the project findings of the researcher/s to the university and the outside community)*


## ABSTRACT

The objective of the study is to evaluate the osteoconductive, osteoinductive and biocompatibility properties of a locally produced dense hydroxyapatite (HA) as bone replacement material in mandibles. The study was carried out on 8 New Zealand White rabbits. Defects were created in the mandible of a rabbit model whereby the right side was implanted with HA while the left side was left empty to act as a control. The dense HA blocks with the pore size of < 5μm promotes bone ingrowth as early as 4 weeks when viewed by the topographic method. Both the implant and control sites were evaluated clinically and histologically at 4, 12, 20, 22 weeks interval. Enhancement of osteoconduction was evident by the presence of abundant capillaries, perivascular tissue and osteoprogenitor cells of the host accounting for the new bone filling the gap and remodelling taking place. This study demonstrated that the dense HA exhibits excellent biocompatibility as noted by the complete absence of reactive cells. HA also promotes osteoconduction. It appears that this locally manufactured HA has the potential to be a valuable replacement material for maxillofacial reconstructive surgery and orthopaedic surgery.

## ABSTRAK

Tujuan penyelidikan ini dijalankan adalah untuk mengkaji ciri-ciri osteokonduktif dan osteoinduksif dan juga penyesuaian biologi ke atas hidroksiapatit (HA) mampat buatan tempatan sebagai bahan pengganti tulang di dalam rahang bawah. Kajian ini dilakukan ke atas 8 ekor arnab New Zealand White. Kecacatan dibentuk pada rahang bawah di mana bahagian kanan rahang di implan dengan HA dan bahagian kiri rahang dibiarkan tanpa rawatan dan bertindak sebagai kawalan. Blok HA yang mampat dengan saiz porosnya < 5μm didapati bersifat merangsang pertumbuhan tulang seawal 4 minggu seperti yang dapat dilihat dalam kajian ini menggunakan kaedah topografik. Selepas pembedahan, kedua-dua bahagian, implan dan kawalan dinilai secara klinikal dan histologi pada minggu ke 4, 12, 20 dan 22. Rangsangan osteokonduktif telah dibuktikan dengan kehadiran kapilari, tisu perivaskular dan sel osteoprogenitor yang bertambah pada hos. Ini kerana terdapat tulang baru yang memenuhi ruang tulang rahang tersebut dan pemodelan semula berlaku. Kajian ini menunjukkan bahawa blok HA yang mampat adalah bersesuaian secara biologi seperti yang dilihat apabila tiada sel reaktif yang hadir. HA juga merangsang osteokonduktif. Material ini adalah salah satu bahan gantian yang berharga untuk pembedahan maksilofasial dan rekonstruktif dan juga dalam bidang ortopedik pada masa kini.

4) Sila sediakan Laporan teknikal lengkap yang menerangkan keseluruhan projek ini. [Sila gunakan kertas berasingan]
*Kindly prepare a comprehensive technical report explaining the project (Prepare report separately as attachment)*

Senaraikan Kata Kunci yang boleh menggambarkan penyelidikan anda :
*List a glosssary that explains or reflects your research:*

| Bahasa Malaysia | Bahasa Inggeris |
|---|---|
| Bahan Gantian Tulang | Bone replacement material |
| ..... | ..... |
| osteokonduktif | osteoconductive |
| ..... | ..... |
| osteoinduksif | osteoinductive |
| ..... | ..... |

5) **Output Dan Faedah Projek**
*Output and Benefits of Project*

(a) * Penerbitan *(termasuk laporan/kertas seminar)*
*Publications (including reports/seminar papers)*
*(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).*
*(Kindly state each type, title, author/editor, publication year and journal/s containing publication)*

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Published in The Medical Journal of Malaysia; indexed in PubMed (attached)
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(b) **Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten atau impak kepada dasar dan masyakarat.**
*Other benefits such as product development, product commercialisation/patent registration or impact on source and society*

This study shows that this locally manufactured HA (Prof. Radzali Othman, School of Materials & Mineral Resources Engineering, USM) has the potential to be a valuable replacement material for maxillofacial reconstructive surgery and orthopaedic surgery.

This study also is part of the development of REKAGRAF, Reformulated Calcium Phosphate (SyntheticBone) for Human Tissue Transplantation, that won MOSTE 2003 Gold Award.

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* Sila berikan salinan
* *Kindly provide copies*

(c) **Latihan Gunatenaga Manusia**
*Training in Human Resources*

i) Pelajar Siswazah : .....n/a.....
*Postgraduate students:*
*(perincikan nama, ijazah dan status)*
*(Provide names, degrees and status)*

*ii)* Pelajar Prasiswazah *:* .....*n/a*.....
*Undergraduate students:*
*(Nyatakan bilangan)*
*(Provide number)*

iii) Lain-Lain : .....n/a.....
*Others:*

6. **Peralatan Yang Telah Dibeli :**
*Equipment that has been purchased:*

.....n/a.....

7. **KOMEN JAWATANKUASA PENYELIDIKAN PUSAT PENGAJIAN**
*Comments of the Research Committees of Schools/Centres*

Memenuhi syarat-syarat Geran USM
Dimajukan kepada RCMO melalui
JK Pelantar Penyelidikan Klinikal

13/02/07.

TANDATANGAN PENGERUSI
JAWATANKUASA PENYELIDIKAN PUSAT PENGAJIAN
*Signature of Chairman*
*[Research Committee of School/Centre]*

13/02/07.

TARIKH
*Date*

JABATAN BENDAHARI
UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI JANGKA PENDEK
PUSAT PENGAJIAN SAINS PERUBATAN
(304/PPSG/6131208)

PENYELIDIK : DR.NOOR HAYATI BT.A.RAZAK

NAMA PROJEK : "IN-VIVO STUDY OF A LOCALLY -MANUFACTURED HYDROXYPATITE-BASED MATERIAL AS BONE REPLACEMENT MATERIAL

PENYATA PERBELANJAAN BAGI TEMPOH BERAKHIR PADA 31 DISEMBER 2003

| PECAHAN KEPALA | PERUNTUKAN (RM) | PERBELANJAAN 2002 | BAYARAN 2003 | TANGGONGAN | PERBELANJAAN 2003 | JUMLAH PERBELANJAAN | BAKI KESELURUHAN |
|---|---|---|---|---|---|---|---|
| 11000 GAJI DAN UPAHAN | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 14000 ELAUN LEBIH MASA | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 15000 BONUS | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 21000 PERJALANAN & PENGANGKUTAN ORANG | 870.00 | 2,319.00 | 2,269.00 | 0.00 | 2,269.00 | 4,588.00 | (3,718.00) |
| 22000 PENGANGKUTAN BARANG-BARANG | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 23000 PERHUBUNGAN DAN UTILITY | 300.00 | 0.00 | 0.00 | 0 00 | 0.00 | 0.00 | 300.00 |
| 24000 SEWAAN | 0.00 | 0.00 | 0.00 | 0 00 | 0.00 | 0.00 | 0.00 |
| 25000 BAHAN-BAHAN MAKANAN & MINUMAN | 0.00 | 0.00 | 40.00 | 0.00 | 40.00 | 40.00 | (40.00) |
| 26000 BEKALAN BAHAN-BAHAN MENTAH & BAHAN PEMBAIKAN | 0 00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 27000 BEKALAN BAHAN-BAHAN LAIN | 13,115.00 | 5,102.80 | 5,823.80 | 0 00 | 5,823.80 | 10,926.60 | 2,188.40 |
| 28000 PENYELENGARAAN & PEMBAIKAN KECIL YANG DIBELI | 0.00 | 0.00 | 0.00 | 0 00 | 0.00 | 0.00 | 0.00 |
| 29000 PERKHIDMATAN IKTISAS & LAIN-LAIN PERKHIDMATAN & HOSPITALITI | 500.00 | 1,104.10 | 781.40 | 0.00 | 781.40 | 1,885.50 | (1,385.50) |
| 35000 LAIN-LAIN HARTA MODAL | 3,250.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3,250.00 |
| JUMLAH BESAR | 18,035 00 | 8,525.90 | 8,914.20 | 0 00 | 8,914.20 | 17,440.10 | 594.90 |

# An in Vivo Study of a Locally-Manufactured Hydroxyapatite-based Material as Bone Replacement Material

**N H Abdul Razak, K A AL-Salihi, A R Samsudin**

School of Dental Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

**Summary**

Defects were created in the mandible of a rabbit model whereby the right side was implanted with hydroxyapatite (HA) while the left side was left empty to act as control. Both the implant and control sites were evaluated clinically and histologically at 4, 12, 20, 22 weeks. Decalcified sections were studied under confocal laser scanning microscope. No reactive cells were evident microscopically in all sections. There was bone ingrowth as early as 4 weeks when viewed by the topographic method. Enhancement of osteoconduction was evident by the presence of abundant capillaries, perivascular tissue and osteoprogenitor cells of the host. At 22 weeks, the implanted defect showed mature bone formation filling almost the whole field. This study demonstrated that the dense HA exhibits excellent biocompatibility as noted by the complete absence of reactive cells. It also promotes osteoconduction.

## Introduction

Synthetic HA has excellent biocompatibility, but it has limited application due to its low toughness and flexural strength. In order to improve the mechanical properties (impact resistance, and tensile strength), metals and other elements were added to HA. The properties of the powder precursors have been studied by controlling important parameters such as particle size and shape, particle distribution and agglomeration[1]. The tissue response to different materials will vary with the chemical composition and micro-and macro-structure of the synthetic material. Composites formed by HA ceramic in combination with zirconia have been proven not to produce any local or systemic adverse reactions or any cytotoxic effects in various in vivo studies[2]. The objective of this study is to evaluate the biocompatibility, osteoconductive and osteoinductive properties of a locally produced dense value-added HA in a rabbit model.

## Materials and Methods

The test material was a value- added dense HA in the form of blocks measuring 6mm x 6mm x 6mm prepared by the School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia. The experimental models were eight New Zealand White rabbits. Defects were created in the mandible whereby the right side was implanted with HA while the left side was left empty to act as control. Both the implant and control sites were evaluated macroscopically and histologically at 4, 12, 20, 22 weeks interval. Decalcified sections were studied under confocal laser scanning microscope.

## Results

There was no incidence of extrusion of the implanted material throughout the study period in all operated sites, neither was there superficial nor deep infection. Macroscopic examination showed that at 4 weeks the HA implant appeared fixed to the host bone edges. By 12 weeks, the margins of the HA implant appeared resorbed in some areas. By 22 weeks, the HA block was not visible. Bone formation and maturation in the implant site was ahead of the control site at all the time intervals of 12, 20 and 22 weeks. Bone deposition was found at the bone implant interface with the earlier and

less mature stages of bone development being found towards the centre of the implant. At 22 weeks, the implanted defect showed mature bone formation filling almost the whole field.

## Discussion

Chemical composition, physical form, and appropriate application are good starting points when evaluating any new biomaterial for potential use[3]. The material under investigation is a locally produced dense HA zirconium ceramic. The present study is therefore a preliminary study into the biocompatibility and the ability of this material to induce bone formation. The study demonstrated that the dense HA blocks exhibit excellent biocompatibility comparable to the published results. Enhancement of osteoconduction in the implant group was evident by the presence of abundant capillaries, perivascular tissue and osteoprogenitor cells of the host accounting for the new bone filling the gap and remodelling taking place. Bone ingrowth into the implant was evident as early as 4 weeks, when the specimen was viewed by topography. The pattern of new bone growth beginning with an ingrowth of cellular loose connective tissue, which is replaced later by a dense connective tissue and matured bone, indicates the osteoconductive property of the implant material. However, it is difficult to confirm osteoinduction, which would be better studied in undecalcified sections. Some researchers suggest that a more desirable bioresorbable implant would be one that would allow for initial permeation and maturation of bone, followed then by resorption and replacement of the implant on the assumption that this type of pattern would allow for increased strength and/or stability at the implant site during the longer healing period[4]. The material in this study showed similar characteristics and this would probably indicate its use in load-bearing clinical situations. From this preliminary in vivo histological study, it appears that this locally manufactured dense HA block has the potential to be a valuable replacement material.

## References


1. Muralithran G, Ramesh S. The effects of sintering temperature on the properties of hydroxyapatite. Ceramic Int 2000; 26: 221-30.
2. Piconi C, Maccauro G. Zirconia as a ceramic biomaterial. Biomaterials 1999; 20: 1- 25.
3. Costantino PD, Friedman CD, Lane A. Synthetic biomaterials in facial plastic and reconstructive surgery. Facial Plastic Surgery 1993; 9(1): 1-15.
4. Jarcho M. Calcium phosphate ceramics as hard tissue prosthetics. Clin Orthop Rel Res 1981; 57: 259- 76.




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Summary

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Links

**An in vivo study of a locally-manufactured hydroxyapatite-based material as bone replacement material.**

**Abdul Razak NH**, **Al-Salihi KA**, **Samsudin AR**.

School of Dental Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.

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PMID: 15468847 [PubMed - indexed for MEDLINE]

## ABSTRACT

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## ABSTRAK

Tujuan penyelidikan ini dijalankan adalah untuk mengkaji ciri-ciri osteokonduktif dan osteoinduksif dan juga penyesuaian biologi ke atas hidroksiapatit (HA) mampat buatan tempatan sebagai bahan pengganti tulang di dalam rahang bawah. Kajian ini dilakukan ke atas 8 ekor arnab New Zealand White. Kecacatan dibentuk pada rahang bawah di mana bahagian kanan rahang di implan dengan HA dan bahagian kiri rahang dibiarkan tanpa rawatan dan bertindak sebagai kawalan. Blok HA yang mampat dengan saiz porosnya < 5μm didapati bersifat merangsang pertumbuhan tulang seawal 4 minggu seperti yang dapat dilihat dalam kajian ini menggunakan kaedah topografik. Selepas pembedahan, kedua-dua bahagian, implan dan kawalan dinilai secara klinikal dan histologi pada minggu ke 4, 12, 20 dan 22. Rangsangan osteokonduktif telah dibuktikan dengan kehadiran kapilari, tisu perivaskular dan sel osteoprogenitor yang bertambah pada hos. Ini kerana terdapat tulang baru yang memenuhi ruang tulang rahang tersebut dan pemodelan semula berlaku. Kajian ini menunjukkan bahawa blok HA yang mampat adalah bersesuaian secara biologi seperti yang dilihat apabila tiada sel reaktif yang hadir. HA juga merangsang osteokonduktif. Material ini adalah salah satu bahan gantian yang berharga untuk pembedahan maksilofasial dan rekonstruktif dan juga dalam bidang ortopedik pada masa kini.

## INTRODUCTION

The problem of reconstructing a bone defect in the maxillofacial region resulting from malformation, disuse atrophy / resorption, infection, trauma or an oncological resection, presents an extremely challenging task (Redondo et al, 1995). An autogenous bone graft is the best solution in most instances, but it involves a second operation on the donor site with additional surgical morbidity. The possibility of using osseous replacement materials with similar end results is therefore very much welcome. For the past two decades a barrage of new biomaterials have been developed. Many of these materials have been developed over a relatively short period of time to meet the increasing demand by surgeons, thus producing materials that may not meet the requirement of an ideal biomaterial.

Bone substitute materials may be classified as either synthetic or of biological origin. Synthetic agents include certain metal alloys, ceramics and synthetic polymers. Materials of biological origin may be homografts or xenografts. The constituents may be bone, bone derivatives (collagen, bone morphogenic protein, and other bone matrix proteins), and natural polymers (collagen). Despite reassurance on the lack of allergenic potential or transmission of infection of the homografts and xenografts, there is nonetheless caution on their usage (Costantino et al, 1993). Synthetic materials / allografts, on the other hand, do not pose such problems, but their bone inductive potential may be less potent. Synthetic materials are either biodegradable or non-biodegradable, whereas compounds of biological origin will biodegrade completely in the body (Hollinger and Battistone, 1986). The tissue response to different materials varies with the chemical composition and micro- and macro-structures of the synthetic material. (McGrath, 1989).

Ceramics contain metallic and non–metallic elements. They possess high stability and resistance to chemical alteration. Generally, they are harder than metals or polymers, high in compressive strength, are poor electrical and thermal conductors and are translucent. Ceramics for bone replacement is one of the most active areas of biomaterial research today. Like an autologous cancellous bone graft, a resorbable ceramic serves as a scaffold for new bone growth and is eventually resorbed by living tissue (McGrath 1989, Yamaguchi et al 1995).

The advantage of a resorbable material is that there will be no long-term instability or compatibility problem. Another advantage is that it is an osteoinductive material and may therefore promote bone regeneration.

The disadvantage is strength degradation during the remodelling process, which may cause mechanical implant failure and the unknown consequences of releasing high concentration of ion from these reactive substances (Muralithran and Ramesh, 2000).

Hydroxyapatite (HA) forms the principal mineral component of bone and comprises 60% to 70% of the calcified skeleton. Its chemical composition is $Ca_{10}(PO_4)_6(OH)_2$, and is sometimes referred to as calcium phosphate tribasic. Synthetic HA was first prepared in 1951 and was studied by Ray and Ward as implants in surgically created defects in the long bones and iliac wings of dogs and the skull of cats and monkeys. Since then, many other studies have been done leading to the present understanding of this biomaterial (Costantino et al, 1993).

Synthetic hydroxyapatite is widely used in practical application as a bone replacement material. It has received considerable attention over the past two decades as an implant material due to its excellent biocompatibility. All forms of HA have excellent biocompatibility and when placed in contact with viable bone, result in osteoconduction and osseointegration, but there is no evidence that it is osteogenic. HA does not cause a chronic inflammatory response, toxic reactions, or a foreign body giant cell reaction (Costantino et al, 1993). It encourages bonding between body tissues and implant surface (Muralithran and Ramesh, 1999).

An explanation of the basic biological profile of these implant materials lies in their chemical nature: all having the same ions and similar ratio of calcium to phosphate as that of the natural bone and teeth. Because of this, these materials, when implanted in bone, are capable of participating in calcium phosphate solid-solution equilibrium at their surface. The requisite calcium and phosphate ions needed to establish these equilibriums may be derived from the implant or surrounding bone, or both (Jarcho, 1981). It is therefore an ideal candidate for clinical application either in the form of fully dense sintered material or as a coating material on a bioinert metallic implant.

Although it would seem that the chemical composition of the implant would be of primary importance, its physical form is equally critical in determining biocompatibility. In fact, an implant with an excellent biocompatibility on a chemical level can fail just because its physical form is not appropriate. Thus, chemical composition, physical form and appropriate application are good starting points to focus on when evaluating any new biomaterial for potential use (Costantino et al, 1993).

Although hydroxyapatite is a promising implant material, the greatest stumbling block to its wider application and utilisation is the brittleness of the material (as in other ceramic materials in general) and its low strength for load-bearing applications (Hollinger and Battistone, 1986). Its use under load-bearing application has been restricted by the low toughness and low flexural strength of the ceramic body (Muralithran and Ramesh, 2000).

Dense, porous or particulate forms have been developed. The particulate hydroxyapatite lacks form and cohesive strength; therefore it tends to dislodge and migrate under externally applied forces during the healing period. Attempts have been made to overcome this problem by combining the hydroxyapatite particles with a collagen fibre, gelatine matrix in block form or with a fibrin (Bakos et al, 1999).

Ceramic HA in blocks, is available in two forms: dense or porous. The selection (by the researchers involved in the production of the material) of nonporous rather than porous forms of HA for the present applications, was based on a critical review of animal and clinical studies reported during a decade of experience. The porous forms have interconnecting micropores of 100-300μm. The porous microarchitecture of hydroxyapatite favours cellular migration, revascularisation and bone deposition in its interior (osteoconduction). The deposition of bone into the implant promotes mechanical fixation with the natural bone (Redondo et al, 1995). However it is fragile, fractures easily and is difficult to contour. Porous HA implants, with compressive strengths of about 1000psi, are weaker than dense HA. The prolonged time required for bone growth into porous HA blocks may also limit its use in clinical situations, whereby stability must be achieved within a two-month period (Kent et al, 1986). It is also recognised that porous HA has a greater potential to become

infected than dense HA as a result of the wicking effect of the interconnecting pores (Kent et al, 1986). Dense HA implants with compressive strengths greater than 25,000 psi, have served as permanent implants, showing no tendency to bioresorb even after prolonged periods of implantation.

In order to improve the mechanical properties (impact resistance, and tensile strength) of sintered hydroxyapatite, the properties of the powder precursors have been studied by controlling important parameters such as particle size and shape, particle distribution and agglomeration (Muralithran and Ramesh, 2000).

Zircon has been known as a gem since ancient times. The name of the metal, zirconium originated from two Persian words Zar (Gold) and Gun (Colour). zirconia, the metal dioxide (ZrO2), was identified in 1789 by a German chemist Martin Heinrich Klaproth. Low quality zirconia is used as an abrasive in huge quantities. Tough, wear resistant, refractory zirconia ceramics are used to manufacture parts operating in aggressive environments. Good chemical and dimensional stability, mechanical strength and toughness were the origin of interest in using zirconia as a ceramic biomaterial (Piconi and Maccauro, 1997).

Partially stabilised zirconia is a new ceramic material with unique properties. It has high fracture toughness, is highly radiopaque and easy to cut or prepare for a superstructure. The ivory colour of zirconia ceramic is also favourable due to its similarity to the colour of the natural tooth. Ichikawa (1992) had quoted that Hulbert et al reported zirconia ceramic as showing excellent tissue compatibility. However, mechanical properties in vivo may be changed longitudinally because of the degradation of zirconia ceramic at 200°C in air and 60°C in water. Thus, although zirconia ceramic is recognised to be strong and tough, there are concerns that it might show deterioration with time due to possible phase transformation particularly in wet conditions. Cubic-phase of zirconia is stable but brittle. Stress-induced transformation of the tetragonal phase to the monoclinic has been shown to increase the fracture toughness. During this transformation, energy is liberated, which is absorbed by the phase-transforming zirconia. This phenomenon is known as stress-induced phase transformation, and through this phase it had been shown to have relatively high mechanical strength (Shimizu et al, 1993).

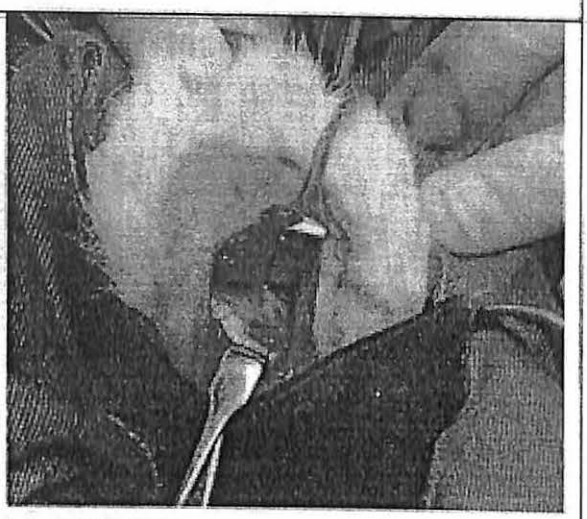
Zirconia ceramics have better mechanical properties than other ceramic biomaterials relating to its fine grained, metastable microstructure. Tetragonal Zirconia Polycrystals (TZP) or Zirconia-yttria ceramics are widely used by manufacturers for total hip replacement (Cales et al, 1994). At present, improvement of the performances of TZP for total hip replacement is ongoing and new applications in dentistry are also emerging.

Composites formed by hydroxyapatite ceramic in combination with zirconia have been proven not to produce any local or systemic adverse reactions or any cytotoxic effects in various in vivo studies (Piconi and Maccauro, 1999). This material showed no decrease in strength after ageing up to 1 year, which is in agreement with the study done by Shimizu et al in 1993.

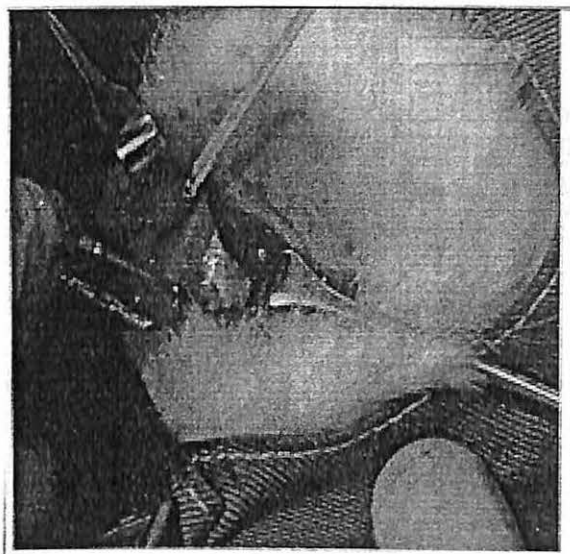
The material used in this study is an advanced material created from the admixing of zirconia and other additional components to a hydroxyapatite base. The HA is hot-pressed and then sintered at a temperature of 1300°C. This is to increase the toughness and strength of hydroxyapatite ceramic.

**Figure 1: Surgical procedures**

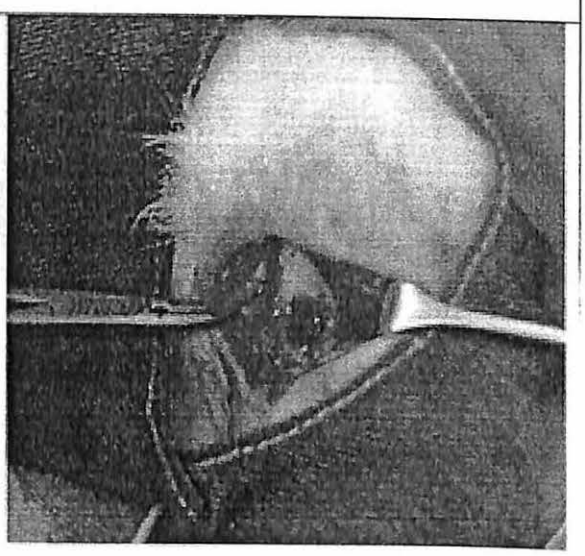
(a) Surgical instruments



(b) Mandible exposed



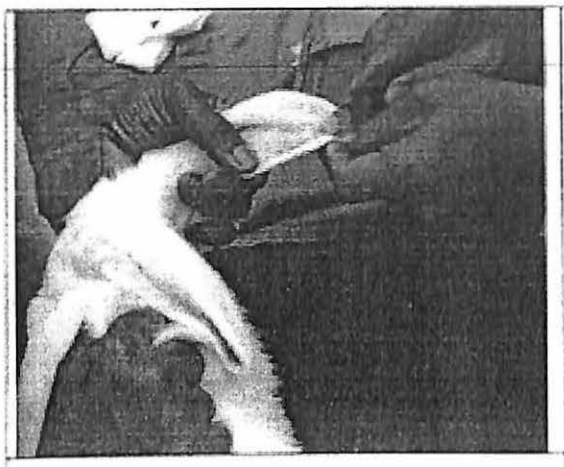
(c) Cutting a defect on buccal cortical bone



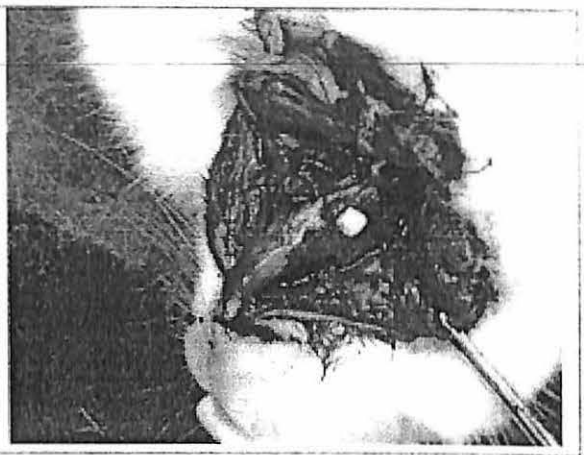
(d) Dimension of defect

(e) HA block in the defect

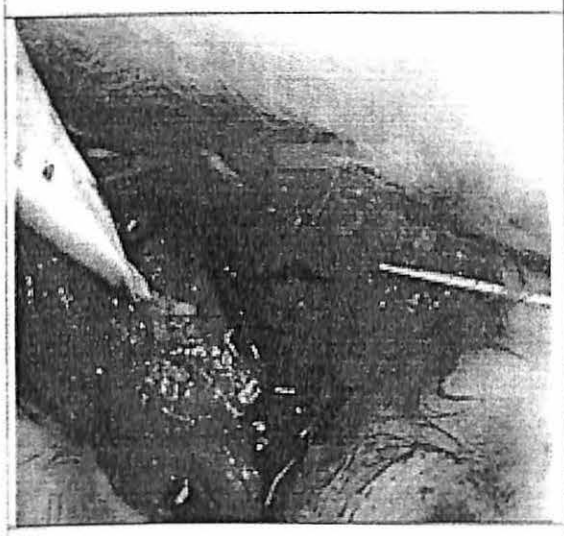
(f) Primary closure



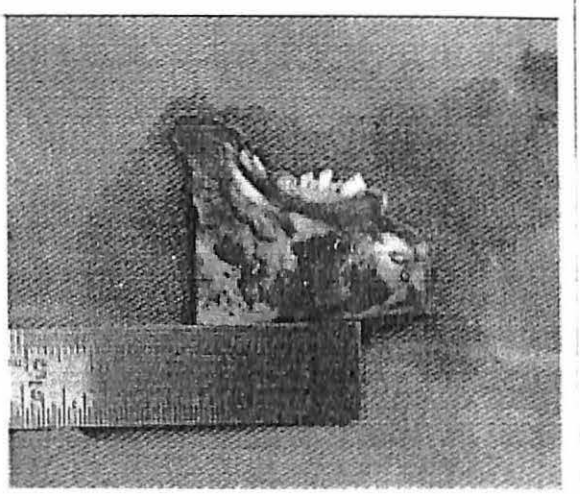
(g) Euthanasia with intra-venous Pentobarbitone



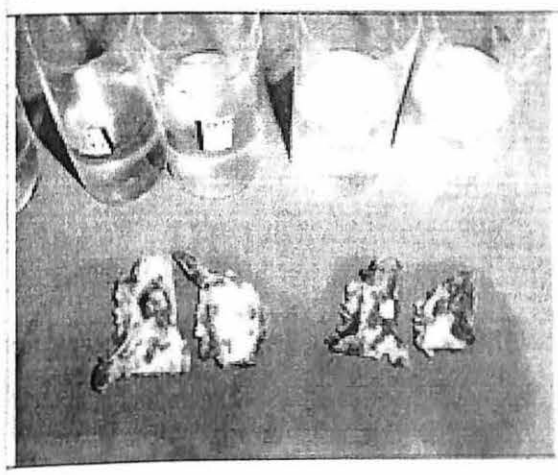
(h) HA block at 12 weeks



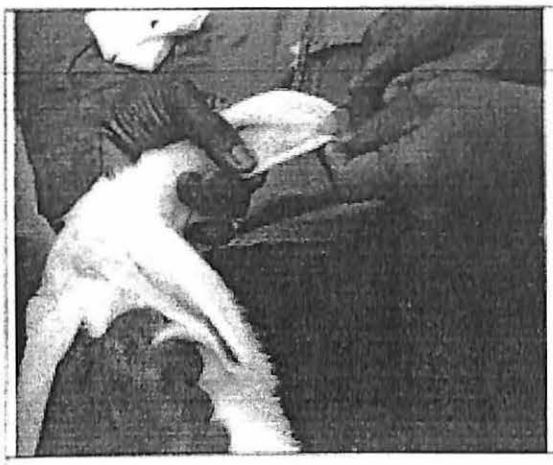
(i) site of HA block at 20 weeks



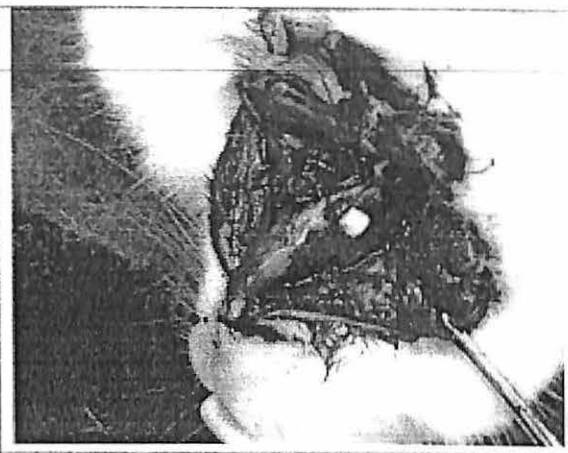
(j) Harvested specimen



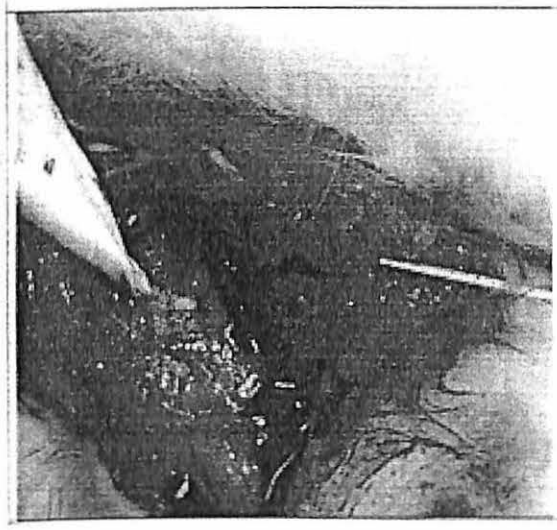
(k) Harvested specimen to be kept in 10% Formalin Saline



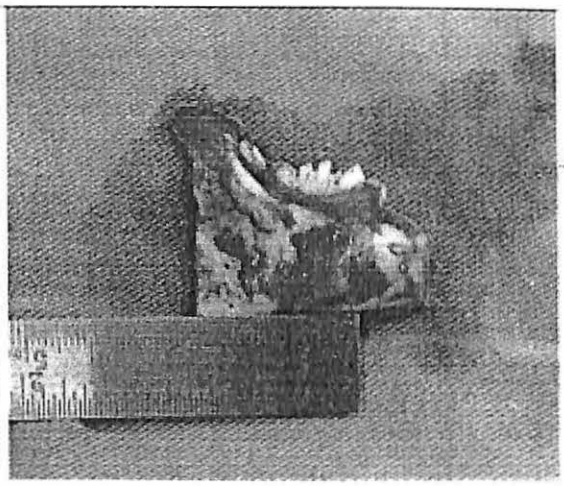
(g) Euthanasia with intra-venous Pentobarbitone



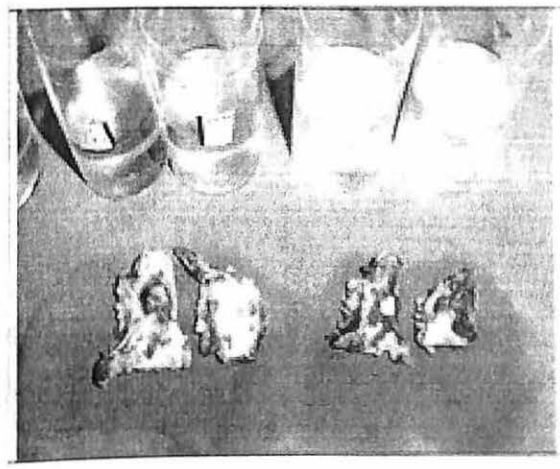
(h) HA block at 12 weeks



(i) site of HA block at 20 weeks



(j) Harvested specimen



(k) Harvested specimen to be kept in 10% Formalin Saline

post-operative infection, an intramuscular injection of Amoxycillin at a dose of 100mg in 0.5ml ampoules was given for 4 days.

Two rabbits at each time, were sacrificed at 4, 12, 20, 22 weeks following implantation using a lethal dose of Pentobarbitone intramuscularly. The mandibular bone at both the operative sites were cut using a saw and removed.

**Histological preparation**

The harvested right and left mandibular sections were fixed in 10% formalin in saline. The sections were then decalcified with 5% nitric acid followed by the process of grossing where the appropriate sections were selected and cut at a thickness of 3mm buccolingually and were processed and embedded in paraffin wax independently. The tissue blocks were sectioned at a thickness of 4μm using the microtome and mounted on slides. The slides were then stained with hematoxylin and eosin.

For histological analysis, two slides from each side of the mandible, were examined. The slides were viewed with a confocal laser-scanning microscope. The images from these sections were transferred from the microscope to a computer screen.

## RESULTS

Neither superficial nor deep infection was observed macroscopically in these experimental animals. There was also no incidence of extrusion of the implanted material throughout the study period.

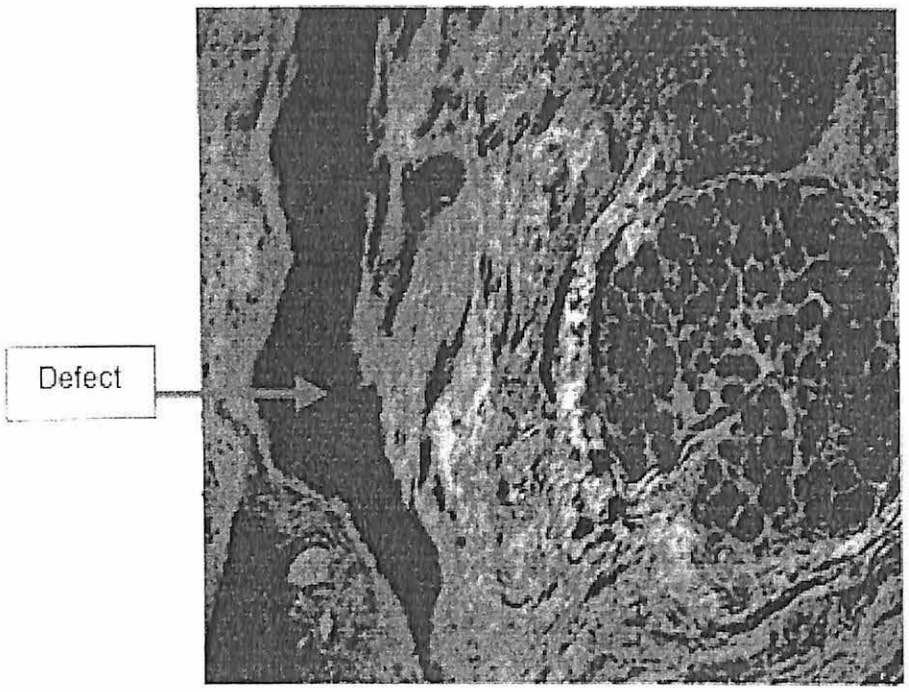
Clinically, at 4 weeks post-implantation, the defect in the control did not appear to have undergone any changes macroscopically. The HA implant in the right mandible appeared fixed to the host bone edges and the contour of the implant remained intact. By 12 weeks, the control defect appeared to be covered by fibrous tissue and in the implanted defect, the HA appeared fixed to the host bone and its margins appeared resorbed in some areas. By 22 weeks, the control defect was completely covered by bone whereas in the implanted defect, the HA block was not visible clinically.

Histological studies showed that bone repair had progressed through a programmed sequence of maturation steps closely resembling the pattern of bone development and growth regardless of whether bone grafts or substitutes were present or not.

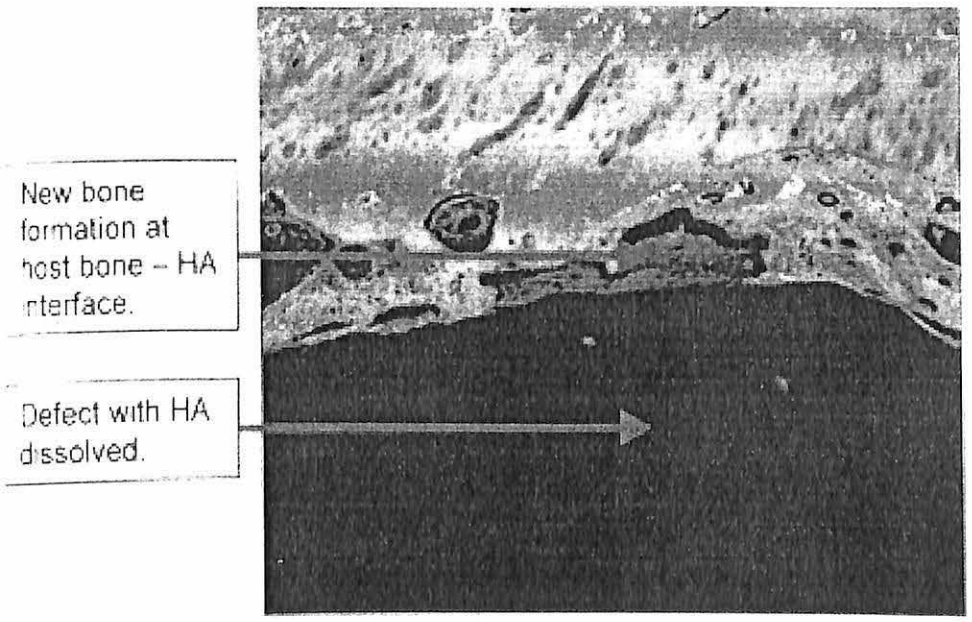
There was enhancement of osteoconduction in the implant group. This was evident by the three dimensional process of growth, namely, the presence of abundant capillaries, perivascular tissue and osteoprogenitor cells of the host accounting for the new bone filling the gap and remodelling taking place.

At 4 weeks after creation of the defects in the mandibles, there were no fibroblasts seen at the periphery of the defect in the control. The dark space represents the defect space with the host bone regenerating. There was no evidence of any new blood vessel formation, indicating that there was no new bone formation (Fig.2a). In the defect with HA, the dark area represents the defect with the HA dissolved during the decalcification process. There was evidence of fibroblasts with fibrous tissue formation around the HA implant. Some mesenchymal cells and new blood vessels

Figures 2




2 (a) – Control site at 4 weeks showing host bone regeneration
No new bone formation. H&E x 10




2 (b) – HA site at four weeks showing bone ingrowths into defect H&E x 10

were seen at the periphery (Fig.2b). When viewed by topography, there were bone ingrowths into the implant (Figure 3).

At 12 weeks after implantation, in the control, there was a plate-like mesenchyme with richly vascularised connective tissue and lots of osteoblasts. Small ossification centres or trabeculae were seen as dense eosinophilic, homogenous mass surrounded by osteoblasts. Very few osteocytes were seen in the lacunae (Fig. 4a). On the implanted side, there was formation of primitive cancellous bone with richly vascularised connective tissue occurring in the gap between the implant and the host bone. Few ossification centres or trabeculae were seen as they coalesced to form cancellous bone. There were many osteoblasts, osteocytes in lacunae and osteoclasts. A layer of periosteum was seen and there were collagenous fibres running in random directions with evidence of woven bone formation (Fig. 4b).

At 20 weeks post-defect, immature bone growth can be seen without any mature Haversian canal in the control. There was proliferation of mesenchymal cells and neovascularisation (Fig. 5a). In the implanted defect, mature bone was seen in many areas of the section with numerous immature Haversian system, indicating an ongoing process of bone maturation. There were new bone ingrowths coalescing in an attempt to fill the defect (Fig 5b).

At 22 weeks, the control showed new bone formation with fibrosis surrounding it. There were only a few areas of mature bone (Fig.6a). Photomicrograph of the implanted defect showed mature bone formation filling almost the whole field. However, there were still areas of dark spaces indicating that the HA implant had not been completely resorbed. Some mesenchymal cells were seen (Fig. 6b).

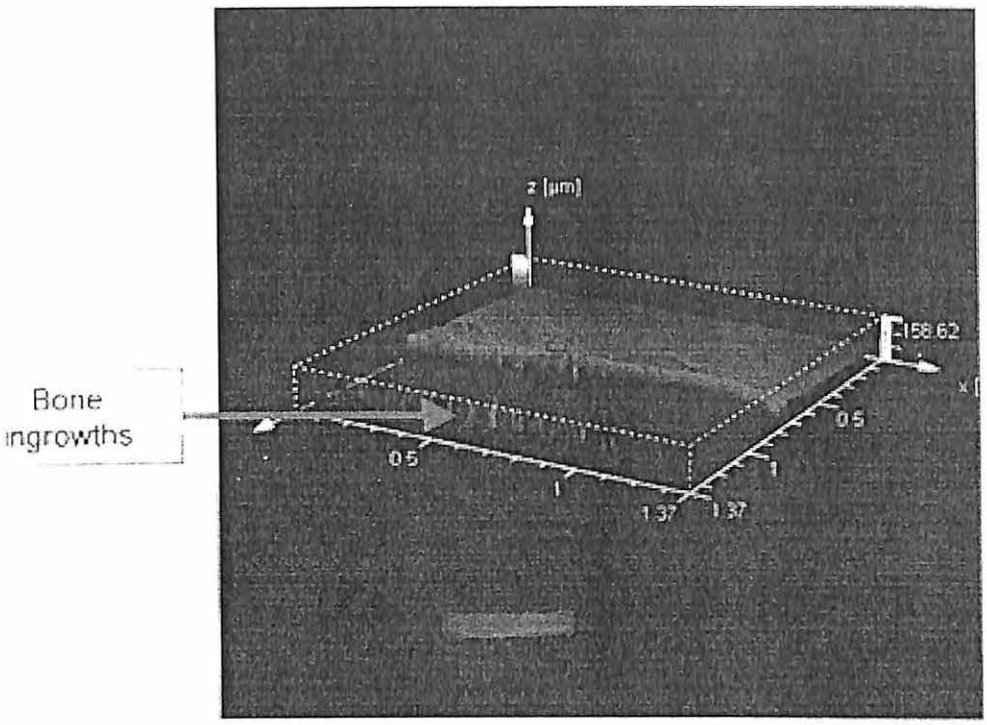
## DISCUSSION

A multitude of materials have been tried as bone substitutes, but many of these materials were found to provoke antigenic or foreign body reactions of varying severity or occasionally to become infected (Rawlings, 1993). It is now widely recognised that calcium phosphate ceramics such as hydroxyapatite or tricalcium phosphate are suitable bone substitutes in orthopaedic, reconstructive and maxillofacial surgery due to their good biocompatibility and extensive bone conductivity, allowing direct bonding between bone and ceramics (Kurashina et al, 1997).

Due to the inherent weakness of the ceramic HA, attempts at modifications to its chemical structure to improve its physical properties such as toughness and flexural strength should be ongoing. The new material should be not only strong, but be biocompatible and osteoconductive. In addition, it had also been shown that physical form is also an important determinant of the success of an implant material. Costantino et al (1993) stated that focusing on chemical composition, physical form, and appropriate application is a good starting point when evaluating any new biomaterial for potential use. The material under investigation is a locally produced dense HA zirconium ceramic. The present study is therefore a preliminary study into the biocompatibility and the ability of this material to replace bone.

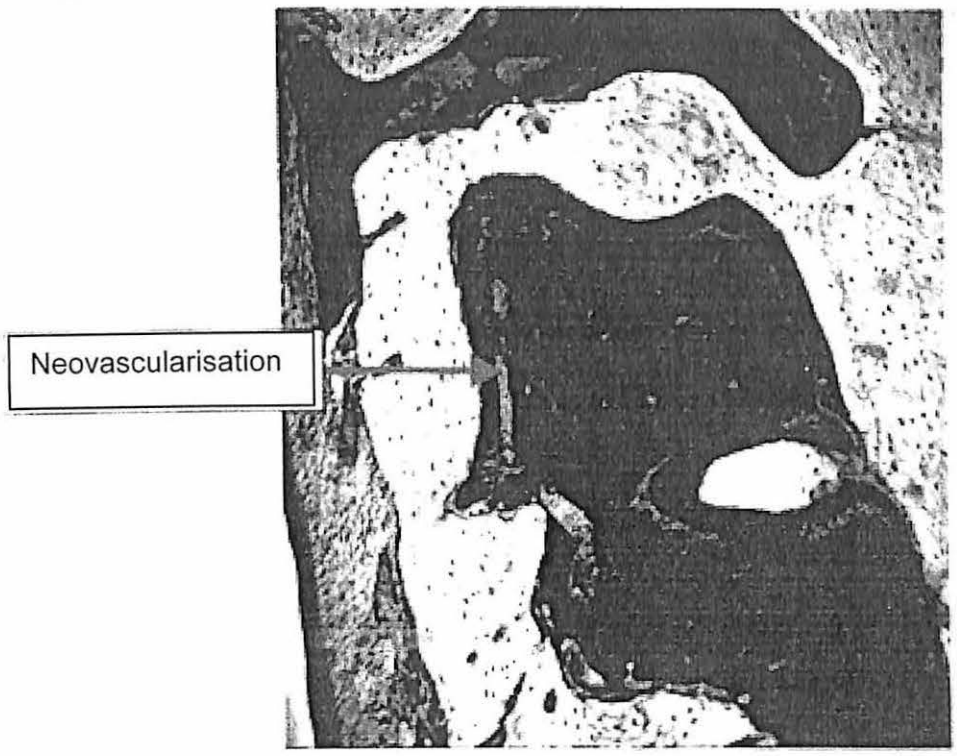
Dense HA-cemented titanium implants and HA-coated titanium implants are currently being used for dental implants. Dense HA is chemically stable and non-resorbable in vivo but it has its limitation of being brittle. Studies contrary to this have been done and they found that dense HA implants with bone and titanium

Figure 3

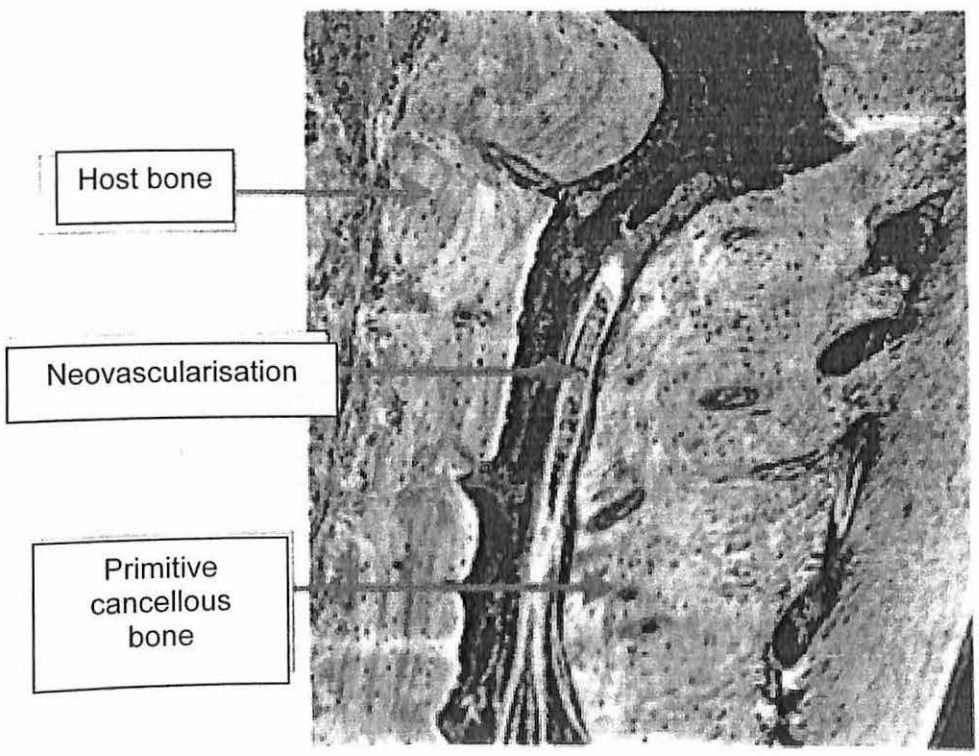



HA site at 4 weeks showing bone ingrowths into HA. (3-D topography)

**Figures 4**

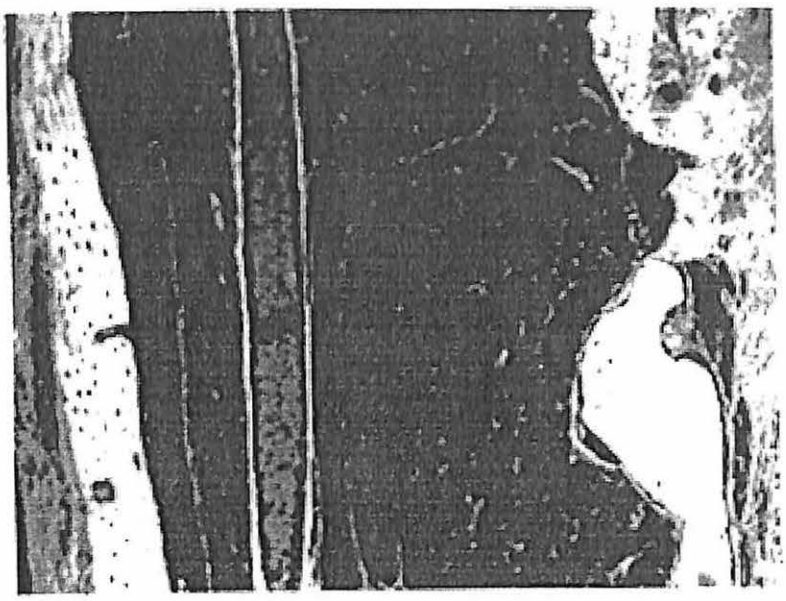



4(a) – Control site at 12 weeks, showing mesenchymal cells, richly vascularised connective tissue and osteoblasts along the defect margin. H&E x 10

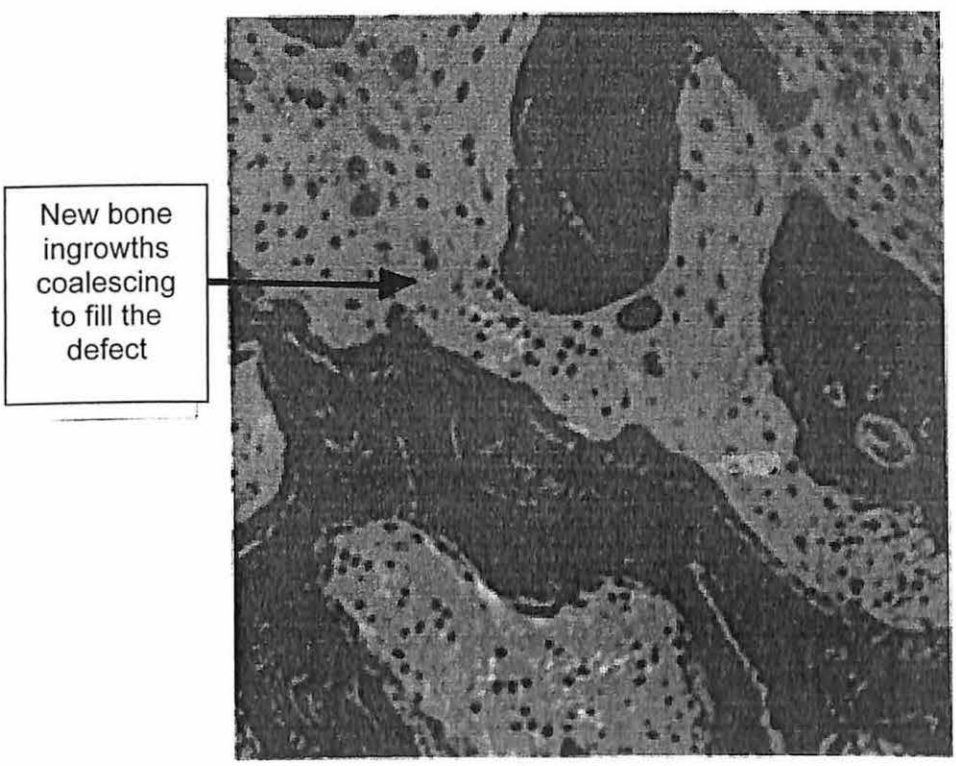



4(b) – HA site at 12 weeks showing primitive cancellous bone with richly vascularised connective tissue in the gap between the imlant and the host bone. H&E x 10

**Figures 5**

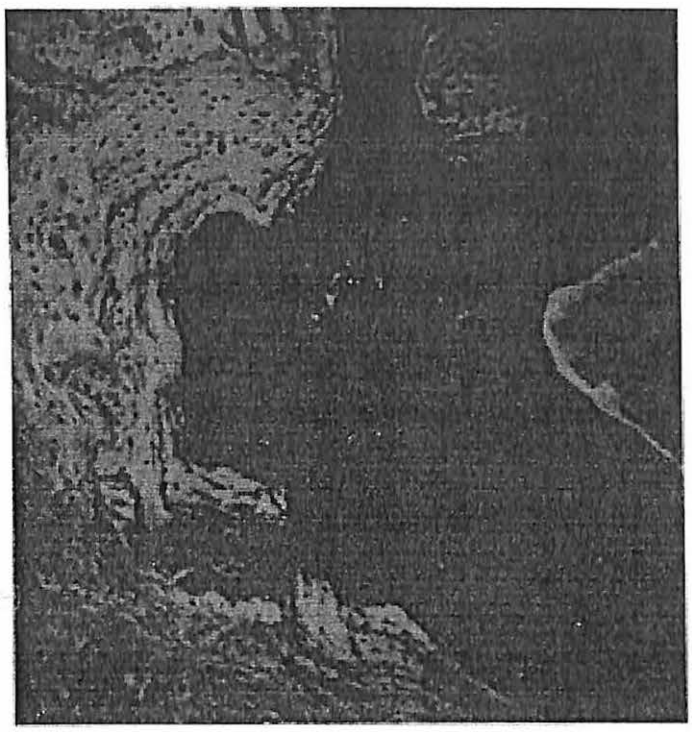


5(a) – control site at 20 weeks showing immature bone growth. Evidence of proliferation of mesenchymal cells and neovascularization. H&E x 10

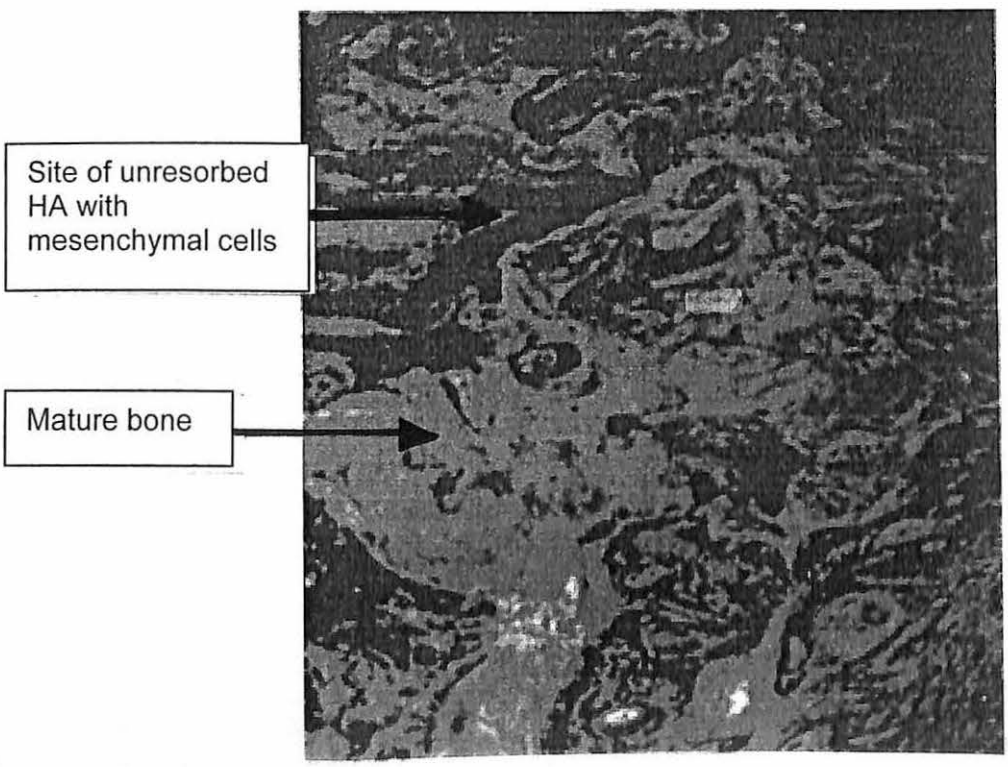



5(b) – HA site at 20 weeks showing mature bone growth at several areas with immature Haversian system, indicating an ongoing process of bone maturation.

## Figures 6



6(a) – control site at 22 weeks, showing new bone formation with fibrosis surrounding it. Only a few areas of mature bone are seen.




6(a) – HA site at 22 weeks, showing mature bone filling almost the whole field. Some mesenchymal cells were seen.

It has been shown that calcium phosphate bioceramics are well tolerated by the body; they cause neither inflammation nor systemic toxicity. Jarcho (1981) had concluded that the results of various studies done indicated that the calcium and phosphate derived from these implants enter the body pool and are utilised or removed in a normal fashion. There is neither foreign body response nor any evidence of rejection (Rawlings, 1993). The present dense HA zirconium ceramic did not provoke any foreign body reactions or show histological indication of infection throughout the 22 weeks period indicating that it has good biocompatibility to the host.

Within 1 day of implantation of a biomaterial, the healing response is initiated by the action of monocytes and macrophages. Fibroblasts and vascular endothelial cells in the implant site proliferate and begin to form granulation tissue. This may be seen as early as 3-5 days following implantation. New small blood vessels are formed by a process of neovascularisation or angiogenesis. Proliferation of fibroblasts in developing granulation tissue is active in collagen synthesis leading to formation of the fibrous capsule (Anderson, 1996).

Takeshita et al (1997) did a study on bone formation around dense HA implants using light microscopy, image processing and confocal laser scanning microscopy. He found that the percentage of bone contact of HA is superior to titanium throughout the experimental period (5, 7, 14, 28, 84 and 168 days). Ferraro (1979) reported on the histological finding of purified processed ceramic CaPO4 blocks, implanted in the body of the mandible of dogs. These implants were of pore sizes ranging from 100 to 300μm. He noted that there was no real evidence of new bone or osteoid formation in the controls even after one year. The margins of the defect show sclerotic bone. The present study shows that there was bone regeneration in the control but the bone formation and maturation lag behind that of the implanted site.

The present study demonstrates that the implanted study material promotes bone growth as early as 4 weeks. This is an advantage over the porous ceramic blocks, which requires a prolonged time for bone growth into the implant.

Bone grafts during their avascular period may become infected. It is also recognised that porous implant materials, whether HA, other ceramics, or polymers, have a greater potential to become infected than do non-porous materials. Throughout the study period, there was no evidence of infection or extrusion of the implanted material. Several other studies reported similar results. Rawlings 1993 reported that Zide et al in 1987 did a study on the repair of frontal bone defects using dense HA particles with or without autogenous bone. Throughout the follow up period of 3½ years, they found no evidence of inflammation, rejection or resorption.

Jarcho in 1981 had stated that the minimum pore size required for effective ingrowth of bone into porous ceramic structures is approximately 100μm. Gauthier et al (1998) showed that macroporosity is conducive to osteoconduction, cell colonisation and bone ingrowth and that a reduction in macroporosity may have a negative effect. He further concluded that the influence of pore size is greater than the percentage of porosity. The pore size of the dense HA is <5μm (Figure 7). However, bone ingrowth is evident in this study as early as 4 weeks when viewed by topographic method. Tetracycline injection would create a fluorescent layer in newly formed bone

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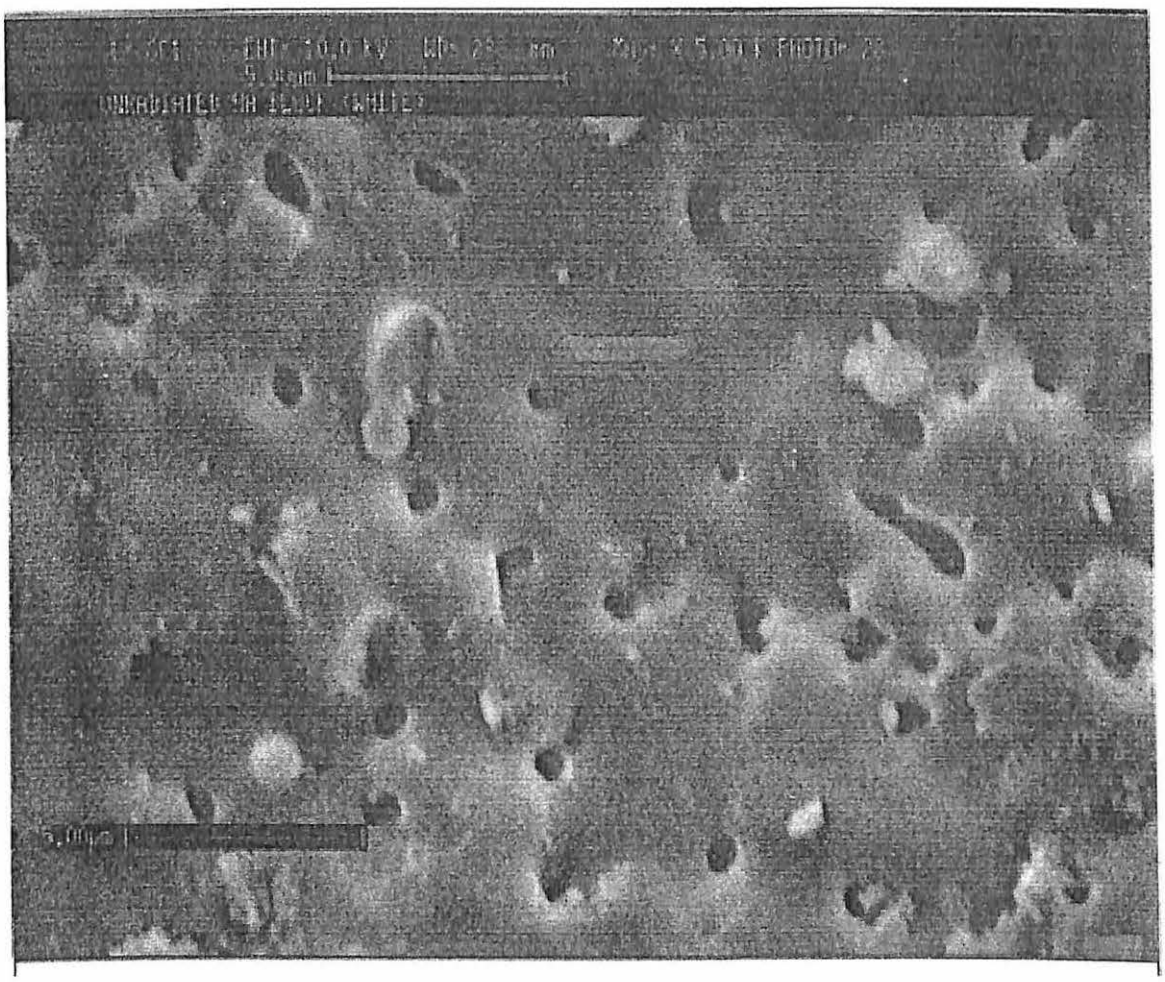

**Figure 7:** Scanning electron microscopy of the dense hydroxyapatite used in this study. The pore size is less than 5 µm

that can be detected with fluorescent microscopy to detect bone-remodelling activities. (Gauthier et al, 1998).

Holmes and Hagler (1988) in a histometric study of porous hydroxyapatite as a bone graft substitute in cranial reconstruction, found that more bone was present in the fields near the cranial cortex than the mid-portion of the implant and that the hydroxyapatite matrix surface covered by bone is also greater nearer the cortex. Chang et al (1996) also mentioned the effect of anatomical location upon bone growth around an implant. Histologically, the periosteum contains many more blood vessels and osteoblasts than the endosteum. Thus, they concluded that, after implantation, bone formation is more abundant in the periosteal region than in the endosteum. Ferraro (1979) found in his study that bone replacement occurred in a concentric manner with the earlier and less mature stages of bone development being found towards the centre of the implant. Most of the bone was deposited in the bone implant interface. The present study has similar finding in that the bone deposition is found at the bone implant interface.

There are two schools of thought regarding the most desirable bioresorption characteristics. One suggests that an implant material that allows for a more or less rapid creeping substitution by healing bone would be more desirable since the implant site would be restored to normal in a minimal amount of time. Others suggest that a more desirable bioresorbable implant would be one that would allow for initial permeation and maturation of bone, followed then by resorption and replacement of the implant on the assumption that this type of pattern would allow for increased strength and/or stability at the implant site during the longer healing period (Jarcho, 1981). The dense HA used in this study has similar characteristics as the latter, and this would probably indicate its use in load-bearing clinical situations.

In addition, a tissue composition high in bone coverage of the implant matrix, documents the strength of the material (Holmes and Hagler, 1988). In the present study, bone formation is in direct apposition to the hydroxyapatite matrix without intervening soft tissue. There was early bone ingrowth (by 4 weeks). By 22 weeks mature bone formation is more abundant in the implanted site than the defect without the implant, and there was high bone coverage of the implant matrix. This may be indicative of the implants being a strong material.

## CONCLUSION

As this study was performed on rabbits, which have shorter life span, the assessment of bone growth and the effects of the implant on host tissues over a long period cannot be established. Nonetheless, the results showed new bone formation around the implant materials from as early as 4 weeks. The new bone formation began with an ingrowth of cellular loose connective tissue, which is replaced later by a dense connective tissue and then matured bone. This may suggest that the implant material is not only osteoconductive but also osteoinductive.

The study also demonstrated that the dense hydroxyapatite blocks exhibit excellent biocompatibility as noted by the complete absence of reactive cells.

From this preliminary in vivo histological study, it appears that this locally manufactured sintered, dense hydroxyapatite block has the potential to be a valuable replacement material for maxillofacial reconstructive surgery and

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