

Application of Dental Tricalcium Phosphate Implants in Osseous Defects of Rabbit Tibia: A Light and Electron Microscopic Study

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ABSTRACT : The use of bioceramics is currently being employed due to limited availability of autografts and added surgical morbidity of donor sites. Among these, tricalcium phosphate is the least toxic and the most osteogenicity. Osseous defects on tibia of twelve rabbits were created and biologic response to dental preparations of tricalcium phosphate were observed by light microscopy on the first, second, fourth and sixth week post-implantation and electron microscopy on the sixth week. An autologous control site was made on the contralateral tibia and compared with the experimental site.

The ceramic was noted to elicit minimal inflammatory response and allowed the ingrowth of fibrovascular tissue into its pores with subsequent deposition of bone. The stages of reparative process were qualitatively comparable to the autograft site.

INTRODUCTION

Approaches to reduce bone defects include the use of biologic tissues, synthetic materials or combinations of these. No single type of implant or graft is ideally suited for all circumstances.¹ The use of bioceramics is being explored to obviate the limited availability of autografts and the additional surgical trauma in its procurement. Most studies on the use of bioceramics are in dental applications. Among these, tricalcium phosphate or TCP was rated by Cutright et al.² in an animal study, to have the highest osteogenic potential and the lowest tissue toxicity. The use of TCP on mandibular defects of dogs studied by Nery³ showed that this material was histologically tolerated. Nery in 1978 and Baldock⁴ in 1985 reported conflicting results on the use of TCP on naturally occurring human

periodontal defect. It was only in the last decade that extensive studies were done on the use of ceramics on long bones, mostly on hydroxyapatite crystals and TCP in animal model.⁵⁻⁸ Presently, the only locally available form of TCP is dental pellets used for alveolar defects.⁹ The study seeks to test the applicability of this dental preparation on long bones. Its objectives are to observe the biologic response of rabbit tibia and to compare this response to an autologous bone graft.

MATERIALS AND METHODS

Twelve New Zealand rabbits aged 5 to 6 months and weighing 1 to 1.5 kgs. were anesthetized with intramuscular ketamine, the rear legs shaved and the tibia exposed through a longitudinal incision. A 1.5 x 1.5 cm section of the tibia and the surrounding periosteum were removed such that the proximal end of the defect was 3 cm distal to the knee joint. Commercial preparation of sterilized TCP was grounded and implanted into the defect. The same incision and metaphyseal opening was made on the contralateral tibia and the defect was filled with bone removed from the experimental site as well as from the same site. The graft was held in place by the surrounding muscles which were sutured and stability was provided by an intact fibula and a cylinder cast around the thigh. Cefazolin was given 1 hour pre-operatively and 2 more doses post operatively.

The animals were observed for gross signs of infection. Three, two, three and four rabbits were sacrificed on the first, second, fourth and sixth week respectively. The whole tibia was removed. Specimens for microscopy were fixed in 10 per cent formalin, decalcified in nitric acid and stained with

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hematoxylin and eosin. Only two rabbits on the sixth week were used for electron microscopy, which were fixed in glutaraldehyde and embedded in silver. Biologic response was evaluated using the following parameters: inflammation, bone deposition on graft surface and reparative response of the host. The control or autograft, and the experimental or ceramic sites were then compared.

RESULTS

All wounds healed uneventfully and walking was normal after several days. Upon harvesting the graft, there was no sign of gross infection on both experimental and control sites. No consistent difference could be identified between the control and the experimental groups. The junction of the original defect and the graft was easily identified. Even on the sixth week gross granules of the ceramics could still be identified.

LIGHT MICROSCOPIC APPEARANCE

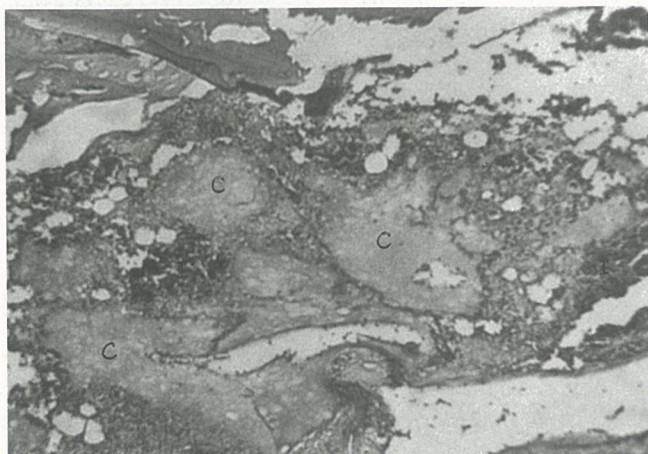


Fig. 1 Implant surrounded by hemorrhage at week 1
C - ceramic I - inflammatory cells

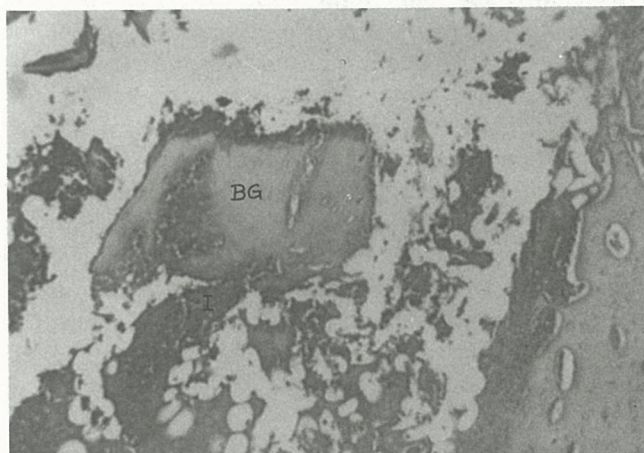


Fig. 2 Dead bone graft in hemorrhagic background at week 2
BG - bone graft I - inflammatory cells

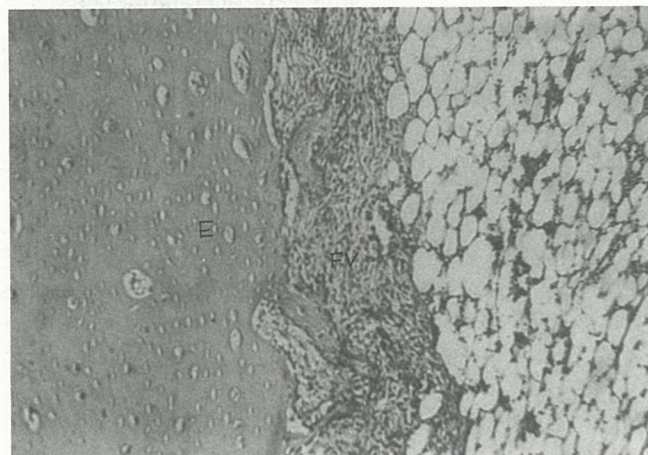


Fig. 3 Increased fibroblastic activity at endosteum
E - endosteum FV - fibrovascular stroma

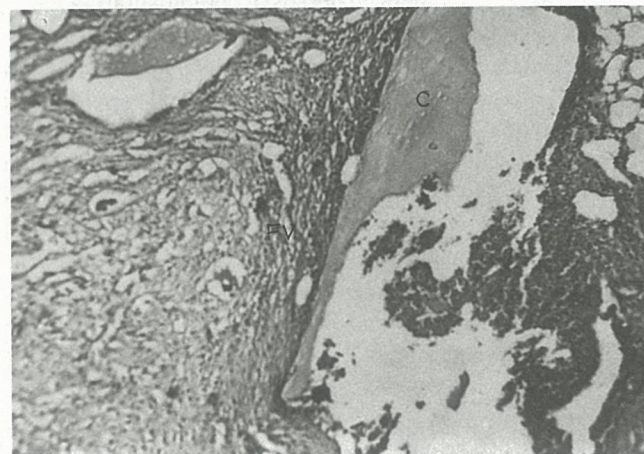


Fig. 4 Increased fibrovascular tissue around implant at week 2
C - ceramic FV - fibrovascular stroma

Specimens taken from the implant site after one week showed islands of ceramic material surrounded by fibrino-hemorrhagic tissue. There was a mild to moderate inflammatory response with minimal necrosis of adjacent marrow tissue (Figure 1). Specimen from the control site after one week showed fragments of lamellar bone with empty lacunae indicative of dead bone graft. They were also surrounded by fibrinohemorrhagic material with minimal inflammation (Figure 2). Both the experimental and control specimen at one week showed increase in fibrovascular tissue at the endosteum. Prominent osteoblasts lined the bone surface of the endosteum. These findings were compatible with bone repair activity and was generally present all through out the study (Figure 3). At two weeks, the ceramic implants were still surrounded by hemorrhagic materials with minimal inflammation and tissue necrosis present. The area adjacent to the implant was fibrovascular (Figure 4). Cortical defect was still unbridged but periosteum showed increased cellularity and vascularity. In the control, fragments of dead bone were still surrounded by hemorrhagic material but with minimal inflammation

compared to the experimental group. Periosteum was the same as in the experimental site. In the ceramic site at the fourth week the cortical defect was bridged by bony spicules. Islands of ceramic implants were admixed with woven bone (Figure 5). New bone deposition and prominent osteoblast lining were present on the ceramic surface. Some islands of ceramic material were completely surrounded by bone. Occasional osteoclasts were present on the ceramic surface. Inflammation was absent (Figure 6). In the control site the cortical defect was also bridged by bone spicules as in the experimental study. Remnants of dead bone grafts were still present. New woven bone was seen deposited on the surface of the graft. Inflammation was also absent (Figure 7). At sixth weeks the process occurring at the fourth week were essentially ongoing. In the experimental site osteoblastic rimming of the implant and ingrowth of fibrovascular tissue into the implant were seen. Bone deposition on the implant was also present (Figure 8 & 9). The corresponding control showed the continuing process of bone repair. Bone grafts were not recognizable. Formation of new bone undergoing en-

dochondral ossification.

ELECTRON MICROSCOPIC APPEARANCE

Under the scanning electron microscope, portions of the ceramic graft were shown to be covered with networks of fibrous connective tissue which on higher magnification showed ingrowth into the pores between the ceramics (Figure 10 & 11). The autologous graft site likewise showed fibrovascular stroma ingrowth with minimal inflammatory reaction (Figure 12).

DISCUSSION

The understanding of the morphology of graft incorporation has not changed significantly.^{1,10,11} After implantation, hematoma forms around the graft and the death of the graft incites an inflammatory reaction. This host tissue reaction is transformed into a fibrovascular stroma over the next few weeks bringing with it osteoclasts and osteoprogenitor cells. The initial host cell activity is bone resorption of the graft by osteoclast followed by osteoid deposition by osteoblast. Re-

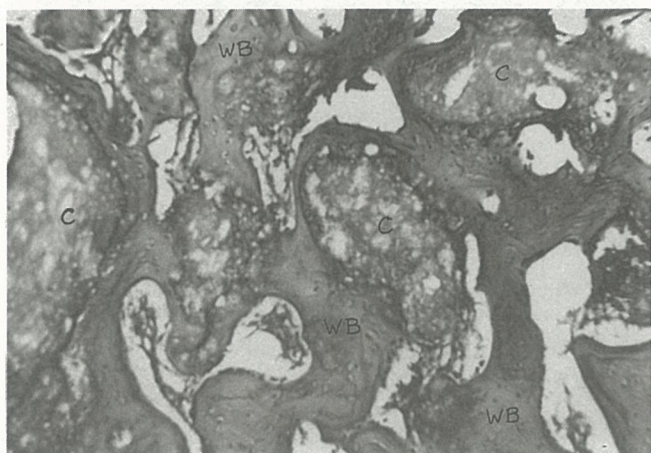


Fig. 5 Incorporation of ceramic into the bone at week 4
C - ceramic WB - woven bone

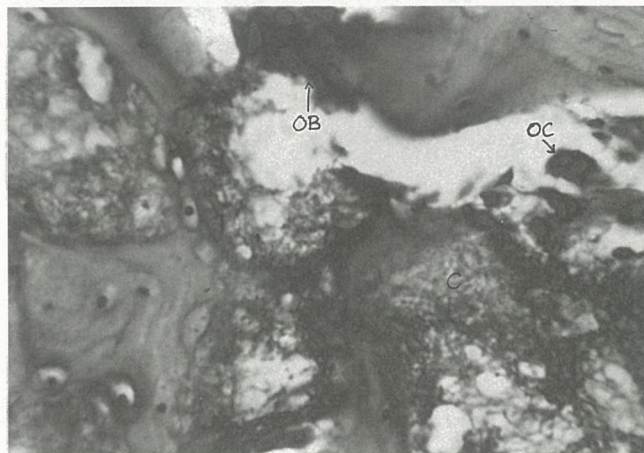


Fig. 6 Osteoblasts on surface of ceramics while being incorporated into the bone
C - ceramic OB - osteoblast OC - osteoclast

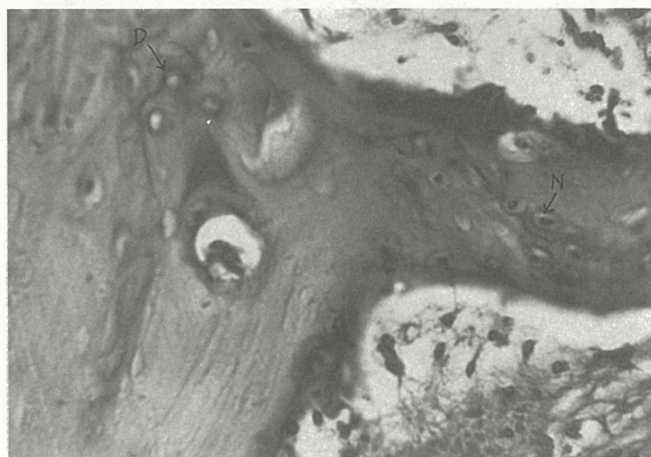


Fig. 7 New bone deposited in dead bone graft
D - dead bone graft N - new bone



Fig. 8 Implant lined by osteoblast
C - ceramic OB - osteoblast

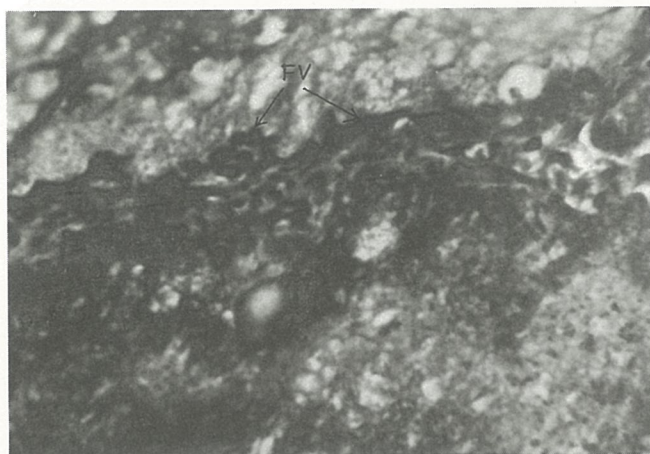


Fig. 9 Ingrowth of fibrovascular tissue into ceramic implant
FV - fibrovascular stroma C - ceramic

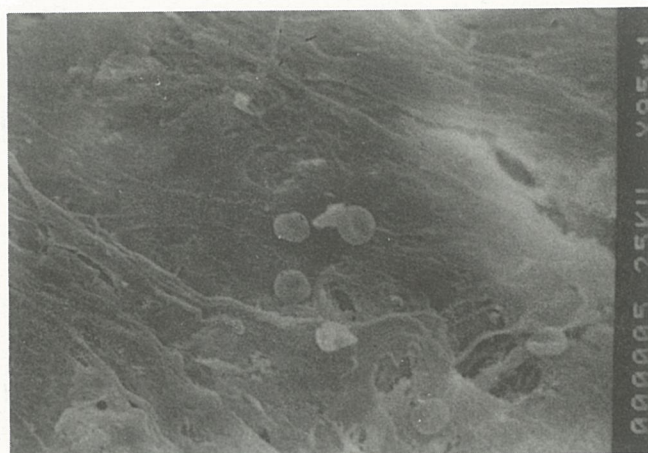


Fig. 10 Fibrous connective tissue covering ceramic

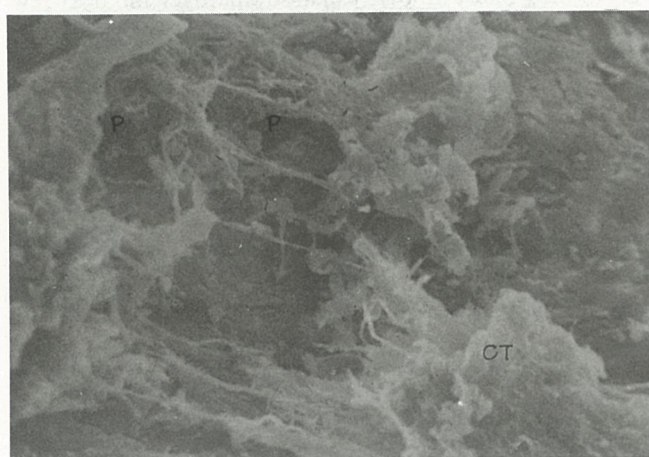


Fig. 11 Ingrowth of fibrovascular tissue into implant pore
P - pore CT - connective tissue

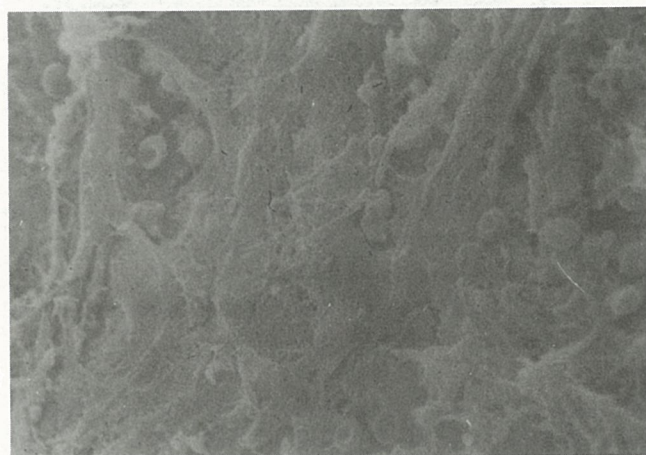


Fig. 12 Fibrovascular stroma ingrowth with minimal inflammatory reaction in autologous graft site

modelling of the new bone then takes place. These general stages of bone graft up took except remodelling were observed in both experimental and control groups, which were qualitatively comparable. The dental preparation of TCP was well tolerated in the environment of long bones in rabbits. The inflammation present was mild, disappeared by the fourth week and did not appear to interfere adversely with bone repair. The beneficial properties of autografts include osteoconduction and osteoinduction.^{1,10} The former refers to the property of the graft to provide a scaffold on which new bone is deposited, the latter refers to the property of the graft to stimulate ingrowth of osteogenic cells usually secondary to bone matrix proteins. Although a TCP implant lacks the cellular and inductive components of an autograft, Holmes⁷ suggested that coralline not a hydroxyapatite was significant impediment to incorporation. The osteoconductive property on TCP was clearly demonstrated in the study. Osteoblasts are seen lining the surface of the ceramic with deposition of new bone trabeculae. The resorbability of this preparation

can only be inferred by the presence of osteoclast on the ceramic surface. Eggli⁶ has shown that TCP is resorbed by osteoclast. The osteoinductive property of TCP could not be inferred by the study design. Morre et al⁸ reported that while TCP was not an osteoinductive, osteogenesis was enhanced when it was mixed with cancellous autograft. The demonstration of biocompatibility of this dental preparation in long bones gave it the potential for application as bone graft or as bone additive in filling cystic bone defects.

CONCLUSION

Dental forms of tricalcium phosphate ceramic when implanted on osseous defects of rabbit tibia elicited minimal inflammatory response and allowed the ingrowth of fibrovascular tissue into its pores with subsequent deposition of new bone. The stages of reparative process was qualitatively comparable to the autograft site on the contralateral tibia.

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