



UNIVERSITI PUTRA MALAYSIA

**THE USE OF MALAYSIAN NATURAL CORAL AND CALCIUM
PHOSPHATE CEMENT AS BONE GRAFT SUBSTITUTE IN
RECONSTRUCTIVE BONE SURGERY OF SHEEP**

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FPV 2005 12

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By

FADILAH BINTI AHMAD

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirements for the Degree of Master of Science**

June 2005



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirements for the degree of Master of Science

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Chairman : Md. Zuki Bin Abu Bakar, PhD

Faculty : Faculty of Veterinary Medicine

Bone defects, which often occur as a result of various diseases have been reconstructed with autograft or allograft. However, their use is always associated with limited supply and to overcome this, biomaterials are considered as an alternative. Synthetic biomaterial such as calcium phosphate cement (CPC) available in the market has been used in bone defect. Another possible source is natural biomaterial such as natural coral.

Therefore, the present study was designed to evaluate the natural coral and compare it to the commercially available CPC post-implantation in sheep femoral bone. Twenty one adult male sheep, weighing between 15 – 20 kg were used in this study. The animals were divided into two groups: 9 animals in group 1 (CPC) and 12 animals in group 2 (coral).



Coral blocks (*Porites spp.*) were prepared and processed according to the protocol established by the Tissue Bank of the Malaysian Institute for Nuclear Technology Research (MINT). Commercially available CPC implant (Rebone Gutai, Shanghai Rebone Biomaterials Co., Ltd, China) was used as comparison. A bone defect (2.5 cm x 0.5 cm x 0.5 cm) was created surgically on the proximal part of femur before it was replaced by the implants.

Radiographs were obtained immediately after the surgery and at 2, 4, 8 and 12 weeks post-implantation. Ultrasonographic examinations were carried out at 1, 2, 4, 8 and 12 weeks post-implantation using ultrasound machine (TOSHIBA Capasee II) connected with 7 MHz frequency transducer. Weekly blood samples were collected from all animals via jugular vein for calcium and phosphate analyses. The sheep were sacrificed at 2, 4, 8, and 12 weeks post-implantation and the implant sites were examined grossly. Samples of the implant site were taken for histological examination.

Radiograph taken at 2 weeks post-implantation revealed mild loss of coral implant architecture and occasional development of radiolucent zone. At 4 weeks post-implantation, coral implant margins became indistinctive, prominent radiolucency zone and the intrinsic architecture was difficult to appreciate. The central part remained more radio opaque, indicating that resorption was proceeding centripetally. At 8 weeks and 12 weeks post-implantation, the coral implant was completely resorbed. In contrast, there

was no change in CPC implant at weeks 2, 4 and 8 post-implantation. At 12 weeks post-implantation, a slight fuzzy appearance had developed at the margins of CPC implant indicating slower progress of resorption. However, the CPC implant remained visible on the radiograph throughout the study period.

Ultrasonographic examination for up to 4 weeks post-implantation revealed that the coral implant was still visible at the implant site. However, by 8 weeks post-implantation, the implant was fully resorbed and soft tissues were observed at the implant site. Meanwhile, for the CPC implant, the ultrasonographic examination demonstrated that the implant was still clearly visible for up to 12 weeks post-implantation.

The serum calcium levels found in both animals implanted with coral and CPC during the study period revealed no significant different, except for weeks 1 and 10 in coral group where the level was significantly increased. While for the serum phosphate level there were significant increased at weeks 1 and 3 for CPC group and at week 11 for coral group. However, by week 12, both serum calcium and phosphate levels were back to normal as measured before implantation. These indicated that the coral and CPC implant were biodegradable.

Microscopically, the natural coral implanted into bone tissue showed rapid resorption and progressive replacement by new bone. At 12 weeks post-implantation, the implant site was almost completely closed and surrounded by new bone. Meanwhile, the CPC implant demonstrated a marginal bone formation at the end of the 12 weeks study.

Scanning electron microscopy observation of the coral implant at 2 weeks post-implantation showed that the implant was irregularly eroded on the surface, but the morphology of the pores was conserved. At 4 weeks, coral implant deteriorated and the shape of the pores changed, indicating increased coral degradation. At 8 and 12 weeks, no more coral was detected and the implant site was filled with dense collagenous extracellular matrix. Meanwhile, the CPC implant was characterized by deformity and broken surface of the implant. Some areas revealed granular appearance due to attachment of the cells.

In conclusion, results of the present study showed that natural coral implant was rapidly resorbed and was almost completely filled by new bone formation at the end of the study. In contrast, the CPC implant has a very slow resorption rate and remained clearly visible by week 12 post-implantation. Thus, the coral implant could be a possible and good candidate for bone graft.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**PENGGUNAAN BATU KARANG SEMULAJADI DAN KALSIUM FOSFAT
SIMEN SEBAGAI PENGGANTI TULANG DI DALAM PEMBEDAHAN
PEMBAIKPULIH TULANG: SATU KAJIAN PERBANDINGAN**

Oleh

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Kebiasaannya kecacatan tulang berlaku disebabkan oleh pelbagai penyakit dapat dibaikpulih dengan menggunakan autograf dan allograft. Walaubagaimanapun, di dalam penggunaannya terdapat beberapa risiko seperti bekalan yang terhad. Untuk mengatasi masalah ini penggunaan bio bahan dijadikan sebagai alternatif. Calcium Phosphate Cement (CPC) merupakan sintetik biobahan yang terdapat dipasaran banyak digunakan sebagai pengganti tulang. Selain itu, biobahan semulajadi seperti batu karang juga berpotensi dijadikan sebagai pengganti tulang.

Oleh itu, di dalam ujikaji ini batu karang semulajadi telah diimplan pada tulang femur bebiri untuk dinilai keberkesanannya dan dibandingkan dengan CPC. Sejumlah dua puluh satu kambing bebiri jantan dewasa (berat



diantara 15-20kg) telah digunakan dalam ujikaji ini. Biri-biri ini dibahagikan kepada dua kumpulan iaitu: kumpulan satu (CPC) mengandungi 9 ekor bebiri, manakala kumpulan 2 (batu karang) terdiri daripada 12 ekor bebiri.

Blok batu karang telah disediakan dan diproses berdasarkan kaedah yang telah dibangunkan oleh Bank Tisu Institut Nuclear Technology Malaysia (MINT). CPC (Rebone Gutai, Shanghai Rebone Biomaterials Co., Ltd, China) digunakan sebagai komersial pengganti tulang. Defek yang bersaiz (2.5smx0.5smx0.5sm) telah dibuat pada proximal femur kiri bebiri dan diganti dengan implan.

Radiograf telah digunakan sebagai penilaian pada minggu ke 0, 2, 4, 8 dan 12 selepas implan. Ultrasonograf pula dilakukan minggu ke 1, 2, 4, 8 dan 12 selepas implant dengan menggunakan Ultrasound Mesin (TOSHIBA Capasee II) disambungkan dengan 7MHz frekuensi transduser. Sampel darah diambil melalui vena jugular bebiri pada setiap minggu. Bebiri dimatikan pada 2, 4, 8 dan 12 minggu bagi kumpulan batu karang manakala pada 4, 8 dan 12 minggu bagi kumpulan CPC. Sampel tulang bagi kedua-dua kumpulan diambil dan diperiksa untuk kajian histoloji.

Bagi kajian radiograf, pada minggu ke-2, batu karang telah menunjukkan perubahan pada struktur implan dan pembentukan sedikit zon 'radiolucent'. Pada minggu ke-4, perubahan yang ketara pada permukaan implan dan

peningkatan pembentukan zon 'radiolucent' dikenalpasti. Didapati keadaan 'radio opaque' yang ketara pada bahagian tengah implan menunjukkan kadar penyerapan berlaku bermula dari luar permukaan implan ke dalam. Pada minggu ke 8 dan 12, batu karang sepenuhnya diserap. Walaubagaimanapun, bagi CPC tiada perubahan yang ketara dan implan masih dikenalpasti secara radiografit sehingga akhir ujikaji.

Kajian ultrasonograf pada minggu ke-4, menunjukkan batu karang implan masih berada pada kawasan defek. Pada minggu ke- 8 implan telah diserap sepenuhnya dan tisu lembut dapat diperhatikan pada kawasan defek. Sementara itu, CPC tidak menunjukkan sebarang perubahan sehingga diakhir ujikaji.

Paras serum kalsium bagi kedua-dua kumpulan tidak menunjukkan perbezaan signifikan kecuali pada minggu pertama dan 11 bagi kumpulan batu karang. Manakala, bagi paras serum fosfat menunjukkan penambahan yang signifikan bagi kumpulan CPC pada minggu pertama dan 3 dan minggu ke 12 bagi kumpulan batu karang. Walaubagaimanapun, pada minggu ke 12 paras kedua-dua serum kalsium dan fosfat kembali pada paras normal seperti paras sebelum implan. Ini menunjukkan kedua-dua implan adalah 'biodegradable'.

Dalam ujikaji mikroskopik pula, batu karang beransur-ansur diserap dan digantikan dengan tulang baru secara progresif. Pada minggu ke-12, implan telah digantikan dengan tulang baru. Manakala, CPC hanya menunjukkan pembentukan tulang pada sisi implan diakhir ujikaji.

Kajian SEM, menunjukkan permukaan batu karang implan yang tidak rata pada minggu ke-2 tetapi tiada perubahan pada morfologi liang batu karang. Walaubagaimanapun, perubahan ketara berlaku pada minggu ke-4, dimana bentuk liang implan batu karang telah mula berubah disebabkan peningkatan degradasi. Pada minggu ke-8 dan 12, defek telah dipenuhi oleh kolagen extrasellular matrik. Sementara itu, CPC pula menunjukkan mikroporositi dan permukaan granular disebabkan pelekatan sel-sel.

Oleh itu, kesimpulan yang dapat dibuat daripada ujikaji ini menunjukkan batu karang semulajadi mempunyai kadar serapan yang lebih cepat dan diganti dengan tulang baru pada akhir ujikaji. Manakala CPC menunjukkan kadar serapan yang lambat dan masih dikenal pasti hingga minggu yang ke-12. Oleh demikian, implan batu karang berpotensi digunakan sebagai alternatif pengganti tulang.

ACKNOWLEDGEMENTS

All praise to Almighty Allah, the Merciful and the Benevolent. Had it not been due to His will and favour, the completion of this study would not have been possible.

I would like to convey my deepest and sincere gratitude and appreciation to my supervisor, Dr. Md. Zuki Bin Abu Bakar for his valuable guidance, advice, supervision, and support throughout the course of this study.

Sincere appreciation and thanks are also extended to Dr. Loqman Bin Muhamad Yusof, Prof. Dr. Mohd Zamri Bin Saad and Dr. Norimah Bt. Yusof, who are my co-supervisors for their constructive advice, support and continuous encouragement towards the completion of this study. Not to forget Dr. Jalilah Bt. Abu and Dr. Goh Yong Meng for their resourceful comments and suggestions during completion of this thesis.

Special thanks to Prof Rani Bin Shamsudin, Dean of School of Dental Sciences of Universiti Sains Malaysia, for his co-operation and allowing me to use their facilities. I am also most grateful to Dr. Karima Alsalihi and staff of Craniofacial Laboratory for their help during the undecalcified bone preparation.



I would like to acknowledge Puan Asnah Bt. Hasan and staff of MINT for their help and providing the facilities for coral processing. My thanks also to Institute of Biosciences staffs, not forgetting, our University Veterinary Hospital supporting staffs; Mr. Adam (surgery), Pn. Noraini Othman (surgery), Mr. Othman Asnawi (Radiology); from histology laboratory, Mr. Jamil Samad; from post-mortem laboratory, Mr. Naziman Sulaiman and lastly Mr. Mohd Hilmi Othman and Mr. Abdullah Misron for their help throughout the course of this study.

I express my very warmest heartfelt thanks to all my good friends, Ain, Faizah, Roy, Dr. Abdel Hafeez, Dr. Saw Po Po and Dr. Ani Yardi, who helped me a lot in this project.

Finally, I owe a great deal of loving thanks to my family especially my mother and father for all their support and continuous encouragement, and to my husband, for his advice, support and continuous encouragement. May God bless them.

Praise be to Allah, who has given me the strength in completing this thesis.



I certify that an Examination Committee met on 17th June 2005 to conduct the final examination of Fadilah Ahmad on her Master of Science thesis entitled “The Use of Malaysian Natural Coral and Calcium Phosphate Cement as Bone Graft Substitute in Reconstructive Bone Surgery in Sheep” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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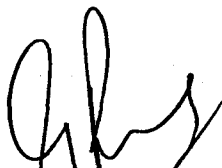
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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

Fad.

FADILAH BINTI AHMAD

Date: *16/08/05*

TABLE OF CONTENTS

	Page
ABSTRACT	iii
ABSTRAK	vii
ACKNOWLEDGEMENTS	xi
APPROVAL	xiii
DECLARATION	xv
LIST OF TABLES	xix
LIST OF FIGURES	xx
LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERMS	xxviii

CHAPTER

I	INTRODUCTION	1
II	LITERATURE REVIEW	5
	Bone	5
	Anatomy of Bone	5
	Cellular Components of Bone	7
	Bone Biochemistry	9
	Bone Healing Process	10
	Bone Grafting and Bone Substitutes	12
	Autograft	12
	Allografts	14
	Xenografts	15
	Biomaterials	15
	Natural Coral as Bone Graft Substitute	17
	Biochemical Compositions of Natural Coral	19
	Architecture and Porosity	20
	Mechanical Properties	21
	Osteoconduction	22
	Osteoinduction	23
	Osteogenesis	23
	Coral Resorption	24
	Biocompatibility	26
	Clinical Applications	27
	Calcium Phosphate Cements (CPCs)	29
	<i>In vivo</i> Characteristic of CPC	32
	Histological Study of CPC	33
	Clinical Applications of CPC	34
	Calcium Metabolism	35
	Phosphate Metabolism	37



III	GENERAL MATERIALS AND METHODS	
	Experimental Animals	39
	Experimental Design	39
	Implant Materials	40
	Natural Coral Processing	41
	Bioburden Test	44
	Media Preparation	44
	Microbiology Quality	44
	Surgical Procedure	44
IV	MACROSCOPIC EVALUATION AND CALCIUM PHOSPHATE ANALYSIS OF MALAYSIAN NATURAL CORAL AND CPC IMPLANTS POST-IMPLANTATION IN FEMUR OF SHEEP	
	Introduction	49
	Materials and Methods	
	Clinical Assessment	51
	Radiographic Evaluation	51
	Ultrasonographic Evaluation	52
	Gross Necropsy Examination	52
	Blood Analysis	53
	Statistical Analysis	53
	Results	
	Clinical Assessment	54
	Radiographic Evaluation	54
	Coral Implant	55
	CPC Implant	56
	Ultrasonographic Evaluation	56
	Coral Implant	56
	CPC Implant	57
	Gross Necropsy	58
	Coral Implant	58
	CPC Implant	58
	Serum Calcium and Phosphate Analysis	59
	Discussion	92
V	MICROSCOPIC EVALUATION OF MALAYSIAN NATURAL CORAL AND CPC POST-IMPLANTATION IN FEMUR OF SHEEP	
	Introduction	98
	Materials and Methods	
	Undecalcified Bone	101
	Scanning Electron Microscopy	102

Results	
Undecalcified Bone	102
Scanning Electron Microscope	105
Discussion	126
VI GENERAL DISCUSSION	132
Conclusion	139
BIBLIOGRAPHY	141
APPENDICES	151
BIODATA OF THE AUTHOR	159



LIST OF TABLES

Table		Page
3.1	Distribution of experimental animals and duration post-implantation.	40
4.1	Radiographic evaluation scoring.	52
4.2	The mean score of the implants from radiographic evaluation.	61
4.3	Results for serum calcium analysis.	63
4.4	Results for serum phosphate analysis.	65



LIST OF FIGURES

Figures		Page
2.1	(a) Diagrammatic view of a segment of compact bone, illustrating its structural units (osteons). The inset shows a more highly magnified view of a portion of one osteon. (b) Photomicrograph of a cross-sectional view of one osteon and portion of others (144x).	7
2.2	Stages in the healing of a bone fracture.	11
3.1	Natural coral of <i>Porites</i> spp.	42
3.2	Natural coral blocks (2.5x 0.5x 0.5 cm).	42
3.3	CPC blocks.	42
3.4	Coral block was shake using shaker.	43
3.5	Coral block was pasteurized in shaking water bath.	43
3.6	The left hind limb was prepared for aseptic surgery.	46
3.7	The skin incision was made on the lateral aspect of the thigh to expose the muscles.	47
3.8	The bone defect (approximate 2.5x0.5cm) was created at the proximal part of the femur.	47
3.9	The implantation of the implant at the bone defect.	48
3.10	Wound closing.	48
4.1	Graph for mean score resorption for radiographic evaluation of coral and CPC post-implantation.	60
4.2	Graph of serum calcium analysis.	62
4.3	Graph of serum phosphate analysis.	64
4.4	Radiograph of the left femur immediately post-implantation shows clear coral structure (arrow). Note that the coral architecture is grossly visible.	66

Lateral view.

- 4.5 Radiograph of the left femur at 2 weeks post-implantation. Note that the coral implant margins become slightly indistinct and intrinsic architecture is still readily apparent (arrow). Slight development of a radiolucent zone in the adjacent bone can also be observed. Lateral view. 67
- 4.6 Radiograph of the left femur at 4 weeks post-implantation demonstrates the coral implant margins have become indistinct (arrow), and a prominent zone of radiolucency has developed in the adjacent bone. Intrinsic architecture is difficult to appreciate. Lateral view. 68
- 4.7 Radiograph of the left femur at 8 weeks post-implantation shows the development of radiolucent zone (arrow) at the coral implant site indicating the implant has been fully resorbed. Lateral view. 69
- 4.8 Radiograph of the left femur at 12 weeks post-implantation demonstrates the radiolucent area (arrow) at the coral implant site as in week 8 post-implantation. Lateral view. 70
- 4.9 Radiograph of left femur immediately post-implantation shows the CPC block is clearly discernible with well define margins. Lateral view. 71
- 4.10 Radiograph of left femur at week 2 post-implantation shows the CPC block that still clearly discernible with well define margins. Lateral view. 72
- 4.11 Radiograph of the left femur at 4 weeks post implantation demonstrates the CPC implant that maintains its initial radiodensity. Lateral view. 73
- 4.12 Radiograph of the left femur at 8 weeks post-implantation demonstrates the CPC implant is still maintain its initial radiodensity. Lateral view. 74
- 4.13 Radiograph of the left femur at 12 weeks post-implantation shows the CPC implant is developing of radiolucent zone in the adjacent bone. Lateral view. 75

- 4.14 Ultrasonographs of the coral implant at week 1 post-implantation. A) longitudinal scan, B) transverse scan. Note that the coral implants (arrow) appear hyperechoic with well define margins. The echogenicity is almost similar to the adjacent bone. 76
- 4.15 Ultrasonographs of the coral implant at week 2 post-implantation. A) longitudinal scan, B) transverse scan. Note that the coral implants (arrow) depict less hyperechoic as compared to week 1 post-implantation with well define margins. No tissue reaction is detected surrounding the implant. 77
- 4.16 Ultrasonographs of the coral implant at 4 weeks post-implantation. A) longitudinal scan, B) transverse scan. Note that the echogenicity of coral implants (arrow) are similar to adjacent bone. The size of coral implant has reduced as indicate by the small gap (small arrow) indicating some resorption occurs. No tissue reaction observed around the implant. 78
- 4.17 Ultrasonographs of the coral implant at 8 weeks post-implantation. A) longitudinal scan, B) transverse scan. Note that coral implant was completely resorbed as shown by the discontinuity of bone surface (arrow). The defects area becomes small and filled by the soft tissue. 79
- 4.18 Ultrasonographs of the coral implant at 12 weeks post-implantation. A) longitudinal scan, B) transverse scan. Note that the coral implant is no more detected at the implant site (arrow) and the margins of the defects almost closed in B. 80
- 4.19 Ultrasonographs of the CPC implant at 2 weeks post-implantation. A) longitudinal scan, B) transverse scan. Note that the CPC implant (arrow) depicts hyperechoic structure with well define margin. The implant is well fix into the defect. 81
- 4.20 Ultrasonographs of the CPC implants at 4 weeks post-implantation. A) longitudinal scan, B) transverse scan. Note that the CPC implant (arrow) appears isoechoic relative to adjacent bone with a smooth and well define margin. 82

4.21	Ultrasonographs of the CPC implant at 8 weeks post-implantation. A) longitudinal scan, B) transverse scan. Note that the CPC implant (arrow) remains similar to the week 4 post-implantation.	83
4.22	Ultrasonographs of the CPC implant at 12 weeks post-implantation. A) longitudinal scan, B) transverse scan. Note that there is no change in the echogenecity and the size of the implant as compared to previous scans. This indicates there is no resorption or very slow resorption occurs.	84
4.23	Photograph of the coral implant (cross section) at 2 weeks post-implantation demonstrates the coral implant (arrow) undergoing resorption process. Note that the coral implant is infiltrated by the soft tissue and blood vessels.	85
4.24	Photograph of the coral implant at 4 weeks post-implantation demonstrate the coral implant (arrow) that is still visible at the implant site.	86
4.25	Photograph of the coral implant site (arrow) at 8 weeks post-implantation reveals the defect area filled with soft tissue. Note that the coral implant has been completely resorbed leaving the size of the defect that is much reduced.	87
4.26	Photograph of the coral implant site (arrow) at 12 weeks post-implantation reveals the much reduce defect size filled with soft tissue.	88
4.27	Photograph of the CPC implant at 4 weeks post-implantation demonstrates the CPC implant (arrow) with distinct margins that is clearly visible.	89
4.28	Photograph of the CPC implant (cross section) at 8 weeks post-implantation reveals the CPC implant with distinct margins and is clearly visible.	90
4.29	Photograph of the CPC implant (cross section) at 12 weeks post-implantation demonstrates the CPC implant that is still clearly visible without marked change.	91
5.1	The harvested bone is sawn into slices using the	108

- diamond saw (a) then the specimen is placed in the tissue cassette (b).
- 5.2 The specimens are dehydrated in increasing grades of ethanol and subsequently infiltrated in Technovit ®7200 VLC-resin. 108
 - 5.3 The blocks are embedded and polymerized in plastic fixation medium at 450 nm wavelength of UV light. 109
 - 5.4 The block is sectioned in vertical plane using a diamond saw. 109
 - 5.5 The slides is grinding and polishing to a final thickness of about 40-60 µm. 110
 - 5.6 The slides with final thickness of about 40-60 µm. 110
 - 5.7 Photomicrograph of the coral implant demonstrates invasion of fibrovascular tissue (F) into the coral pore region. No bone is seen in the pores and coral matrix is partly absorbed (arrow). Masson-Goldner's Trichrome. (Primary magnification: 10x). 111
 - 5.8 Photomicrographs of coral implant showing abundant active osteoblasts (arrows) lined on the partially resorbed surface of the coral matrix (C). Toluidine blue. (Primary magnification: x40). 111
 - 5.9 Photomicrographs demonstrate a) the pores of the coral implant are filled with regenerated bone and new bone (green area) is placed at vacant site, b) coral implant (C) has already lost its structure due to resorption process. Masson-Goldner 's Trichrome (Primary magnification: x10). 112
 - 5.10 Photomicrographs of the coral implant (C) showing the osteoblasts present at the resorbed area (arrow). Toluidine Blue. (Primary magnification: x40). 112
 - 5.11 Photomicrographs of the CPC implant viewed at x10(a) and x4(b) demonstrates the gap between the CPC implant and host bone (HB) that is filled with fibrovascular (F). Note that the presence of gap observed as empty space (arrow). Masson-Goldner 's Trichrome. 113