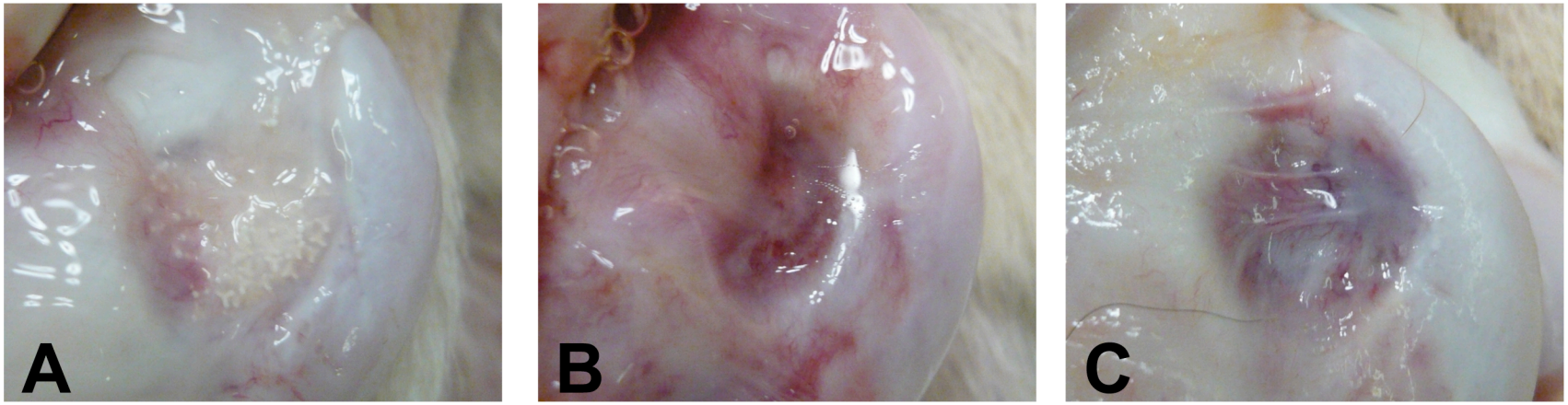

Fig.3-4 Gross appearance of femoral condyle defect in each group at 8 weeks of implantation. (A) Tetrabone®,(B) β-TCP granule, and (C) control groups. In the Tetrabone® group, Tetrabone® was well filled inside the defect and showed smooth surface at the opening. The opening of the defect was concave-shaped and was covered with fibrous tissue in the β-TCP granule (B) and control (C) groups.

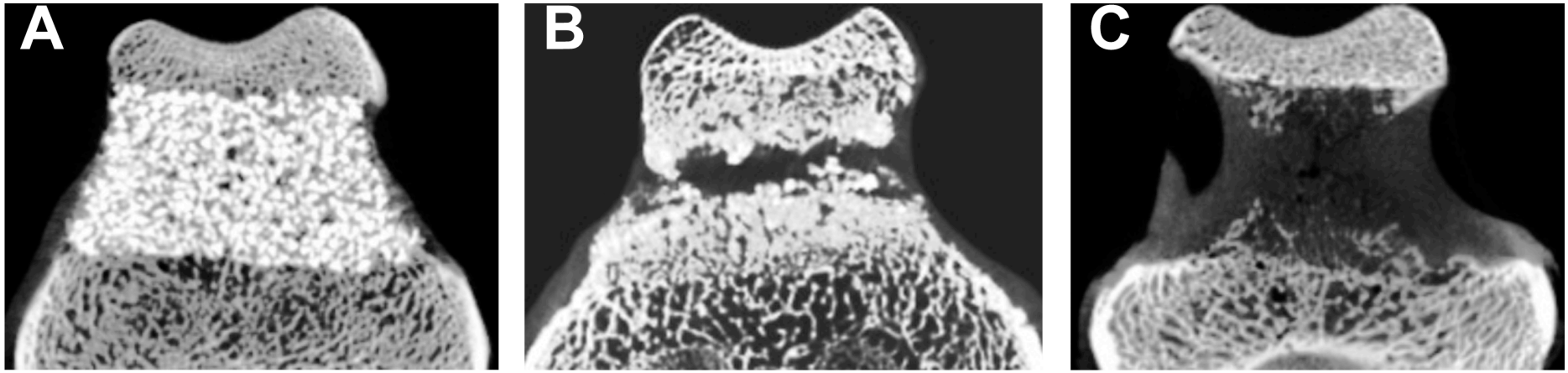

Fig.3-5 The typical transverse micro-CT images of each group at 8 weeks of implantation. (A) Tetrabone®, (B) β-TCP granule, and (C) control groups. Tetrabone® was well connected with new bones inside the defect. The β-TCP granules were resorbed and disappeared at the both ends and the central area of the defect. Some new bones were found in the defect in the control group.

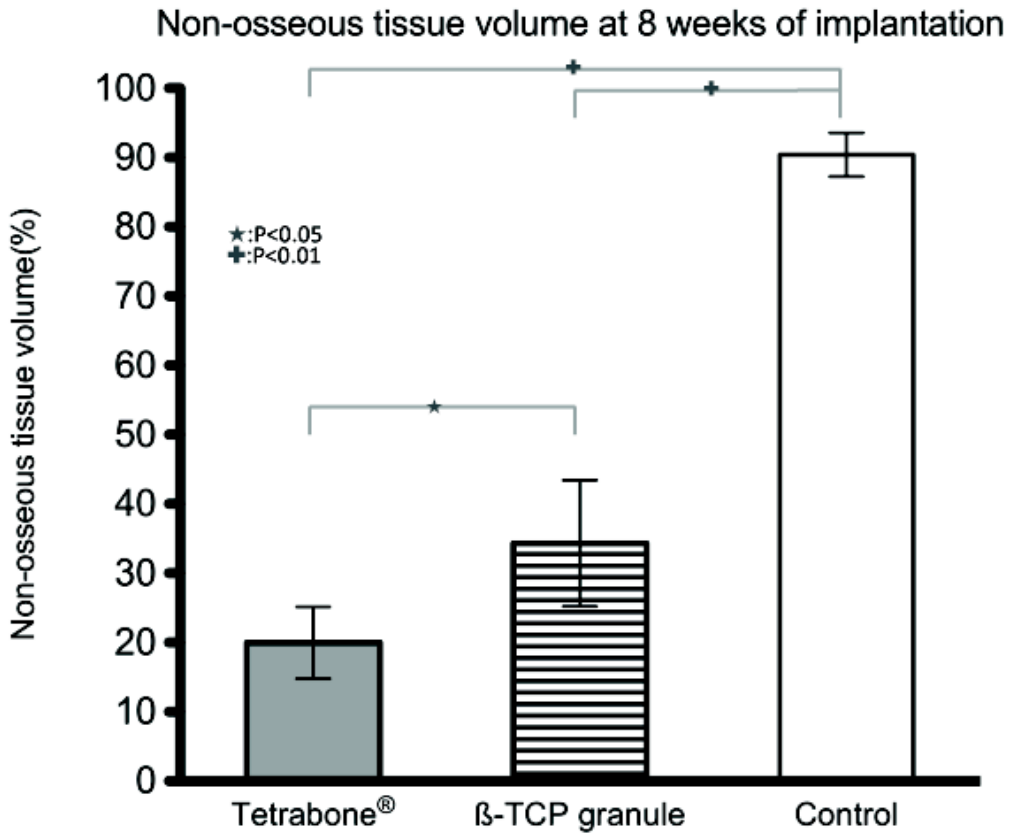

Fig.3-6 The non-osseous tissue volume in the micro-CT images at 8 weeks of implantation. Values are shown as mean ± SD. Each volume was analyzed from reconstructed 3D data and expressed as a percentage in the region of drill hole. The non-osseous tissue volume was significantly higher in the β-TCP granule group than that in the Tetrabone® group. Additionally, the non-osseous tissue volume was significantly higher in the control group than other groups.

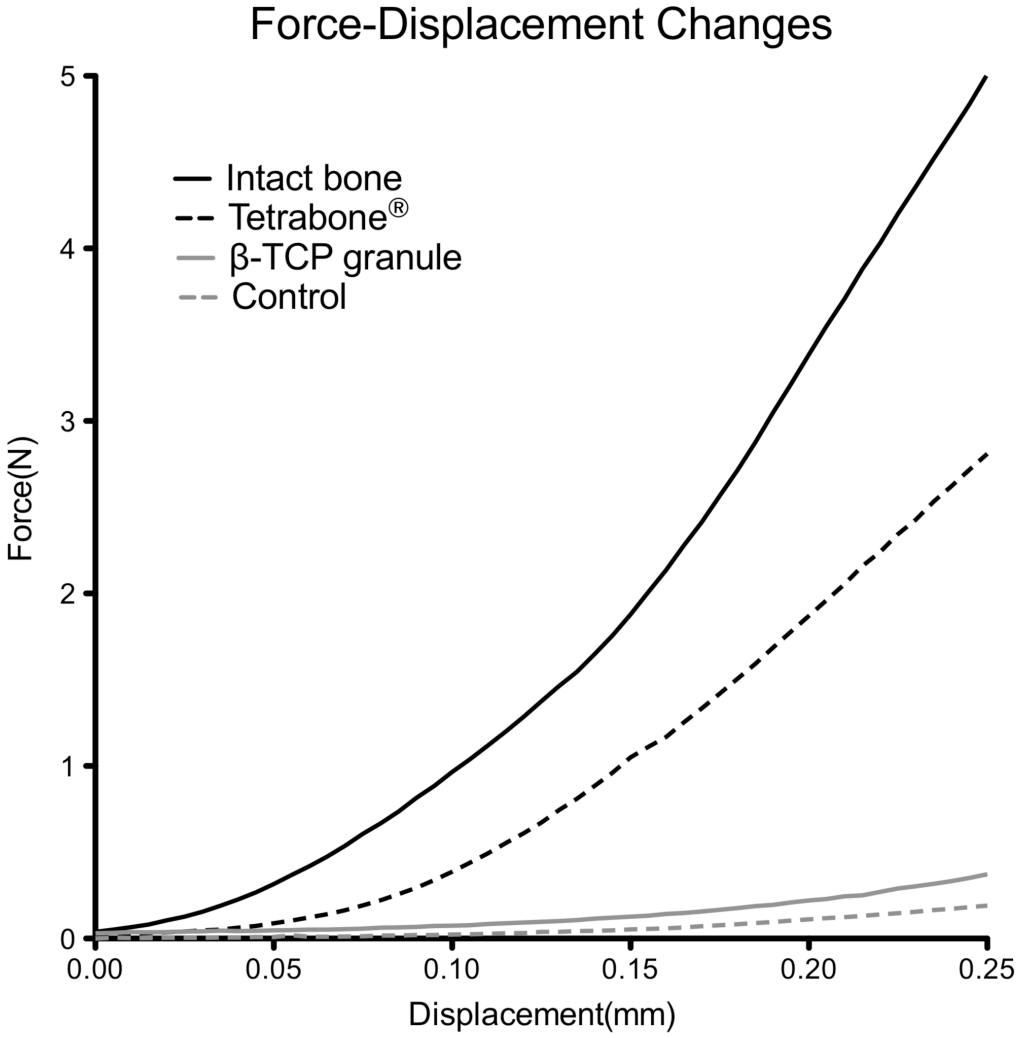

Fig.3-7 The force-displacement curves at 8 weeks of implantation in each group.

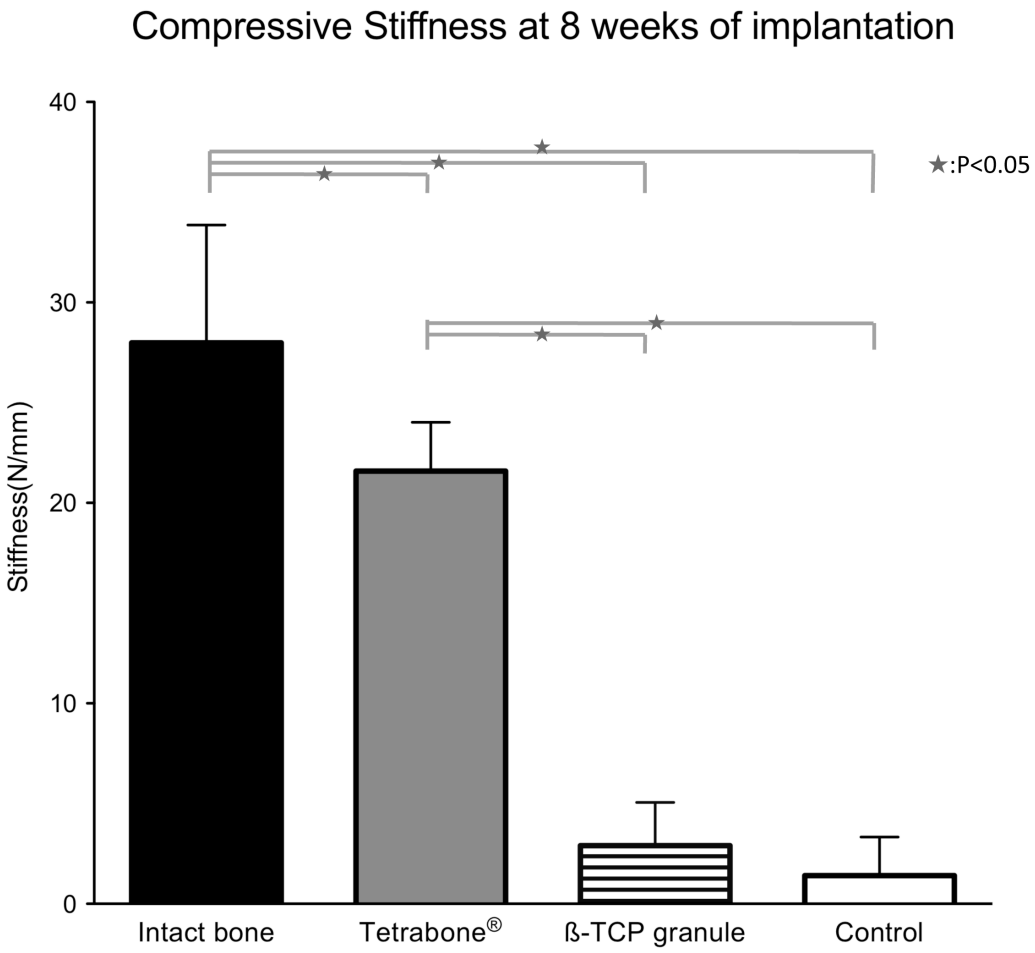

Fig.3-8 The compressive stiffness at 8 weeks of implantation in each group. Values are shown as mean ± SD. The compressive stiffness was significantly higher in the Tetrabone® group than the β-TCP granule and control groups.

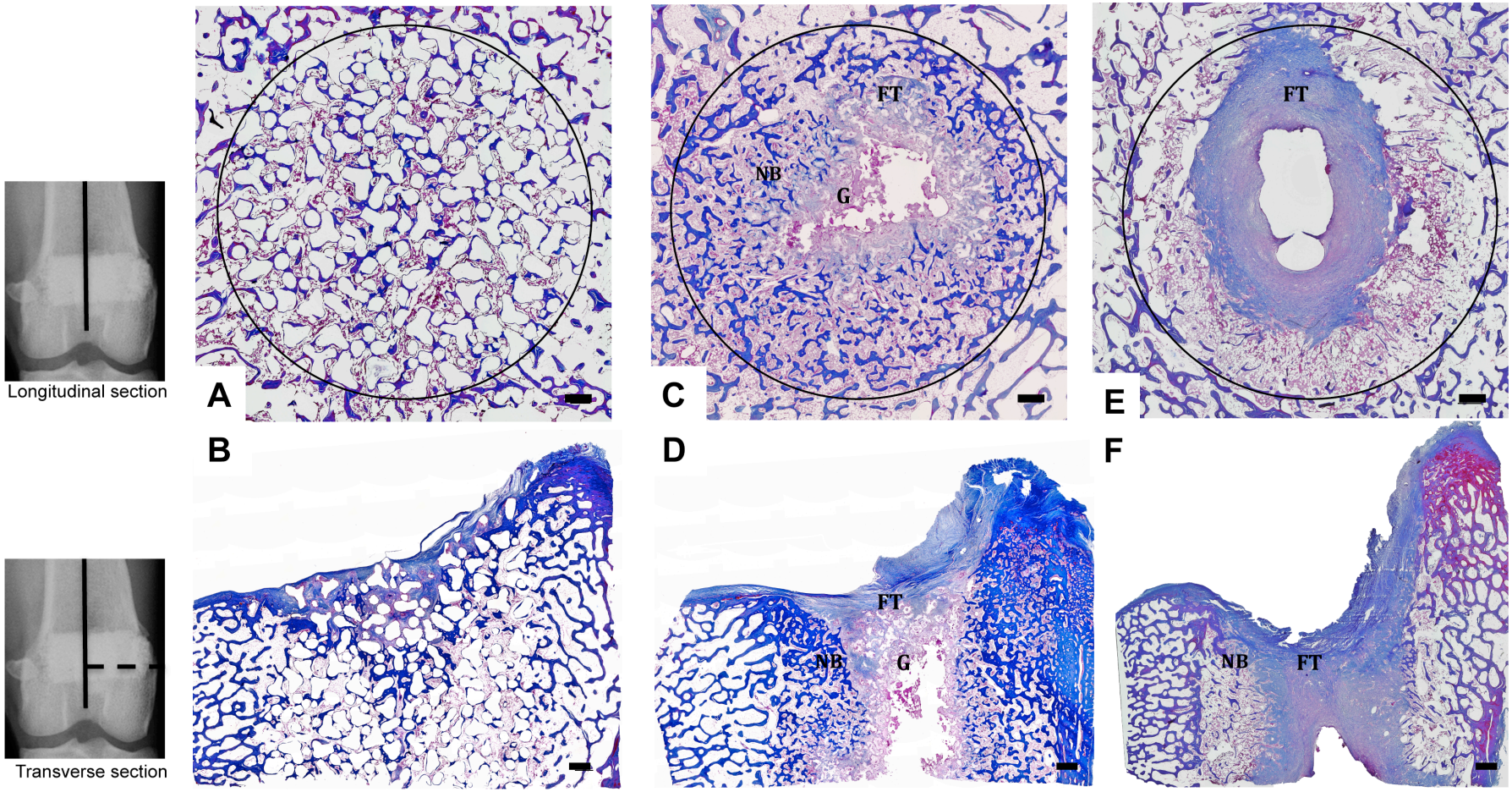

Fig.3-9 The typical histological findings of the Tetrabone® (A, B), β-TCP granule (C, D), and control (E, F) groups at 8 weeks of implantation. The longitudinal section (vertical line, A, C, E) at the center of the defect and the transverse section of the defect (horizontal dotted line, B, D, F) are shown. New bones were found in the intergranular spaces between Tetrabone® granules and fully distributed in the defect area (A, B). The β-TCP granules were resorbed and fibrous tissues were left in the central area of the defect(C, D). The fibrous tissues and an empty space in the center of the defect were found in the defect in the control group (E, F). (NB: new bone, G: β-TCP granule, FT: fibrous tissue; Masson's Trichrome stain, scale bar: 1mm)

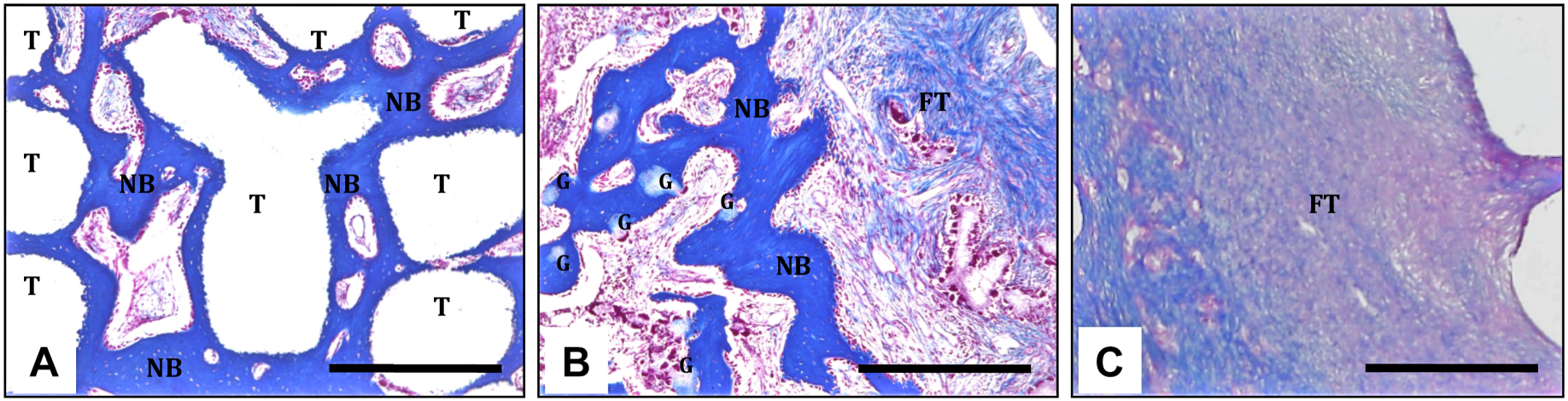

Fig.3-10 The high magnification of the histological findings of the transverse section. (A) Tetrabone®, (B) β-TCP granule, and (C) control groups at 8 weeks of implantation. (T: Tetrabone®, G: β-TCP granule, NB: new bone, FT: fibrous tissue; Masson's Trichrome stain, scale bar: 500μm)

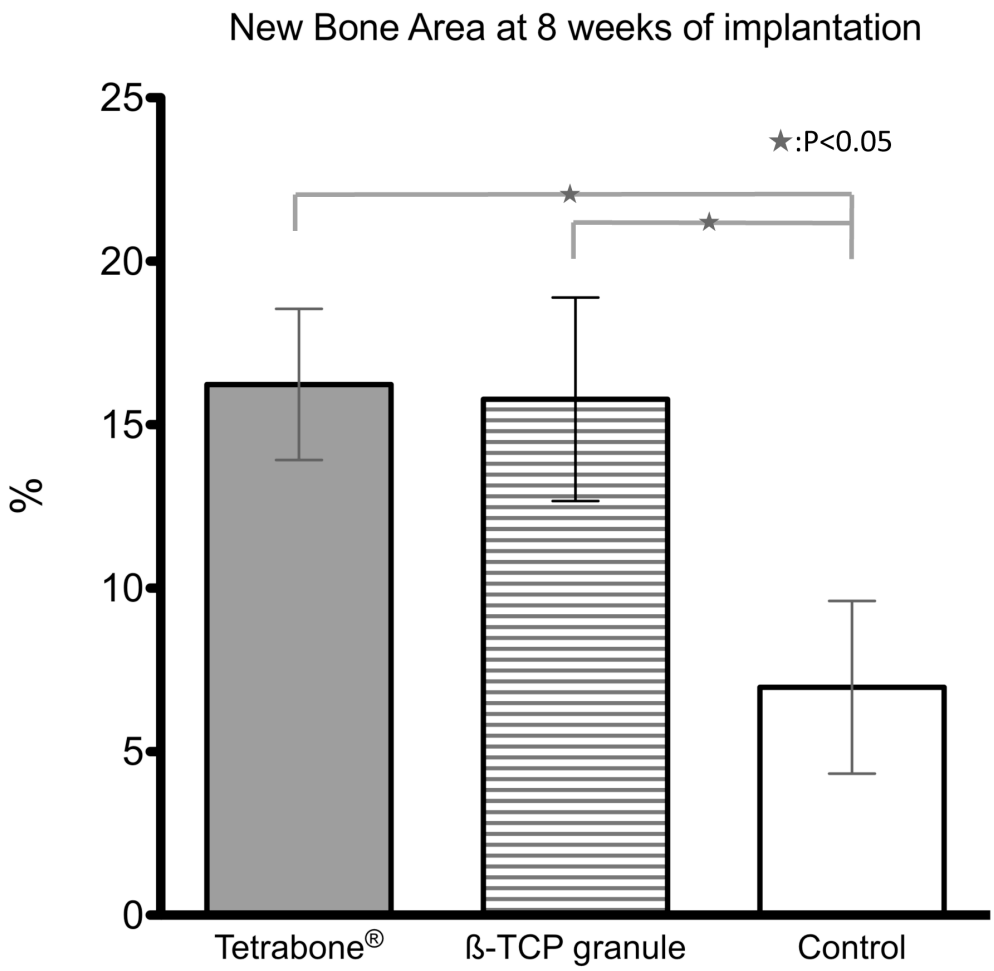

Fig.3-11 The new bone area on the histological sections of all groups at 8 weeks of implantation. Values are shown as mean ± SD. New bone area was significantly higher in the Tetrabone® and β-TCP granule groups than in the control group, however there was no significant difference between the Tetrabone® group and the β-TCP granule group.

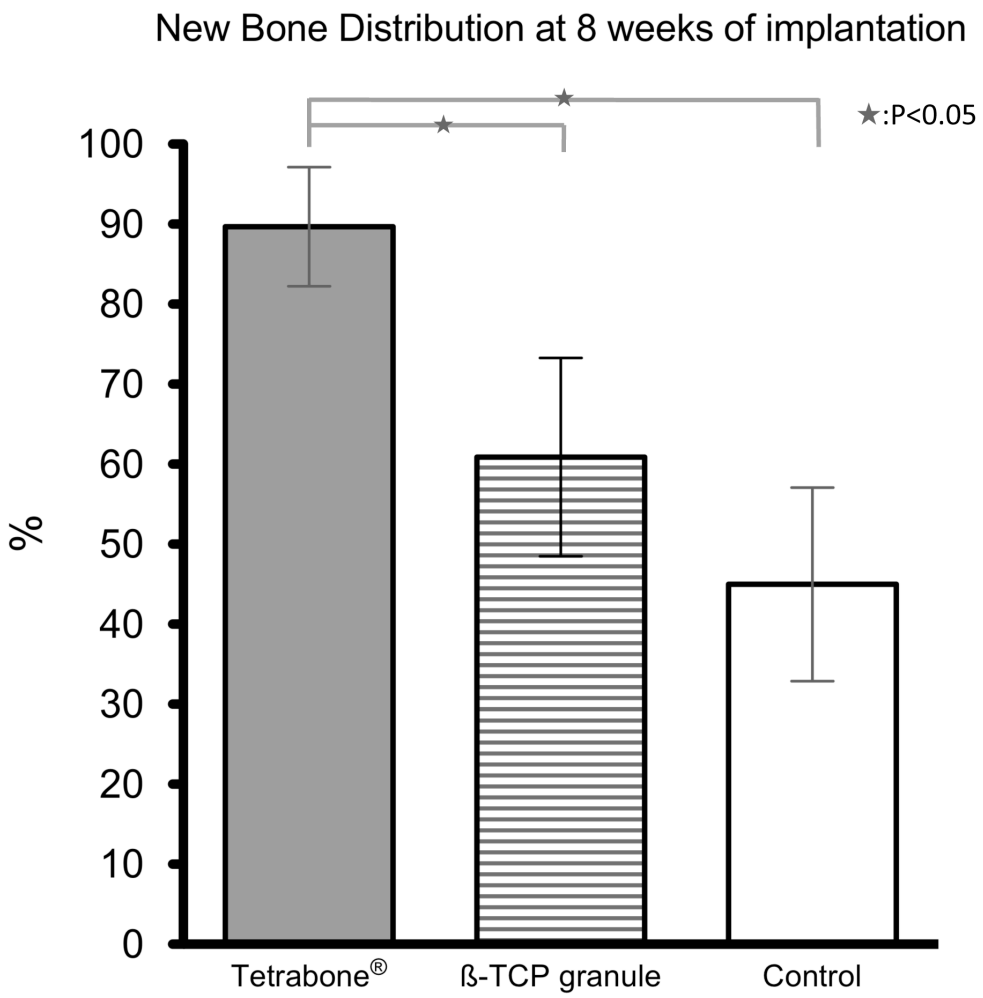

Fig.3-12 The new bone distribution on the histological sections of all groups at 8 weeks of implantation. Values are shown as mean ± SD. The area inside the red line (Fig. 3-1) was defined as new bone distribution volume. New bone distribution was significantly higher in the Tetrabone® group than in the β-TCP granule and control groups.

Conclusion

A variety of artificial bones have been used to repair the large bone defects resulting from fracture, bone tumors, and congenital skeletal deformity. Additionally, an ideal artificial bone should resorbed with an adequately rate but also provide a three-dimensional matrix to support bone ingrowth during resorption. Therefore, tricalcium phosphate (TCP) has been most widely used in the clinical practice.

Some commercially available granular artificial bones of β-TCP showed osteoconductive function, however, insufficient mechanical strength of the defect and rapid resorption of the materials were still need to be conquered. Therefore, we fabricated novel tetrapod-shaped granular artificial bones (Tetrabone®) using micro-particles of α-tricalcium phosphate (α -TCP) by injection molding and succinic acid treatment to form octacalcium phosphate (OCP) on the surface. The tetrapod-shaped structure of that is expected to maintain the shape and mechanical strength of the defect site, and also produce effective intergranular pores for cells and vessels invasion after aggregation. In this thesis, the new bone formation and mechanical properties of tetrapod-shaped granular artificial bones to repair femoral defect in animals were investigated.

In chapter 1, I investigated the long-term effect in new bone regeneration and safety of the Tetrabone® implanted into the femoral condyle defect in rabbits and compared the results with β-TCP granules. There were no side effects in any rabbits receiving Tetrabone® during 26 weeks of experiment, however, granule

leakage was found in the β-TCP granule group. It is indicating the safety of Tetrabone® as an artificial bone.

On gross findings, micro-CT analysis, and histology, Tetrabone® retained well inside the defect until 26 weeks, however, β-TCP granules were resorbed in a shorter time, which might lead the concave shape at the opening of the defect. In addition, new bones area in the Tetrabone® group was more than that in the β-TCP granule group at 13 and 26 weeks. These results suggest that β-TCP granules could not maintain the defect shape before enough new bones were formed, and Tetrabone® had better osteoconductivity than β-TCP granules in the long-term implantation. However, in this chapter, mechanical strength was not evaluated and the control rabbits without any implantation showed repair of the defect.

In chapter 2, I investigated the mechanical properties of the Tetrabone® implanted to the femoral defect in canine cadavers and to compare these characteristics with the β-TCP granules. The canine femoral defect model was used in this chapter. In the femoral bone fragment compression, the ultimate compressive load of the Tetrabone® group was almost half of the intact bone and was significantly higher than that of the β-TCP granule and control groups. The elastic modulus was significantly higher in the Tetrabone® group than that of the β-TCP granule and control groups. In the defect insertion testing, the slope of force–displacement curve was higher in the Tetrabone® group than other groups, and the compressive stiffness was significantly higher in the Tetrabone® group than other groups. In conclusion, it was confirmed that Tetrabone® implanted in the canine femoral defect model showed better mechanical properties than β-

TCP granules *in vitro*. However, further research on the usefulness of Tetrabone® *in vivo* should be needed.

In chapter 3, I investigated the *in vivo* effect of Tetrabone® on new bone formation and mechanical properties using a canine femoral large defect model and compared the effects with those of β-TCP granules. After 8 weeks of implantation, no clinical side effect was observed in all experimental groups. On gross findings, the end of the defect was concave in the β-TCP granule and control groups, but in the Tetrabone® group, the defect were kept filled up with implants. On radiography, CT, and micro-CT analysis, the center of the defect in the β-TCP granule group was shown as dark appearance, suggesting the earlier resorption of the β-TCP granules and no new bone formation, however, Tetrabone® retained tightly in the defect at 8 weeks of implantation, showing no resorption. Additionally, compressive stiffness of the defect in the Tetrabone® group was maintained almost 80% of the intact bone after 8 weeks of implantation and was significantly higher than that in the β-TCP granule group. These results suggest the better mechanical strength of the defect implanted with Tetrabone® than β-TCP granules.

In the evaluation of new bone regeneration, new bone distribution was significantly higher in the Tetrabone® group than that in the β-TCP granule group, though new bone area and new bone volume were similar in both groups. The new bone tissues in the Tetrabone® group were fully distributed in the defect area, however, in the β-TCP granule group, granules were resorbed and the new bone tissues were partially distributed mainly adjacent to the defect margin. These results indicated that the interconnectivity of the intergranular

pores were effective for new bone invasion in the Tetrabone® group and Tetrabone® had better osteoconductivity than the β-TCP granule.

In conclusion, Tetrabone® showed slower resorption rate and better process of new bone formation than β-TCP granules. Tetrabone® provides much higher mechanical strength and better osteoconductivity of the defect than β-TCP granules. These results encourage the application of Tetrabone® for the large bone defects in the clinical practice.

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