論 文 の 内 容 の 要 旨

獣医学　専　攻
平成２０年度博士課程 入学
氏　　名　劉　以立
指導教員名　佐々木　伸雄

論文題目　Osteoconductivity and mechanical properties of tetrapod-shaped granular artificial bones in animal femoral defect models
（動物大腿骨欠損モデルにおけるテトラポッド型顆粒人工骨による骨形成能ならびに力学的特性）

Repair for large bone defects due to fractures and cancers is a huge challenge as these defects do not spontaneously heal and require additional treatments such as bone graft and/or implants to enhance new bone regeneration. Many kinds of bone grafts have been used to repair large bone defects such as autograft, allograft, and artificial bones. Autograft is regarded as the excellent scaffold for bone regeneration. However, there are several problems in autograft, including morbidity of the donor site, insufficient amount of bone graft that can be harvested, and additional surgery is required. Allograft is another choice for bone graft, but it could induce immunologic reaction in the recipient and the risk of transferring diseases.

Recently, a large number of artificial bones with various compositions are commercially available. Calcium phosphate materials, such as hydroxyapatite (HA) and tricalcium phosphate (TCP), have been widely used as artificial bones in orthopedic, maxillofacial, and plastic surgeries due to their high biocompatibility and osteoconductivity. However, HA has a low degradation rate and takes a longer period to be replaced by new bone tissues *in vivo*. On the contrary, TCP has been widely used since it is replaced with host bone tissues.

TCP is subdivided into alpha and beta form by its crystallinity. The granule form of β-TCP is commercially available, but it is irregular in shape and size and is fragile in mechanical strength, therefore its use is restricted for non-load-bearing sites. α-TCP has larger mechanical strength with the surface of larger crystals. α-TCP is a gradually degradable osteoconductive material and is suggested to be a better choice in TCP artificial bones. Currently octacalcium phosphate (OCP) has become an important artificial bone, and is supposed to be a precursor of biological apatite such as bone and tooth. OCP could stimulate osteoblastic cell differentiation *in vitro*, and exhibited biodegradability, osteoconductivity, and osteoinductivity *in vivo*. In addition, it was reported that the cement comprised of OCP and α-TCP had higher compressive strength.

Another factor influencing on new bone formation and mechanical strength is the macrostructure design. The artificial bone with adequate connective interpores was expected as an ideal bone graft, because these interpores may provide the space for better bone ingrowth capability. Bone growth within granules must increase the mechanical strength with new bone regeneration in these interconnected pores.

Our group developed a novel tetrapod-shaped granular artificial bone (Tetrabone®) made from a mixture of α -TCP and OCP with the size of 1mm. Both rupture strength of a single particle and elastic modulus of aggregated particles of Tetrabone® were significantly higher than those of β-TCP granules *in vitro*. Therefore, the purpose of this thesis was to clarify the new bone formation and mechanical properties of Tetrabone® when repairing the femoral defect in animal models.

In chapter 1, I investigated the long-term effect of implantation of Tetrabone® into the femoral condyle defect in rabbits on new bone regeneration and its safety, and compared with those by β-TCP granules. Eighteen male New Zealand white rabbits were used. The bone defect (5mm in diameter and 8mm in depth) was made at both lateral femoral condyles. Then, the defects were filled with Tetrabone® (N=15) or β-TCP granules (Osferion®) (N=15) or left empty (control: N=6). Rabbits were euthanized at 4,13, and 26 weeks after surgery. After euthanasia, the condyles were collected and examined on gross observation, micro-CT, and histology. There were no clinical side effects in any rabbits receiving Tetrabone® during 26 weeks of the experimental period. However, granule leakage was observed in the β-TCP granule group at 4 weeks of implantation.

Tetrabone® was retained well inside the defect with less granular absorption at 26 weeks of implantation. The opening of the defect was covered with connective

tissues and concave-shaped in the β-TCP and control groups. β-TCP granules were rapidly resorbed at 4 weeks, which might lead the concave shape at the opening of the defect due to the fragile property of the granule at 13 and 26 weeks of implantation. In addition, new bone area in the Tetrabone® group was more than that in the β-TCP granule group at 13 and 26 weeks. These results suggested that β-TCP granules could not maintain the shape of the defect due to its rapid resorption, especially in the central area of the defect without new bone formation. It was indicated that Tetrabone® had better osteoconductivity than β-TCP granules in the long-term implantation.

In chapter 2, I investigated the mechanical properties of Tetrabone® implanted into the femoral defect of canine cadavers and compare with the β-TCP granules. Fourteen beagle dog cadavers were used and rectangular trabecular bone blocks with the shape of 14mm X 14mm X 8mm and the defect of a 10 mm cylindrical hole in the center were made from each distal femoral condyle. The defects were filled with artificial bones and divided into 3 groups; Tetrabone® group (N=8), β-TCP granule group (Osferion®) (N=8), and control group (PEG gel) (N=8). Additionally, 4 other femoral bone blocks without the cylindrical hole were used as an intact control.

The femoral bone blocks were mounted on Instron® mechanical test system (Instron-3365) to determine the load-deformation changes of the specimens in vertical compression until fracture, and the ultimate compressive load and elastic modulus were calculated from the mechanical test recordings. The ultimate compressive load of the Tetrabone® group was almost half of the intact bone and was significantly higher than those of the β-TCP granule and control groups. The elastic modulus was significantly higher in the Tetrabone® group than those of the β-TCP granule and control groups.

In the defect insertion testing, 15 femoral condyles with a tunnel defect (10 mm in diameter) were made and divided into the same 3 groups. The specimens were fixed on a rheometer with bone cement, and the rod (5 mm in diameter) was vertically inserted into the exposed surface of the graft material (10mm in diameter). The force-displacement changes were measured and the slope of the initial linear portion of the force-displacement curve was defined as compressive stiffness. 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 into the canine femoral defect model showed better mechanical properties than β-TCP granules *in vitro*.

In chapter 3, I investigated the effect of Tetrabone® implanted into the femoral

defect in dogs by evaluation of new bone formation and mechanical strength. Seven male beagle dogs were used and a tunnel defect (10 mm in diameter) was made at the both femoral condyles. The tunnel defects were filled with Tetrabone® (N=5), β-TCP granules (N=5), or without filling (control group) (N=4). All dogs were euthanized at 8 weeks after surgery.

No clinical side effects were observed in all experimental dogs. On gross findings, the opening of the defect was concave in the β-TCP granule and control groups, while smooth in the Tetrabone® group. 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, whereas Tetrabone® was retained in the defect at 8 weeks of implantation. Compressive stiffness of the defect in the Tetrabone® group was maintained almost 80% of the intact bone and was significantly higher than that of the β-TCP granule group. These results suggested the better mechanical strength of the defect implanted with Tetrabone® than with β-TCP granules after 8 weeks of implantation.

On histology, new bone distribution was significantly higher in the Tetrabone® group than that in the β-TCP granule group, though new bone area was similar in both groups. The new bone tissues in the Tetrabone® group were fully distributed in the defect area, while in the β-TCP granule group, granules were resorbed and the new bone tissues were partially distributed around the defect margin. These results indicated that the interconnectivity of the intergranular pores were effective for new bone invasion in the Tetrabone® group. In addition, Tetrabone® had better osteoconductivity than β-TCP granules.

In conclusion, although Tetrabone® was resorbed at a much slower rate than β-TCP granules, Tetrabone® provided much higher mechanical strength and better osteoconductivity in the defect than β-TCP granules. These results encourage the clinical application of Tetrabone® for the large bone defects at load-bearing sites in the clinical practice.