

# 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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By

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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 lost 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 postimplantation, 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 postimplantation, 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 postimplantation 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 postimplantation. Thus, the coral implant could be a possible and good candidate for bone graft.



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### 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 gunakan 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 frekuansi 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 radiograft sehingga akhir ujikaji.

Kajian ultrasonograf pada minggu ke-4, menunujukkan 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 siknifikan kecuali pada minggu pertama dan 11 bagi kumpulan batu karang. Manakala, bagi paras serum fosfat mnunjukkan penambahan yang siknifikan 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 menujukkan 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 impan 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.



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