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## 【24】

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## 論 文 内 容 の 要 旨

## [Introduction]

While bone tissue has a high potential for self-repair and regeneration, pseudoarthrosis or loss of function is likely to occur when a fracture defect that needs to be bridged is large. Metal or ceramic materials have been widely used for bone tissue repair, however, it is still hard to rebuild tissue with its original morphology. As for the scaffold-based bone regeneration therapy, there are still some limitations for repairing large defects since mechanical strength of the scaffold cannot be maintained during the healing period due to biodegradation.

Hydroxyapatite (HAp) is the first choice as a source material for bone reconstructive scaffolds because it is the main component of bone and demonstrates excellent cellular and host tissue affinity. Zirconia ( $\text{ZrO}_2$ ), a stable inorganic material with high biocompatibility and mechanical properties, can be an additive to strengthen HAp. We therefore considered that the achievement of bone reconstruction and regeneration led by composite materials of HAp and  $\text{ZrO}_2$  would be an effective approach to the treatment of large bone defects. The purposes of this study were to fabricate porous  $\text{ZrO}_2$ /HAp composites and evaluate their effectiveness for bone tissue repair.

## [Materials and Methods]

### 1. Scaffold fabrication and characterization

Porous ZrO<sub>2</sub>/HAp composite scaffolds were fabricated by a polyurethane sponge method. Briefly, the sponge block was dipped into the different ratios of ZrO<sub>2</sub>/HAp slurry (50/50, 60/40, 70/30, 80/20, 100/0 wt%) and compressed slightly to remove excess slurry on the foam. Then, the specimen was heated to 700°C for 3 h to burn out the sponge block and binder, and sintered at 1500°C for 5 h. The scaffolds prepared were characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD) analysis, and mechanical testing. The porosity measurement was conducted based on Archimedes' Principle.

### 2. *In vitro* evaluation of biocompatibility

To assess the cellular affinity, adhesion and proliferation of osteoblast-like MC3T3-E1 cells grown on ZrO<sub>2</sub>/HAp scaffolds with 91% porosity was evaluated by SEM. Protein adsorption study was carried out to estimate the organic matrix affinity of the scaffolds fabricated. Several gene expressions and alkaline phosphatase activity of the cells cultured on the ZrO<sub>2</sub>/HAp scaffolds were also evaluated.

### 3. *In vivo* evaluation of bone formation

A ZrO<sub>2</sub>/HAp (70/30 wt%) scaffold was implanted in critical-size bone defects of SD rat calvaria. Bone reconstruction and/or regeneration were evaluated by sectional staining, micro-CT, and SEM and energy-dispersive X-ray spectroscopy (EDS) analysis. Scaffolds containing bone marrow-derived stromal cells (BMSCs) were also examined, with animals undergoing no implantation of any scaffold serving as a negative control.

## [Results and Discussion]

The porosity of ZrO<sub>2</sub>/HAp scaffolds ranged from 72 to 91%. The compressive strength of the scaffolds increased from 2.5 to 13.8 MPa as the ZrO<sub>2</sub> content increased from 50 to 100 wt%. The osteoblastic cell adhesion, cell proliferation, protein adsorption, and alkaline phosphate activity and the expression levels of osteogenesis-related genes were significantly improved by culturing on the scaffolds composed of 70 wt% or less of ZrO<sub>2</sub> compared with the scaffold made of ZrO<sub>2</sub> alone. In terms of mechanical strength and biocompatibility, combination of ZrO<sub>2</sub>/HAp at the ratio of 70/30 wt% was found to be optimum.

Previous studies indicated that the implantation of a variety of cell-scaffold combinations can lead to better bone regeneration than implantation of scaffolds without cells. Therefore, ZrO<sub>2</sub>/HAp (70/30 wt%) scaffolds having optimum porosity, mechanical strength, and biocompatibility, were implanted with or without BMSCs into rats. HE and von Kossa staining after 6 weeks indicated that bone formation in the repair sites where ZrO<sub>2</sub>/HAp scaffolds had been implanted was better than the control, and implantation of the BMSCs-loaded scaffold demonstrated the greatest repair. Immunofluorescent staining revealed that OPN and Col I expression at the repair sites increased in the order of the control, ZrO<sub>2</sub>/HAp scaffold, and BMSCs-loaded scaffold, suggesting enhanced new bone formation in cell-containing ZrO<sub>2</sub>/HAp scaffolds. The results of micro-CT and SEM/EDS analysis, which can accurately quantify cancellous and cortical bones, confirmed that the BMSCs-loaded ZrO<sub>2</sub>/HAp scaffolds promoted full

healing within 6 weeks.

## [Conclusion]

The ZrO<sub>2</sub>/HAp porous scaffolds fabricated in the present study showed excellent mechanical properties and high cellular affinity. The characteristics of these scaffolds can be finely adjusted by modifying the content of ZrO<sub>2</sub> and HAp starting materials. Furthermore, ZrO<sub>2</sub>/HAp scaffolds with highly connected pores could be a promising substrate with which to obtain sufficient levels of reconstruction and regeneration to treat large bone defects.

## 論文審査の結果の要旨

本研究は、生体親和性にすぐれ、かつ機械的強度の高い骨補填材料を開発することを目的に、ジルコニアとハイドロキシアパタイトの多孔質複合体を作製し、骨組織再生における有効性を *in vitro* および *in vivo* で検討したものである。

その結果、ジルコニア/ハイドロキシアパタイト混合比の調節により、複合体の強度や細胞親和性を制御でき、また、この多孔質複合体を骨髄由来間葉系幹細胞と組み合わせることによって効率的な骨組織の再生を達成できることが示された。

以上の研究成果は、骨組織の再建と再生を促す新しい生体材料を提示する重要な知見であり、本研究は博士（歯学）の学位授与に値するものと認める。