# Effect of Tricalcium Phosphate on New Bone Formation: An Experimental Study in Beagles

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Key words: tricalcium phosphate, bone formation, bone histomorphometry, speed of bone healing

## Abstract

Tricalcium phosphate was implanted into standardized defects in the maxilla and mandible of beagles. The tricalcium phosphate provoked new bone formation in the defects, which healed 0.02 mm/day faster than the untreated controls.

## Introduction

A large number of oral and maxillofacial surgery procedures result in bone defects, which pose problems with reconstruction. For the treatment of these defects, various natural and synthetic materials such as bone, cartilage, hydroxyapatite and tricalcium phosphate (TCP) have been used. For a material to be used as a graft or as an implant, it must be easily and cheaply obtainable, easily used, requiring no special equipment, and biocompatible. In addition, the material to be placed in bone should eliminate the defect by filling it completely until new bone forms. It should also be osteoinductive and resorbable, allowing its position to be filled by the newly formed bone as soon as possible.

Over the last two decades, hydroxyapatite and TCP, both calcium phosphate ceramics, have gained special interest because they attach directly to bone, binding both calcium and phosphate and preventing fibrous ingrowth[1,2]. Another advantage of TCP and hydroxyapatite is that they do not cause local or systemic toxicity[3].

TCP has been used in animals for reduction of periodontal defects[4], repair of pulpal perforations[5], closure of cleft palates[2] and in the reconstruction of alveolar ridges[6]. It has also been used in humans to fill periodontal defects[7] and for regeneration of periapical defects[8]. However, there is still a lack of agreement on the efficiency of TCP for inducing new bone formation[9,10] and there have been only a limited number of studies on the use of TCP in maxillary and mandibular bone defects[2,11]. The rate of ossification of defects filled with this substance has also not been studied fully.

The main purpose of the present study was to assess whether TCP (Synthograft, Miter, Inc., Indianapolis, IN) could be used for the healing of maxillary and

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mandibular bone defects. We also compared the speed and quality of the filling obtained with TCP with that of control defects.

## Materials and Methods

This study was performed on 6 healthy beagle dogs with permanent dentition. The animals were anesthetized with 50 mg/kg sodium thiopentane in saline solution injected intravenously. They were maintained under continuous IV injection of 5% dextrose until completion of the operation.

In each quadrant of the maxilla and mandibula, the area between the canine and the apex of the first premolar was exposed. In each exposed area, four defects were created with a steel surgical burr, 3.2 mm in diameter, until a depth of 4 mm was reached. Alternate defects were filled with TCP without application of pressure, whereas the other defects served as controls (Figs. 1, 2). The wounds sutured with silk. Randomly chosen pairs of animals were killed on the 10th, 20th and 40th days after the operation. Bone segments were resected and fixed in 10% buffered fermalin solution. A total of 48 slices were made, each containing a TCP-filled defect along with a neighboring control. These were embedded in paraffin, sectioned at 5$\mu$m in the horizontal plane and stained with hematoxylin and eosin (Fig. 3).

To detect the rate of new bone formation, bone height was measured with a microscope from the bottom of each defect to the highest new bone level with the aid of a scale for each study period. Then using the "speed: bone height/day" formula, the speed of daily bone formation was calculated. For the quality of new bone formation, the amounts of mineralized bone, connective tissue and new bone formation were determined using the point-counting technique and a graticule[12,13] (Fig. 4).

## Results

No suture-related infection or delayed wound healing occurred in any of the animals. Histologic analyses were performed for the three groups representing different time periods, each containing 32 lesions (16 experimental and 16 control) giving a total of 96. Histologic evaluation criteria were based on the degree of osteoblastic and fibroblastic activity, the degree of new bone formation and mineralization and the presence and type of inflammatory infiltrates.

10th day: Although osteoblastic differentiation and activity were observed around the implant material in the TCP-filled defects, there was no significant histologic difference between the experimental and control defects in terms of fibroblastic proliferation and inflammatory cell infiltration. Features of osteoblastic activity along the borders of the defects were also similar.

20th day: In the untreated controls, judged two-dimensionally, approximately the peripheral one-third of each defect was filled with new bone; the central areas were fibroblastic in character and contained some mononuclear inflammatory cells. More pronounced bone formation was observed in the experimental defects, all of which were about half-filled with new bone.

40th day: Two-thirds to three-fourths of each control defect was filled with

mature new bone (covered at the outer pole with fibrous tissue bundles). Only a few particles of the implanted material remained in the experimental defects, which were almost completely filled with new bone (Figs. 5, 6, 7). In the maxilla, two holes had been drilled accidentally into a tooth. One of these defects had been filled with TCP and showed more pronounced new bone formation (Fig. 8) than the other, which served as a control (Fig. 9).

The results of measurements of bone height are shown in Fig. 10. The proportion of mineralized bone, connective tissue and new bone in defective areas on the 40th day, determined by point-counting, are shown in Table II. There was no significant difference between the extents of healing of the maxillary and mandibular defects. At the end of the study period, filled defect shapes were different in the control and TCP groups, as shown in Fig. 11.

## Discussion

The potential of TCP to induce bone formation has generally been studied in long bones[14,15,16] and periodontal areas[7,11]. Although there is a need for more refined and complete data on the application of this implant material to the jaws, there has also been a scarcity of experimental studies mainly for anatomical reasons which preclude the standardization of studies. Some work has confirmed[3,17] the effect of TCP on bone formation but some authors still continue to doubt it[9,18,19]. BARKHORDER and MEYER[18] strongly emphasized that TCP should be avoided in inflamed areas, since it not only fails to induce bone formation but also increases resorption. In our study, we did not observe any significant inflammatory reaction on or adjacent to the defects filled with TCP. As shown in Fig. 5, TCP served as a matrix for bone formation and as early as day 20, it started to be resorbed, giving way to new bone. FERRARO[19] detected traces of TCP 18 months after its placement in dogs. In CUTRIGHT's[14] work, 95% of the TCP used in tibias of guinea pigs was resorbed in 48 days. However, the period of bioresorption probably changed according to the volume of the defects. As stated by JARCHO and SALSBURG[20] and UCHIDA et al.[21], the porous nature of TCP makes both new bone formation and bioresorption easy. Our $4 \times 3.2$-mm defects were comparatively smaller than those used by previous workers, and this probably contributed to the fact that only a very small amount of TCP remained on the 40th day. In our study, bone height measurements were significantly different ($P < 0.05$) in the control and study groups on the 20th and 40th days (Fig. 10).

We calculated that bone formation in our standardized defects filled with TCP was 0.02 mm/day faster than that in controls. The shapes of the study and the control defects were found to be different on the 40th day. The remodelling process seemed to alter the contour of the control defects more than those filled with TCP. In naturally healed controls, owing to resorption at the edges of the defects, the bone heights attained were rather lower than the original level. The process of new bone mineralization was also more rapid and pronounced in the experimental group than in the controls (Table 1). This observation suggested that use of TCP may be preferable in situations where rapid bone formation is anticipated.

The differences in bone height and the speed of bone formation may have

resulted from the different remodelling process in the naturally healed control group, and the fact that TCP served as a guide and support matrix for new bone

Table 1 Bone histomorphometry results on day 40

| | Mineralized bone % | Connective tissue % | New Bone % | |
|---|---|---|---|---|
| Control | 19 | 54 | 27 | $P<0.05$, significant |
| TCP | 26 | 34 | 40 | |
| Accidental Control | 27 | 61 | 12 | |
| Accidental TCP | 26 | 36 | 38 | |

formation in the experimental group. Similar results were also obtained for the two defects accidentally drilled in a tooth. Whether this was a true osteoinductive effect remains to be investigated using different study designs.

We believe that TCP has a definitely beneficial action and that it seems ideal for use in non-inflamed tissues where there is a need for rapid repair of bone defects.

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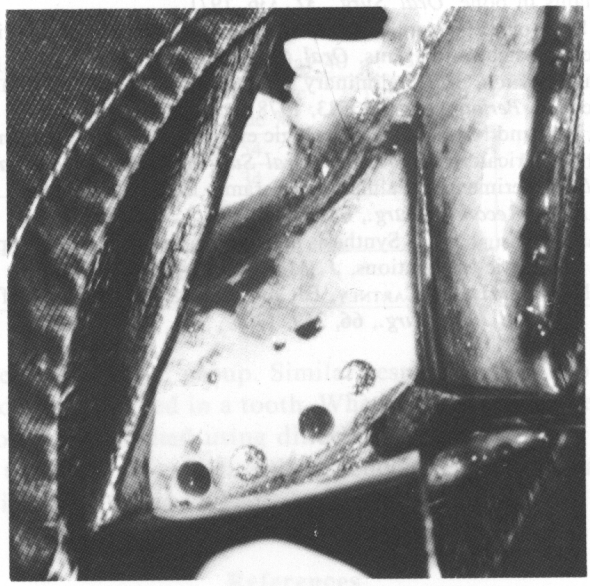

Fig. 1 Intraoral view showing standardized defects in the mandibular bone of a beagle. Two of the defects are filled with tricalcium phosphate and the others have been left to heal naturally.

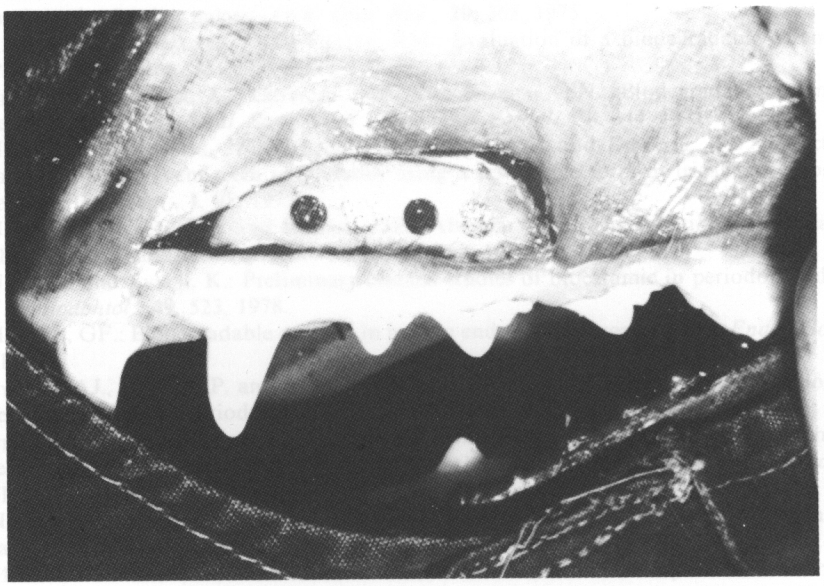

Fig. 2 Standardized defects in the maxillary bone of a beagle

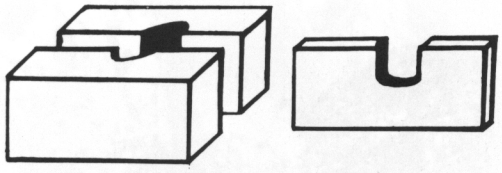

Fig. 3 Defects were sectioned in the horizontal plane proper for bone height measurements.

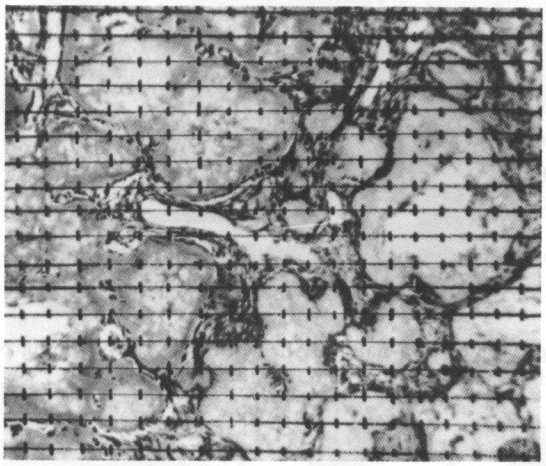

Fig. 4 Graticule placed over the defect area to quantify areas by point-counting

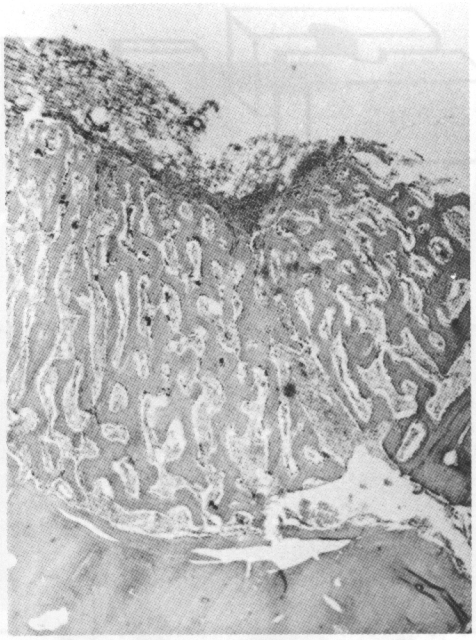

Fig. 5 Histologic appearance of a naturally-healed defect. Remodelling process seems to have lowered the bone height. (Hematoxylin and eosin stain. x75)

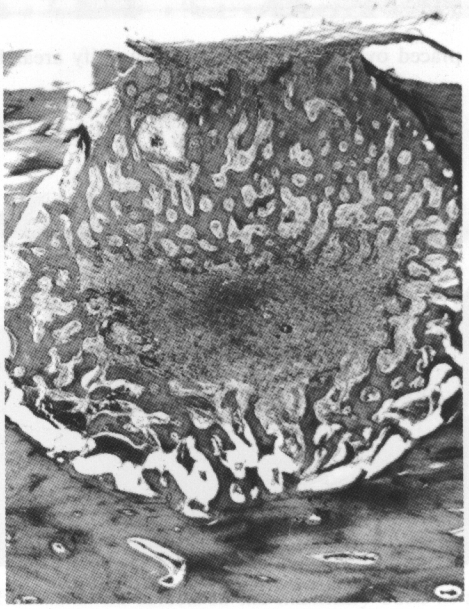

Fig. 6 Histologic appearance of a defect filled almost completely with new bone from the experimental group on the 40th day. (Hematoxylin and eosin stain. x75)

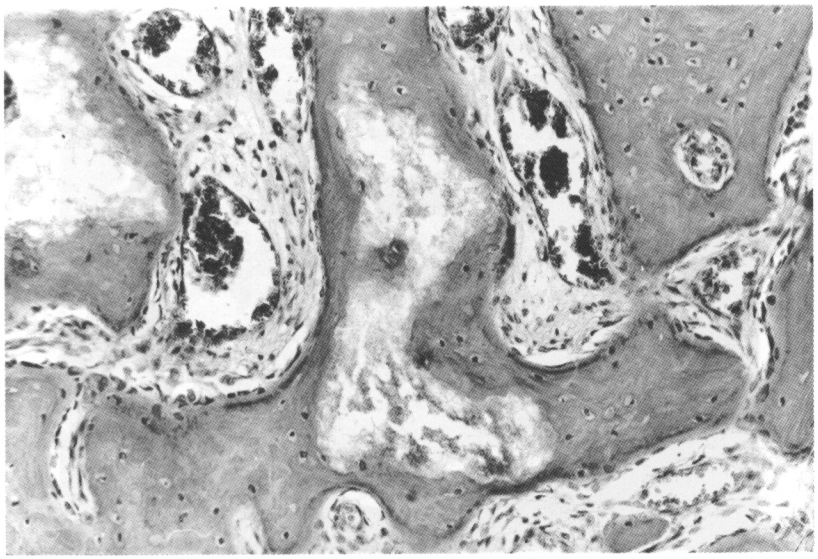

Fig. 7 New bone both around and inside tricalcium phosphate particles on the 40th day in the experimental group. (Hematoxylin and eosin stain. x500)

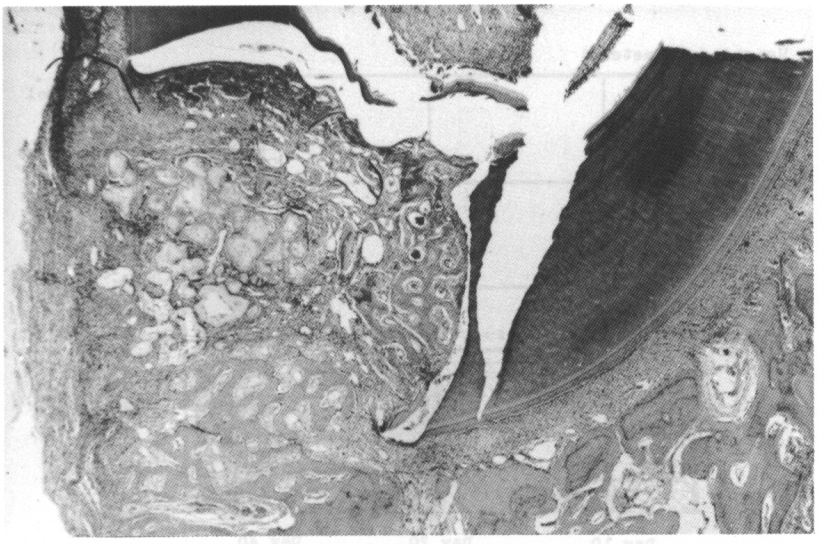

Fig. 8 New bone formation in accidentally drilled tooth of an experimental animal on the 40th day. (Hematoxylin and eosin stain. x75)

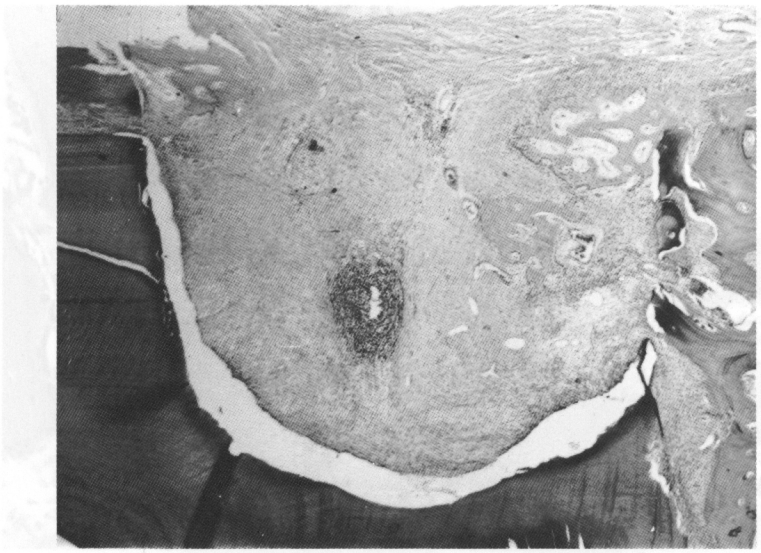

Fig. 9 Bone formation in accidentally drilled tooth of a control animal on the 40th day. (Hematoxylin and eosin stain. x75)

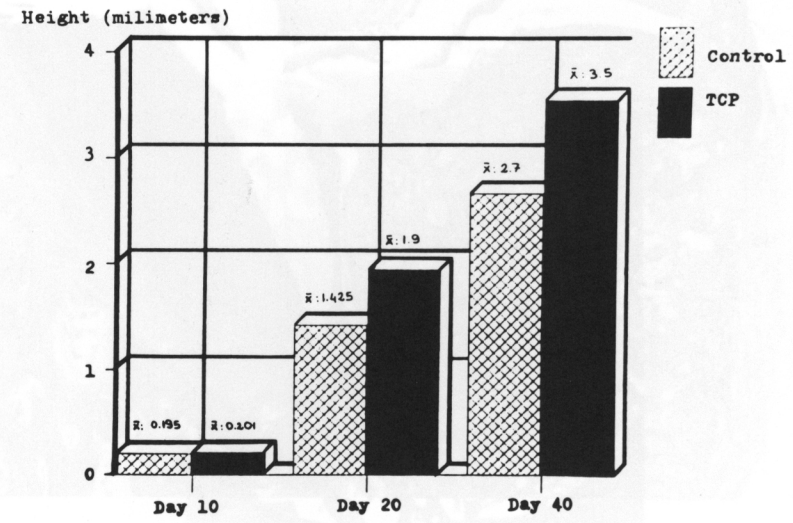


Fig. 10 Heights of newly formed bone in standardized defects 4 mm deep.
Day 10, control (x: $0.195 \pm 0.0163$) and experimental (x: $0.201 \pm 0.0111$) groups. $P < 0.10$, insignificant.
Day 20, control (x: $1.425 \pm 0.129$) and experimental (x: $1.9 \pm 0.137$) groups. $P < 0.05$, significant.
Day 40, control (x: $2.7 \pm 0.141$) and experimental (x: $3.5 \pm 0.155$) groups. $P < 0.05$, significant.

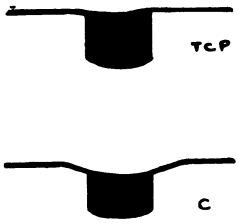


Fig. 11 Shapes of healed defects differed between the TCP and control groups.