

Article ID: 1003 - 6326(2003)01 - 0065 - 04

Osteogenesis process of tricalcium phosphate ceramics in vivo^①

DAI Hong-lian(戴红莲), LI Shi-pu(李世普), YAN Yu-hua(闫玉华),
LI Xiao-xi(李小溪), JIA Li(贾莉)
(Biomaterials and Engineering Research Center,
Wuhan University of Technology, Wuhan 430070, China)

Abstract: To investigate the osteogenesis of calcium phosphate ceramics, β -TCP ceramics were implanted into the condyle femur of rabbits, and tetracycline was injected timely. Specimens were host at 1, 2, 3, 4, 5, 6 months after implanted. The new bone formation and osteogenesis process were observed by the histomorphology, fluorescent microscope, SEM and EPMA. The results demonstrate that, osteogenesis is active, there are abundant osteoblasts on the surface of osteoid, mesenchymal cell hyperplasia and incursion is found in materials after 1 month. After 2 months, there is blood vessel formation and macrophage soakage within materials. Bone island appears and connects by bone bridge after 3 months. β -TCP ceramics degrade and are dispersed by new formation bone. Woven bone turns into bone lamella by rebuilding and calcification. The materials entirely change their original shape and combines with bone tissue as a whole after 6 months. The typical structure of spongy bone forms. It is confirmed that β -TCP is a degradable biocompatible artificial bone material which can incorporating in life.

Key words: β -tricalcium phosphate; osteogenesis; fluorescence; biodegradation

CLC number: R 318.08

Document code: A

1 INTRODUCTION

Losses of bone substance for congenital, tumoural or traumatic reasons need a long time treatment with bone grafts^[1]. To avoid the drawbacks of autografts (blood loss, haematoma, pain, risk of infection) or heterografts (rejection, infection, viral risk), various synthetic bone substitutes are now proposed. Among them, calcium phosphate ceramics(CPC) appear suitable^[2], since their chemical composition is very close to the mineral phase of natural bone. Such materials have been applied clinically as a bone substitute and shown to be biocompatible and good bioaffinity as well as osteointegrative^[3,4]. When implanted in osseous sites, these materials can bind directly with bone without an intervening fibrous layer^[5,6]. CPC, which are usually used for this purpose, are made of beta-tricalcium phosphate(β -TCP), hydroxyapatite or their mixture^[7]. Though there is significant experience in application of CPC, the nature and mechanism of osteogenesis, namely the process of bone tissue formation on CPC in the biological environment resulting in physicochemical integration of ceramics with living bone, are not still clear^[8]. Thus the problem of osteogenesis must be studied in close connection with the ceramic state at different stages of implantation. It is well known that β -TCP ceramics have better bioactivity than other CPC. It had excellent biocompatibility and biodegradable capability. In

this work the process of bone tissue formation in porous β -TCP in vivo was studied in order to investigate its osteogenesis.

2 EXPERIMENTAL

2.1 Implant materials

In order to obtain porous β -TCP ceramics, the β -TCP powder (average size of 1 μ m) prepared by wet chemical precipitation method, was mixed with high-temperature binder and pore-forming material, foamed by the resin foaming method and sintered in air at 900 °C for 2 h. The porosity was 40% - 50%, and the mean pore size ranged from 100 to 800 μ m, the density was 1.05 - 2.00 g·cm⁻³ and the average compression strength ranged from 15 to 30 MPa. The structure was found that the crystalline grains are connected necked, and all the pores are interconnected. Cylindrical block with d 5 mm \times 8 mm was implanted in bone defects.

2.2 Surgical procedure

Twenty-four New Zealand white rabbits, weighing 2.0 - 2.5 kg were divided into six groups according to different stages of implantation. Animals under general anesthesia were subjected to a standard operative procedure in the area of both condyle femurs. Bone defects(d 5 mm \times 8 mm) were created with a dental drill perpendicular to

① **Foundation item:** Project(G1999064701) supported by the National Key Fundamental Research and Development Program of China

Received date: 2001 - 12 - 06; **Accepted date:** 2002 - 01 - 28

Correspondence: LI Shi-pu, Professor.

the long axis of the femoral shaft. Following press-fit of the ceramic blocks were inserted into the defects. Each animal was implanted with two cylinders. In each group, one rabbit was not be implanted acted as a control. One rabbit from each experiment group and control group were injected twice with 15% tetracycline($30 \text{ mg} \cdot \text{kg}^{-1}$) liquor before sacrificed. After 1, 2, 3, 4, 5 or 6 months, as well as the control group, the implants and the surrounding tissue were excised and placed into 10% buffered formalin solution or 2.5% buffered glutaraldehyde solution.

2.3 Histologic study

For undecalcified sections, six fixed specimens from each group were dehydrated in an ethanol series, and then embedded in methylmethacrylate. Three specimens were cut into 5 μm sections in thickness perpendicular to the long axis of the specimen, and stained with toluidine blue. The others tetracycline-labeled were cut into sections of 10 μm to observe under fluorescent microscope. The rest specimens refrigerated in glutaraldehyde solution, was washed in an ultrasonic cleaner, dehydrated in 95% alcohol and examined using SEM and electron probe X-ray microanalyzer.

3 RESULTS

According to the roentgenogram, the space between ceramics and bony tissue became vague after one month. After five months, ceramics and bony tissue were integrated and the external form of the material became incomplete. After six months, some part of the materials were disappeared and the residual became to be fragments or particles. After implantation, the implant inserted into the medial cortex of rabbit's femur was tightly united with the host bone in all cases, without any sign of movement at the interface. The portion of the porous β -TCP exposed outside of the cortex was partially covered with new bone after one month.

Light microscopy showed that, β -TCP has no early adverse effects, inflammation and foreign body reaction. One month after transplantation, the interface was visible, there were abundant osteoblasts on the surface of osteoid, mesenchymal cell hyperplasia and incursion was found in materials. New bone that showed an irregular woven bone structure appeared on the surfaces and in pores of implanted β -TCP. The new woven bone often formed masses in shape together with chondrocytes and mesenchymal cells, lined by a layer of osteoblast on their surfaces, and generally located at periphery of the masses. New capillaries appeared in the center of masses(Fig. 1). Osteoids and thickened trabeculae of bone almost

completely filled the outside of ceramic pores after two months. More deep pores were filled by woven bone and partially mineralized matrix. There was blood vessel formation and macrophage soakage within materials, and the osteoid turned into lamella bone through calcification. After three months, thickened trabeculae and dense plates of lamellar bone were deterred in all pores of the ceramic block. The bone ingrowth patterns resembled Haversian canal structures instead of woven bone structures by rebuilding. Bone-island appeared and connected by bone-bridge. A great deal of medullary tissue has grown into the lacunae of the materials after four months. After six months, the residual materials are surrounded by bony tissue(Fig. 2). Prior to and during bone formation, the number of osteoclast-like multinucleated giant cells decreased with the increase of already mineralized areas of the pore inner surface.

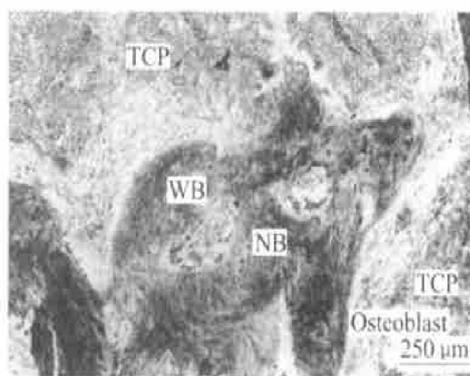


Fig. 1 New bone(NB) formation at periphery of ceramics(TCP) and irregular woven bone(WB) pattern after one month implantation

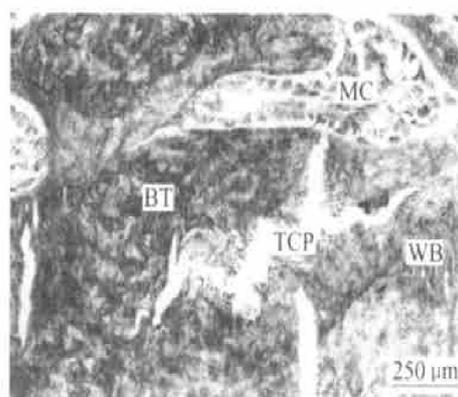


Fig. 2 New bone(NB) showing osteon structure forming bone trabecul(BT) and narrow cavity(MC) after six months implantation

Fluorescent microscope observation indicated that, osteogenesis is active; double fluorescence bands that are the symbol of new bone forming are found evidently and densely on the interface between β -TCP ceramics and

bone after one month. As the time went on, β -TCP particles were surrounded by fluorescence which became more markedly, and fluorescence appeared clearly among β -TCP particles in the center of defect section. β -TCP ceramics degraded and were dispersed by new formation bone (Fig. 3). The great mass of materials was substituted by bone tissue. While at the same period, the control group did not appear fluorescent phenomenon.

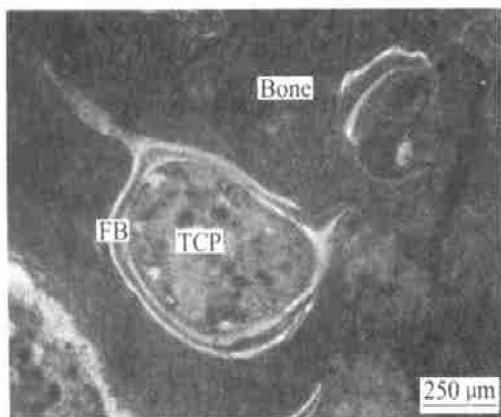


Fig. 3 Double fluorescent cycle directly embraced β -TC particles in center of defect area after six months implantation,

SEM morphology demonstrated that, the interface among particles changed obviously after implantation. The original connection among particles broke up. The particles were separated and their external form became irregular or incomplete. Five months after implantation, we found that the crystal granules of material have already altered their formation under EPMA. Unlike regular crystal shape these granules' pointedness has died away and their edge has been softened. Meanwhile the material's granules began to get touch with the bone firmly (Fig. 4) and life element emerged inside the material (point a) such as C, N, S and so on (Table 1). New bone formation binding with grafted material closely increases with time (Fig. 5). The materials entirely lost their original shape and combined with bone tissue as a whole after six months. Some particles could be found in the Haversian system of new bone. The typical structure of spongy bone formed.

Table 1 Element analysis of point by EDXA

Element	Mass fraction/ %	Mole fraction/ %
O	28.61	46.98
Na	1.22	1.39
Mg	0.74	0.80
P	25.63	21.75
S	0.32	0.26
Ca	43.17	28.3
C	0.31	0.52
Total	100	100

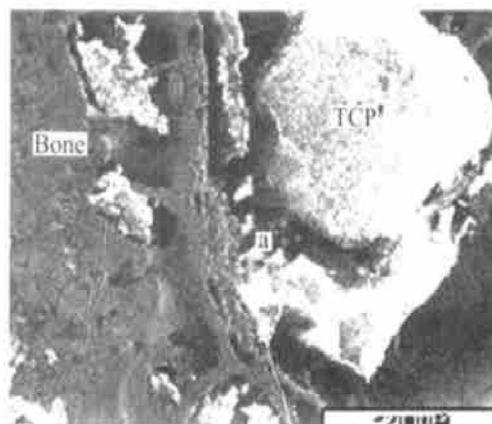


Fig. 4 EPMA photograph of material's granule after six month implantation

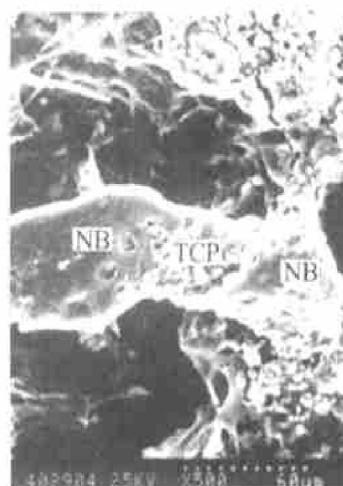


Fig. 5 SEM photograph of new bone tissue directly connect with β -TCP ceramics after six month implantation

4 DISCUSSION

The results showed that the synthetic porous β -TCP ceramics are capable of inducing osteogenesis. This ability depends on biodegradation and bioactivity of the ceramic, which are improved by little crystal size, low-temperature sintering, phase composition, porous structure and the addition of binder. The biodegradation process is caused by the action of living systems. The dissolution process in body fluid and the absorption process mediated by cells. On one hand, the particles of β -TCP ceramics can be dissolved continuously or be degraded by cells (e. g. phagocytes or osteoclasts)^[9], which stretch out tiny processes to wrap the grains and phagocytize them into their body, then phagolysosome in plasma release hydrolytize enzymes to the particulates and make the originally alkaline internal circumstance acidulous. This helped β -TCP ceramics to be degraded. On the other hand, the acid products of metabolism (e. g. lactate and citrate) and acid hydrolytic enzymes in tissue fluid, parts of implanta-

tion area become acidulous and it will promote the degradation process of β -TCP.

The products (including calcium ions and phosphate ions), which are degraded from the implanted materials, enter blood circulation immediately and take part in the metabolism of organs and tissues and are excreted with urine and feces. Most of them are stored in the calcium pool of body and are reused by bony tissue to form new bone.

Tetracycline that is an innocuous and non-radioactive fluorescence dyestuff can enter into new bone tissue selectively, and combine with the calcium of the hydroxyapatite (HA) in new bone tissue by forming steady chelating-ligand. With excited by a certain wavelength ultraviolet radiation, the undecalcified sections can appear olive fluorescence, which reveals the growth position of new bone tissue. The results of tetracycline-labeled experiment further show that osteogenesis is very active, and β -TCP ceramics are degraded and dispersed by new formation bone. Osteogenesis resulted from the degradation and bioactivity of β -TCP.

The process of inducing osteogenesis on β -TCP ceramics described above can be caused by the cellular elements of host bone and the degradation of porous materials. The slow replacement of β -TCP ceramics by bone tissue is thought to transfer mechanical strength to the bone as it has been shown that the strength of a porous calcium phosphate implant is highly correlated with the amount of pore space that becomes occupied by new bone.

On the basis of the resulting evidence the following model of osteogenesis is proposed. After implantation of the block, the surface layer of the ceramic at the block/bone interface partially degrades in the biological fluid through two ways: the dissolution process in body fluid and the degradation process mediated by cells, and enrichment of the fluid with calcium ions and also with ions of phosphate and hydroxyl groups. In the process, the dissolution rate of the material depends on its phase structure. When the solution becomes over-saturated by basic ionic components, the deposition of a substance HA begins because HA is the most steady salt of calcium phosphate in vivo^[10]. The structure is built from organic groups present in the body fluid, and inorganic elements and groups, including the ones not present in stoichiometric HA. Small over-saturation, low temperatures and impurities can block the growth process, delay the formation of large crystals, and as a result, a nanocrystalline composite material, the bone tissue, is formed. That is to say that the nonliving substance can incorporate in the activity of vital tissue in vivo.

5 CONCLUSIONS

Histological specificities of bone formation and biodegradation of β -TCP ceramics are found, and porous β -TCP ceramics can serve as a filling material for bone defects and can facilitate new bones to grow into its pores and defective area. It not only can play the role of bracket for bone growing but also can degrade and afford osteoblasts enough calcium and phosphate ions. So it will induce the new bone growing, incorporate in the process of bone tissue forming, make full use of bone conduction for the repair of bone defects, finally integrate with the host bone and become a portion of the organic new bone. Thus β -TCP ceramic is a degradable biocompatible artificial bone material which has the ability of inducing osteogenesis. In a word, β -TCP ceramic is a unique substitute material for bone transplantation with wide application perspective.

REFERENCES

- [1] Delloye C. Considerations actuelles sur les greffes [A]. Harmond MF. Calcified Tissues and Biomaterials [C]. Biomat Edn, Bordeaux, France, 1987. 149 - 163.
- [2] Kokubo T, Hayashi T, Sakka S, et al. Vincenzini. Ceramics in Clinical Applications [M]. Amsteden: Elsevier, 1987. 175 - 177.
- [3] Gao T J, Lindholm T S, Kommonen B, et al. Microscopic evaluation of bone implant contact between hydroxyapatite, bioactive glass and tricalcium phosphate implanted in sheep diaphyseal defects [J]. Biomaterial, 1995, 16(6): 1175 - 1179.
- [4] Daculsi G, Passuti N, Martin S, et al. Macroporous calcium phosphate ceramics for long bone surgery in humans and dogs: clinical and histological study [J]. J Biomed Mater Res, 1990, 24(3): 379 - 392.
- [5] Kotani S, Fujita Y, Kitsugi T, et al. Bone bonding mechanism of β -TCP [J]. J Biomed Mater Res, 1991, 25(8): 1303 - 1315.
- [6] Abdel-Fattah W I, Osiris W G, Mohamed S S, et al. Reconstruction of resected mandibles using a hydroxyapatite veterinary bone graft [J]. Biomaterials, 1994, 15(3): 609 - 614.
- [7] Oonishi H, Jassuti T, Ninholm G, et al. Orthopaedic applications of hydroxyapatite [J]. Biomaterials, 1991, 12(2): 171 - 178.
- [8] Zyman Z, Ivanov I, Glushko V, et al. Malyshkina. Inorganic phase composition of remineralisation in porous CaP ceramics [J]. Biomaterial, 1998; 19(7): 1269 - 1273.
- [9] ZHENG Qi-xin, DU Jing-yuan, XIA Zhi-dao, et al. Biodegradation of tricalcium phosphate ceramics by osteoclasts [J]. J Tongji Medical University, 1998, 18(4): 257 - 261.
- [10] Berger G, Gildenhaar R, Ploska U, et al. Short-term dissolution behaviour of some calcium phosphate cements and ceramics [J]. J Mater Sci Let, 1997, 16(13): 1267 - 1269.

(Edited by HUANG Jin-song)