

# In Vivo Study of CORAGRAF: A Preliminary Results

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## Introduction

Natural coral is a bone graft substitute, which has been widely used in maxillofacial, orthopaedic, ORL and periodontal surgery. The capacity of coral to disappear and to be substituted by new bone distinguishes it from non-resorbable materials extensively used in these surgeries. An optimal clinical utilization of coral requires thorough knowledge of factors influencing resorption, particularly regarding the interface between implant and connective tissue, which is larger than the surface in contact with the bone. This study was designed to evaluate coral as bone substitute for reconstruction of critical mandible bone defect in rabbit using histological and scanning electron microscopy (SEM) observations.

## Materials and Methods

Coral blocks (CORAGRAF) 4mm x 4mm x 4mm from sea coral *Porites species* are produced by the National Tissue Bank, Universiti Sains Malaysia (USM). They were immersed in hypochlorite solution, cleaned with ultrasound and rinsed with distilled water before final drying. DHA was prepared in the same size and used as control implant. Eight New Zealand male rabbits at

2 months old were anaesthetized by intramuscular injection of Ketamine and Xylazine. Muscle was blunt dissected to reach the mandible. The defects were created on both sides of the mandible. CORAGRAF was placed in the right side while the left side was implanted with DHA as control. Then the area was closed with resorbable suture. The implants were retrieved at 2 and 4 weeks later. For the undecalcified method, the implants were fixed in neutral buffered formalin solution, dehydrated with alcohol and infiltrated by alcohol/technovit solution. All samples were embedded and polymerized in plastic fixation medium at 450 nm wavelength and sectioned using Exakt band cutting machine. The final thin section (8µm) were grinded and stained with Mayer's H&E. For SEM method, coral implants were dried at 150°C for 24 h and dehydrated. The implants were coated with gold and examined with scanning electron microscopy (Leica Cambridge S360 at 10 KV).

## Results

None of the control DHA implants showed bone formation at 2nd and 4th week or implant -bone integration at 2nd week. However, there was good integration border to the host bone at 4th week. The

DHA material was surrounded by fibrous connective tissue at all period of implantation, and unchanged in size or form with no significant inflammatory reactions. The periphery of the CORAGRAF implant was separated from the surrounding tissue by the connective tissue composed of interiorly by a cellular layer and externally by a fibrous or adipous layer. The centre of the pores was occupied by a fibrous tissue. This tissue contained some vessels and appeared to be more or less dense depending on the area examined. All pores border surrounded by osteoblasts. At 4th week, the centre of the pores of the implant appeared denser and more vascularized. At the interface of the implant and soft tissue, there were good integration with the CORAGRAF. Mineralized bone tissue appeared at 4 weeks of implantation and successively deformed in the shape and size of coral block. SEM observation showed irregular erosion at the surface of the CORAGRAF at 2nd week. At 4 weeks CORAGRAF became more deteriorated and the shape of the pores had changed, indicating an increased coral degradation. The pores surface were almost covered by a dense collagenous extracellular matrix.

## Discussion

Histological examination demonstrated that DHA implant was surrounded by intervening fibrous tissue at 2 and 4 weeks, with good integration border to the host bone at 4 weeks while there was no bone formation in the implant area. Compare with control DHA, the CORAGRAF promoted osteogenesis began on the surface and border of the coral pores and proceeded centripetally toward the centre of the pores with slightly intervening fibrous tissue surrounding the implanted area. New mineralised bone tissue was seen at 4 weeks of implantation and successively deformed in the shape and size of CORAGRAF block. The SEM observation of the CORAGRAF at 2 weeks showed that the implants were irregularly eroded at the surface, but the morphology of the pores was conserved. At 4 weeks the implants were more deteriorated and the shape of the pores had changed, indicating an increased coral degradation. This study revealed that natural coral has shown good biocompatibility, osteoconductivity and biodegradability properties to be used as bone substitute to reconstruct bone defects.