Mechanical Strength and In Vivo Evaluation of Novel Unidirectional Porous Hydroxyapatite Substitute

Watanabe A; Sakane M; Iwasashi M; Suetsugu Y; Funayama T; Abe T; Ochiai N
University of Tsukuba, Tsukuba, Ibaraki, Japan
Biomaterials Center, National Institute for Materials Science, Tsukuba, Ibaraki, Japan
sakane-m@md.tsukuba.ac.jp

Introduction:
The ideal hydroxyapatite (HAp) requires good biocompatibility without the possibility of inflammation or foreign body reaction. Moreover, HAp bone substitute after implantation needs new bone and tissue ingrowth inside HAp for long-term stability. We have developed unidirectional porous HAp which has a purity above 99.9% and a porosity of 75% (1). As regards its microstructure, it has penetrating oval pores 100 ~ 300 μm in diameter (Fig.1). Such a structure may be advantageous not only in migration for osteogenic and angiogenic cells inside HAp but also in mechanical strength in the direction parallel to the unidirectional pore. As we reported previously, the initial compression strength of the HAp in the direction parallel to the unidirectional pore is superior to that of commercially available porous HAp of the same porosity (2).

The objective of this study was to investigate the long-term stability of the mechanical strength of the new material after implantation in the femur of rabbits and to do histological evaluation.

Materials and Methods:
This study was approved by the local ethics committee. Twenty six Japanese white rabbits (BW: 3.0 ~ 3.5 kg) were used in this study. Unidirectional porous HAp was obtained from Kuraray Co., Ltd. Under general anesthesia, a bone tunnel was made by retrograde drilling through the distal femur, and a cylindrical unidirectional porous HAp specimen (6 mm in diameter and 7 mm in height) was implanted in the femur. The direction of the unidirectional pore is parallel to the axis of the femur. The animals were sacrificed 6, 12 and 52 weeks after implantation. Femurs were harvested and HAp inside femurs were cut in 4 ~ 5 mm segment as test pieces using a precision sectioning saw (BUEHLER IsoMet, Illinois, USA). Compression strength was measured in the parallel direction to the unidirectional pore. A mechanical test machine (SIMADZU AG-100kNi, Kyoto, Japan) was used at a cross head speed of 0.5 mm/min. Same-sized samples before implantation used as controls. For statistical analysis, one-factor factorial ANOVA and the Tukey-Kramer test were used, and a P value less than 0.05 was considered statistically significant.

Two femurs of each group were fixed in 4% paraformaldehyde and hematoxylin and eosin staining was performed for histological evaluation.

Results:
The compression strength of control sample (time 0) was 13.4±2 MPa in the direction parallel to the unidirectional pore. In the same direction, the compression strength was 29.5±17 MPa and 39.4±14 MPa at 6, 12 and 52 weeks, respectively. Statistical significance was observed between 0 and 6 weeks, 0 and 12 weeks and 0 and 52 weeks. However, no statistical significance was observed among each time periods (Fig.2).

Histological findings showed a new bone, a new capillary in HAp at 6 weeks. The direction of the new bone was parallel to the long axis of the unidirectional pores. The new bone formation remained at 12 weeks. The remodeled bone and fat cells were existed within the unidirectional pores at 52 weeks (Fig.3). There were no signs of infection, inflammatory reaction, or foreign body reaction during the period examined.

Discussion:
Our results showed long-term mechanical strength and stability of the unidirectional porous HAp. Previous reports suggested that the optimal pore size for osteoconduction was 150 ~ 500 μm in diameter. The properties of interconnected porous biomaterials allow fibrovascular tissue to enter the deeper pore areas smoothly. Unidirectional porous HAp almost meets this criterion. Our results showed increased compression strength after implantation at 6 weeks and the compression strength remained up to 12 weeks. Its microstructure may contribute to an increase in its mechanical strength with new bone formation inside it. At 52 weeks, the compression strength was similar to the strength at 12 weeks. However, the newly formed bone remodeled and bone marrow-like formation was found inside HAp. This indicates that the unidirectional porous HAp plays a role as a scaffold to regenerate bone marrow-like tissue.

In conclusion, the compression strength of the unidirectional porous HAp increases at an early stage after implantation and maintains up to one year. Sufficient osteogenesis and angiogenesis occur inside HAp. Further, in femoral medially canal, unidirectional porous HAp could be a useful scaffold for regeneration of bone marrow structure.

References:
2. Iwasashi M, et al. 54th ORS 2008; poster No.1702