生物陶瓷骨内植入后与组织间的界面研究 *

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摘　要　将 β–TCP 陶瓷植入大白兔的股骨内并定期注射四环素，分别在光学显微镜、荧光显微镜或扫描电子显微镜下观察新骨的形成和成骨过程，研究了 β–TCP 植入体内后与组织间的界面作用以及磷酸钙生物陶瓷的成骨作用. 结果表明，在类骨质表面有大量的成骨细胞，间充质细胞增生和浸入. 植入 β–TCP 陶瓷两个月后，类骨质通过钙化转变为编织骨. 植入三个月后，出现由骨桥连接的“骨岛”， β–TCP 陶瓷降解，并被新骨分散. 植入六个月后，新的骨髓腔形成，编织骨变成板层骨. 八个月后，在哈弗氏骨板上出现材料颗粒，形成典型的松质骨结构. 因此，无生命的钙磷材料在体内可以参与有生命的组织活动.

关键词　无机非金属材料， β–TCP 生物陶瓷， β–TCP 植入，界面，成骨作用，生物降解

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Study on the interface between tissue and bioceramic implanted into bone

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ABSTRACT　The interface of porous β–TCP ceramics implanted in rabbit was investigated in order to study the osteogenesis of calcium phosphate bioceramics. β–TCP ceramics (5 mm diameter and 8 mm height) were implanted in the femur of rabbits, and tetracycline was injected termly. The samples of bone were taken 1, 2, 3, 4, 5, 6 or 8 months after operation, respectively. The new bone formation and osteogenesis process on the interface were observed by using the light microscope, fluorescent microscope, scanning electron microscope and histochemical test. The results showed that bone formation rate increased faster than that of contrast groups. The interface was visible 1 month after operation, and osteogensis was active. There were abundant osteoblasts, mesenchymal cell hyperplasia and incursion on the surface of osteoid. The osteoid turned into woven bone through calcification 2 months after operation. Bone–island appeared and connected by bone–bridge 3 months after operation. β–TCP ceramics degraded and were dispersed by new bone. New marrow cavity formed and woven bone became bone lamella 6 months after operation. The granules of material were found on the Haversian lamella 8 months after operation. The typical structure of spongy bone formed. It can be concluded that the non–living calcium–phosphate materials can participate in the activity of living tissue in vivo.

KEY WORDS　inorganic non–metallic materials, β–tricalcium phosphate, β–TCP ceramics implant, interface, osteogenesis, biodegradation

1. Introduction

Losses of bone substance due to congenital, tumorous or traumatic reasons have been treated with bone grafts[1,2]. Various synthetic bone substitutes are now proposed in order to avoid the drawbacks of autografts (blood loss, haematoma, pain, risk of infection) or heterografts for a long time (rejection, infection, viral risk). Among them, calcium–phosphate ceramics (CPC) appears to be suitable[3,4], since their chemical composition is very close to the mineral phase of natural bone. These materials have been used in clinic as a bone substitute and seem to be biocompatible as well as osteointegrative[5,6]. Being implanted in osseous sites, these materials can bind directly with bone without an intervening fibrous layer[7,8]. CPC, which are usually used for this purpose, are made of beta–tricalcium phosphate (β–TCP), hydroxyapatite or their mixture[9,10]. Though the experience in application of CPC is significant, the nature and mechanism of osteogenesis–the process of bone tissue formation in CPC under the biological environment resulting in physico-chemical integration of ceramics with living bone–are not still clear[11,12]. Thus osteogenesis must be studied in close connection with the ceramic state at different stages of implantation. It is well known that β–TCP ceramics is biodegradable after implantation. The process of bone tissue formation in porous β–TCP in vivo was investigated in this paper.

2. Experiment

2.1 Implant materials

In order to prepare porous β–TCP ceramics, the β–TCP powder (average size: 1 μm), prepared by a wet chemical precipitation process, was mixed with high–temperature binder and pore–forming material, then foamed through the rosin foaming method and sintered in air at 900 ℃ for 2 h. The sintered β–TCP structure was formed by neck connecting at grain boundaries, the grain size is about 2~3 μm. The porous β–TCP has 40%~50% porosity and exhibits interconnecting pores with a mean pore size of 100~800 μm. The β–TCP ceramics (density 1.05~2.00 g·cm^{-3}) exhibits compression strength of 15~30 MPa. The implants in the form of cylindrical block with 8 mm in height and 5mm in diameter were prepared by using this material for bone defect filling.

2.2 Surgical procedure

28 New Zealand white rabbits in both sexes, weighing 2~2.5 kg, were divided into 8 groups according to different stages of implantation. The rabbits were subject to a standard operative procedure in the area of both condyle femurs under general anesthesia. Bone defects of 5 mm in diameter and 8mm in height in the tibiae condyle for implanting ceramics samples were created at low speed under a sterilized saline coolant. The cavity orientation including spongy and compact bone was perpendicular to the longitudinal and sagittal axis of the tibia. After being washed and cleaned with normal saline, the cylindrical β–TCP ceramics were press–fit inserted into the defects, and each condyle received an implant. In each groups, one rabbit was not implanted with materials acting as a contrast group. One rabbit of each experiment group and control group were injected twice (every other 7 days) with 15% tetracycline (30 mg·kg^{-1}) liquor before being sacrificed. The

animals were killed by CO_2 asphyxiating 1, 2, 3, 4, 5, 6 or 8 months after operation, respectively. The implants with surrounding tissue were taken out and fixed in 10% buffered formalin solution or 2.5% buffered glutaraldehyde solution. The same part of control group was treated with above procedures also.

2.3 Histological study

For getting un–decalcified sections, four fixed specimens from each group were dehydrated in an ethanol series, and then embedded in methylmethacrylate. Two specimens were cut into sections with 5 μm in thickness perpendicular to the long axis of the specimen, and were stained with toluidine blue. The other two tetracycline–labeled specimens were cut into sections of 10 μm, and were observed with a fluorescent microscope. The rest specimens, refrigerated in glutaraldehyde solution, were washed in an ultrasonic cleaner, dehydrated in 95% alcohol liquor and examined using a scanning electron microscope.

3. Results and Discussion

According to the roentgenogram, the space between ceramics and bony tissue became vague after one month. Ceramics and bony tissue were integrated together and the external form of the material became incomplete 5 months after operation. After 6 months, some parts of the materials disappeared and the residual became into fragments or particles. 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 β–TCP exposed outside of the cortex was partially covered with new bone one month after being implanted. The strength of implants seemed to be improved gradually. The gradual replacement of β–TCP ceramics by bone tissue is considered transferring mechanical strength to the bone, because it has shown that the strength of a porous calcium phosphate implant is highly correlated with the amount of pore space being occupied by new bone.

Light microscopic observation showed that β–TCP appears to having no early adverse effects, such as 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 were found in materials. New bone with an irregular woven bone structure appeared on the surfaces and in the pores of implanted β–TCP. The new woven bone often formed from matrix, chondrocytes and mesenchymal cells, which was lined by a layer of osteoblast on their surfaces, and generally located at periphery of the materials. New capillaries appeared in the center of bone matrix (Fig.1). The outside ceramic pores were almost completely filled by osteoids and thickening trabeculaes of bone after two months. The more deep pores were filled by woven bone and some mineralized matrix. There was blood vessel formation and macrophage soakage in the materials, and the osteoid turned into lamella bone by force of calcification. Three months after operation, thickening trabeculae and dense lamellar bone grew into all pores of the ceramic. The bone ingrowth patterns resembled Haversian canal structures instead of woven bone structures by rebuilding. Bone–island appeared and was connected by bone–bridge. The lacunae of the materials were filled by a great deal of medullary tissue four months after operation. Six months after operation, the residual materials are encircled with bony tissue (Fig.2). Prior to and during the new bone formation, the number of osteoclast–like multinucleated giant cells decreased with the increasing of mineralized areas of the pore inner surface. New bone grew perfectly and material granules were found on Haversian lamella eight months after operation.

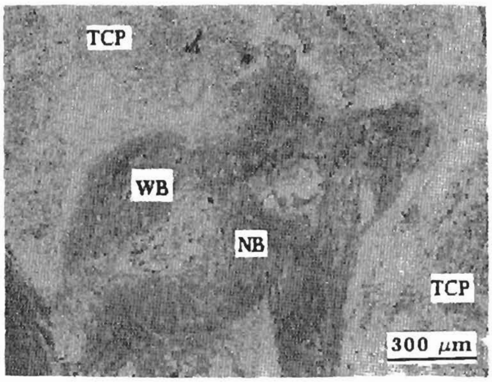

Fig.1 One month after implantation, new bone (NB) formation at the periphery of the ceramics (TCP), an irregular woven bone (WB) pattern is found

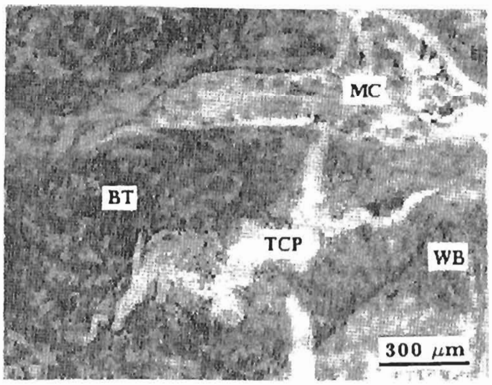

Fig.2 Six months after implantation, the new bone (NB) showing an osteon structure forming bone trabecula (BT), marrow cavity (MC) is found

The above results show that the porous β–TCP ceramics are capable of inducing osteogenesis. This ability depends on biodegradation and bioactive of the ceramics, which are improved by suitable crystal size, low–temperature sintering, phasic composition and porous structure. The degradation of β–TCP ceramics increases greatly because of the addition of binder. β–TCP ceramics is biodegradable after implantation. The biodegradation process is caused by the action of living system, which includes the dissolution process in body fluid and the absorption process mediated by cells. On the one hand, the particles of β–TCP ceramics can be dissolved continuously or be degraded by cells (e.g. phagocytes or osteoclasts)[13,14] which stretch out tiny pseudopod to wrap the grains and phagocytize them into their body, then the phagolysosome in plasma release hydrolase to the particulates and turn the original alkalescent internal environment into acidulous one. This process is benefit to the degradation of β–TCP. On the other hand, because of the acid products of metabolism (e.g. lactate and citrate) and acid hydrolytic enzymes in tissue fluid, parts of implantation area becomes acidulous and it will promote the degradation process of β–TCP.

The degradation of β–TCP result in enriching the fluid with calcium ions, phosphate ions and hydroxyl groups. When the solution becomes over–saturated by basic ionic components, the deposition of a kind of substance–HA begins. Little over–saturation, low temperatures, impurities which can block the growth process do not promote the formation of large crystals, as a result, a nanocrystalline composite material–the bone tissue–is formed. It means that the nonliving

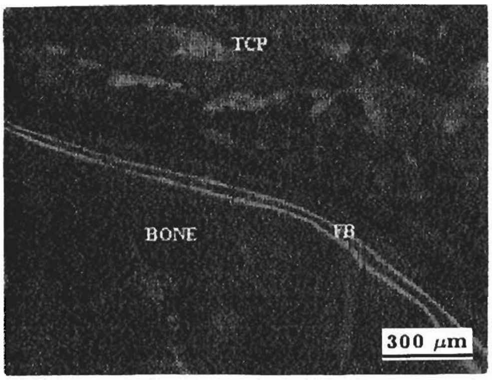

Fig.3 One month after implantation, double fluorescent band (FB) show up at the interface between implanted artificial bone and host bone(8.1681 μm/day)

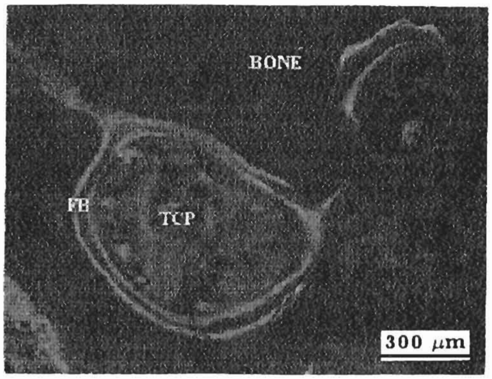

Fig.4 Six month after implantation, double fluorescent cycle directly embraced β–TCP particles in the center of defect area(5.2846 μm/day)

substance can participate in the activity of living tissue in vivo.

Tetracycline is an innocuous and non–radioactive fluorescence dyestuff, which can selectively enter into new bone tissue, and combine with the calcium of the hydroxyapatite (HA) in new bone tissue through forming steady chelating–ligand. After being excitated by a certain wavelength ultraviolet radiation, the un–decalcified sections can appear olivine fluorescence, which reveal the growth position of new bone tissue. The result of fluorescent microscope indicated that osteogensis was actively. The fluorescence bands, the symbol of new bone forming, were found obviously and massively on the interface between β–TCP materials and bone after one month (Fig.3). The new bones grew up with the operation time quickly and β–TCP particles were surrounded by double fluorescence bands which became more obvious. The fluorescence appeared clearly among β–TCP particles in the center of defect section. β–TCP ceramics degraded and were dispersed by new formed bone (Fig.4). There were a large number of material granules on the Haversian lamella which participated in the new bone formation after operation for eight months, while fluorescent phenomenon did not appears for samples of the control group in the same period. The kinetic parameters of them can be detected by image analysis system, distance of double–label (DDL) is the distance between the inner edge of the first gold label line and the inner edge of the second one. Three non–decalcified sections per rabbit were chosen randomly to measure the value of DDL, and then the average value was calculated.

IOB is the interval of Bis–administration, i.e. 7 days. Mineral appositional rate (MiAR) is the amount of mineralized osteoid per day. The calculative equation is as follows[15]: MiAR=$\frac{DDL\times\pi/4}{IOB}$. Detecting data showed that the growth rate in the first month was about 8.164 μm/day, much considerably faster than that of control groups (3.219 μm/day). The growth rate of second month was fastest. The bone formation rate increased comparing with those in the control group, and decreased month by month. Curve of bone–formation rate showed that the rate of new bone formation became similar to the rate of normal bone formation with time going on, which indicated the grafted materials were completely replaced by bone (Fig.5). So, the osteogenesis is result of the degradation and bioactivity of β–TCP.

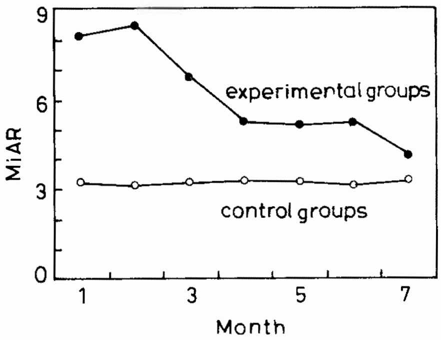

Fig.5 Curve of bone formation rate

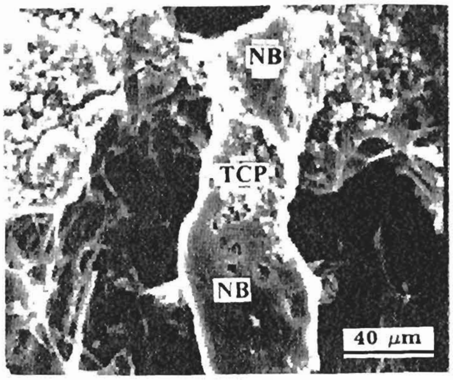

Fig.6 SEM photograph six months after implantation

SEM observation showed that the interface between particles changed obviously after implantation. The original connection between particles broke up. The particles were separated, and the external form became irregular (smaller and smoother). New bone directly connected with the implant was increasing (Fig.6). The materials losted their original shape entirely and combined with bone tissue wholly after 6 months. Some particles could be found in the Haversian system of new bone. The typical structure of spongy bone formed. These results proved that there are two ways through which the materials participate in the bone formation. One is degradation, absorption and recrystallization, the other is rebuilding of their structure.

4. Conclusions

The precondition of the participation of inorganic materials in life activity is the degradable capability of the materials. While materials degraded continuously, the new bone formed and grew intact. The bone was mostly coalesced with material. β–TCP ceramics can participate in life activities through degradation, become a part of vital organization, then achieve biological conversion, is a degradable material and biocompatible with artificial bone, which has the ability of osteogenesis. It is not only an excellent bracket for bone grows, but also an ideal bone substitute.

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