学 位 論 文 の 要 旨

氏名　SHA JINGJING

学 位 論 文 名　Application of a Bioactive/Bioresorbable Three-Dimensional Porous Uncalcined and Unsintered Hydroxyapatite/Poly-D/L-lactide Composite with Human Mesenchymal Stem Cells for Bone Regeneration in Maxillofacial Surgery: A Pilot Animal Study

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著 者 名　Jingjing Sha, Takahiro Kanno, Kenichi Miyamoto, Yunpeng Bai, Katsumi Hideshima and Yumi Matsuzaki

論 文 内 容 の 要 旨

INTRODUCTION

A novel three-dimensional (3D) porous uncalcined and unsintered hydroxyapatite/poly-D/L-lactide (3D-HA/PDLLA) composite demonstrated superior biocompatibility, osteoconductivity, biodegradability, and plasticity, thereby enabling complex maxillofacial defect reconstruction. Mesenchymal stem cells (MSCs)—a type of adult stem cell—have a multipotent ability to differentiate into chondrocytes, adipocytes, and osteocytes. In a previous study, we found that CD90 (Thy-1, cluster of differentiation 90) and CD271 (low-affinity nerve growth factor receptor) double-positive cell populations from human bone marrow had high proliferative ability and differentiation capacity in vitro. In the present study, we investigated the utility of bone regeneration therapy using implantation of 3D-HA/PDLLA loaded with human MSCs (hMSCs) in mandibular critical defect rats.

MATERIALS AND METHODS

All animal experiments in this study were approved by the Animal Care and Use Committee of Shimane University. The Twenty-four male Sprague Dawley rats created the mandibular critical bone defect, and were divided into four groups: a no-transplantation group, a 3D-HA/PDLLA + Hank's Balanced Salt Solution group (HBSS group), a 3D-HA/PDLLA + 1 ×

10^4 hMSCs group (1×10^4 hMSCs group), and a 3D-HA/PDLLA + 1×10^5 hMSCs group (1×10^5 hMSCs group). Except the no-transplantation group, the other groups were transplanted the materials into the defects and were respectively injected 10μl HBSS Solution, 1×10^4 hMSCs, and 1×10^5 hMSCs into the 3D-HA/PDLLA composites. After 2 and 4 weeks, we completed the Microcomputed tomography (Micro-CT) and Villanueva Goldner (VG) staining analysis.

In Micro-CT, the average fusion rate and average fusion depth were used to evaluate the degree of fusion of the material and host bone. The average area of newly formed osteoid tissue was the quantity of newly formed osteoid tissue in each radiograph, which was used to assess the progress of ectopic bone regeneration. The average fusion rate and average fusion depth between the mandibular critical defect on the superior and inferior sides were analyzed separately, to determine whether blood supply and nutrition influenced ectopic bone regeneration.

In VG staining, the samples were observed under a light microscope to evaluate bone regeneration and count nucleated cells (i.e., osteogenically relevant cells, such as osteoblasts, osteoclasts, osteocytes, and macrophages) using ImageJ software in three areas of the material pores (i.e., connective, surrounding, and central pores).

In statistical analyses, the Kruskal–Wallis H test was used to analyze the average fusion rate and average fusion depth. And then, the osteogenesis difference in the mandibular critical defects between the superior and inferior sides was used Wilcoxon Signed-Ranks test. To compare the quantity of newly formed osteoid tissue in Micro-CT and the nucleated cells count in VG staining, one-way analysis of variance and LSD-*t* test were used. All statistical analyses were performed using SPSS statistical software (SPSS Japan Inc., Tokyo, Japan). All differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

1. Micro-CT Evaluation

The implantation of the composite with hMSCs was more abundantly fused with the mandibular bone at 4 weeks. Especially, with the addition of 1×10^5 hMSCs, the compact fusion was observed, and the new bone surrounding the buccal–lingual side was shown. The average fusion rate and depth of the two hMSCs groups were not only higher than those of the composite only but also increased from 2 weeks to 4 weeks after surgery. The average area of newly formed osteoid tissue increased over time in the following order: the no-transplantation group, the HBSS group to the 1×10^4 hMSCs group, and the 1×10^5 hMSCs group. Furthermore, at 4 weeks, there were significant differences in the average fusion rate and depth between the two sides, revealing improved osteogenesis and extent of fusion on the superior side than the inferior side.

2. VG staining results

To evaluate the bone regeneration after implantation, no or slightly regenerated osteoid tissue was observed around the border region between the composite and mandibular bone in the no-implantation and composite-only implantation controls. However, the invasion of newly

formed osteoid tissue in the implant was clearly observed in the group receiving implantation of the composite with hMSCs. New bone had grown into the surrounding pores and osteogenesis was more active, especially in the 1×10^5 hMSCs group at 4 weeks. In all groups, the number of nucleated cells from the connective pores to the central pores decreased gradually throughout all time periods. Furthermore, there was no significant difference in connective pore cell counts among the three groups at 2 weeks; however, at 4 weeks, there were significantly fewer cells in the two hMSCs groups than the HBSS group. There was no significant difference between the number of nucleated cells in the surrounding and central pores; therefore, the results are not shown.

3. Discussion

Micro-CT indicated that implantation of a 3D-HA/PDLLA-hMSC composite scaffold improved the ability to achieve bone regeneration compared with 3D-HA/PDLLA alone. The mandibular alveolar artery enters the mandible through the mandibular foramen and passes through the canal. Its branches nourish the mandible body and ascending branch below the foramen. Similar to the mandible in humans, the defect on the superior side of the mandible in rats was closer to the neurovascular bundle, which received a greater blood supply than the inferior side. Compared to the sufficient blood supply in the mandibular defection superior side, a lack of blood supply in the inferior side caused delayed healing. The use of VG staining revealed the gradual progression of the nucleated cells and new bone from the scaffold border into the central pores, indicating that 3D-HA/PDLLA loaded with hMSCs had good osteoconductivity. Meanwhile, the HBSS group exhibited more nucleated cells in connective pores than either of the two hMSCs groups at 4 weeks, which occurred because a given amount of new bone were generated in the two hMSCs groups. In connective pores, the proliferation rates of nucleated cells in the two hMSCs groups peaked at 2 weeks and then began to decrease, because the decrease provided more space for new bone growth. However, this phenomenon did not occur in surrounding and central pores, because the new bone had not filled enough space at 4 weeks. Therefore, the longer periods are required to achieve satisfying results.

CONCLUSIONS

The 3D-HA/PDLLA-hMSC scaffold in a mandibular defect improved the bone formation compared to 3D-HA/PDLLA scaffold filling alone. The growth of osteogenesis-relevant cells and new bone gradually progressed from connective pores to central pores, demonstrating that the 3D-HA/PDLLA-hMSC scaffold exhibited a better osteoconductivity. Given an adequate blood supply, the 3D-HA/PDLLA-hMSC scaffold effectively aids bone regeneration for boney defect reconstruction in maxillofacial surgery.