学 位 論 文 の 要 旨

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学 位 論 文 名　Bone Regeneration Capacity of Newly Developed Uncalcined/ Unsintered Hydroxyapatite and Poly-L-lactide-co-glycolide Sheet in Maxillofacial Surgery: An In Vivo Study.

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論 文 内 容 の 要 旨

INTRODUCTION

Bioresorbable fixation device systems have received considerable attention in oral and maxillofacial surgery due to their advantages compared with the traditional material, titanium alloys. Among the bioresorbable polymers used to manufacture bone fixation devices, uncalcined/unsintered hydroxyapatite and poly-L-lactide acid (u-HA/PLLA) possesses outstanding features, such as high mechanical strength, biocompatibility, bioresorbability, bone-bonding, osteoconduction, and radiopacity, that suitable to apply in oral and maxillofacial surgery. However, some clinical studies reported that its long degradation time (>5 years) is related to the late body response. Recently, a new generative nanomaterial of uncalcined/unsintered hydroxyapatite and poly-L-lactide-co-glycolide (u-HA/PLLA/PGA) has been developed with the aims of preserving the advantages of existing material (in terms of bioactive/osteoconductive potential) and shortening the resorption time. Because the mechanical and degradation properties of co-polymer PLLA/PGA are presumed to be regulated based on the PLLA:PGA ratio, this new nanomaterial is expected to have properties superior to those of u-HA/PLLA. However, the biocompatibility and bioactive osteoconductivity of this nanomaterial remains unclear. To our knowledge, this is the first animal study to assess the bone regeneration ability of this novel regenerative nanomaterial.

Because of the complex three-dimensional (3D) structural features, reconstructing bone defects in the craniomaxillofacial skeleton can be challenging. u-HA/PLLA material has been confirmed as a feasible choice to reconstruct the maxillofacial region. Accordingly, we expected that the new nanomaterial, which also contains u-HA, would possess similar favorable bone regeneration ability in the maxillofacial area. In this study, we evaluated the

bioactive/osteoconductive bone regeneration capacity and bioresorbability of u-HA/PLLA/PGA material in the maxillofacial bone compared with u-HA/PLLA material by implanting the materials to cover critical defects in rat mandibles.

MATERIALS AND METHODS

A total of 28 Sprague Dawley (SD) male rats (age = 10 weeks, weight = 250-270 g) were divided into three groups: u-HA/PLLA/PGA (n = 12), u-HA/PLLA (n = 12), and sham control (n = 4). Each group was divided into four subgroups of 1, 3, 8, and 16 weeks of follow-up time. At each time point, there were three rats in the u-HA/PLLA/PGA subgroup, three rats in the u-HA/PLLA subgroup, and one rat in the sham control group. A 4-mm-diameter critical-size defect was created at the right-side mandibular angle using a trephine bur from the buccal side to the lingual side. Then, the defect was covered buccally with either u-HA/PLLA/PGA or u-HA/PLLA, as follows: each rat in the u-HA/PLLA/PGA group received one u-HA/PLLA/PGA sheet, whereas each rat in the u-HA/PLLA group received one u-HA/PLLA sheet.

The rats' right hemimandibles were harvested at 1, 3, 8, and 16 weeks and then soaked in 10% neutral buffered formalin before performing micro-CT scanning, hematoxylin-eosin (HE) staining, and immunohistochemical (IHC) stainings using Runx2 antibody, Leptin Receptor (LepR) antibody, and Osteocalcin (OCN) antibody. The amount of new bone formation was assessed on micro-CT data and histomorphometry using Fiji software. The expressions of Runx2 and LepR were evaluated using IHC optical density (OD) score. OCN expression was quantified using the digital H-score. Both methods were completed using Fiji software.

Statistical analyses were performed using SPSS software for Mac OS (version 20.0; IBM Corporation, Armonk, NY, USA). The Mann–Whitney U test was conducted to compare the percentage of new bone (micro-CT and histomorphometry), IHC OD score (Runx2 and LepR), and digital H-score (OCN) between the u-HA/PLLA/PGA and the u-HA/PLLA groups at different time points. An intra-group comparison was also carried out. Values of $p < 0.05$ were considered to indicate statistical significance.

All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University (Approval number: IZ 31-61).

RESULTS AND DISCUSSION

The outcomes of new bone formation in both methods in histomorphometry and micro-CT data indicated that the amount of new bone was similar in both u-HA/PLLA/PGA and u-HA/PLLA groups at each time point except week 3 when the percentage of the bone fraction was higher in u-HA/PLLA/PGA group than in the u-HA/PLLA group. Although the proportion

of u-HA in the new material (10%) is considerably smaller than that in the previous material (40%), the u-HA particles of the new material may be exposed easily to body fluids due to its more rapid degradation process. This promotes the differentiation of osteoblast cells and early bone regeneration in the defect. Therefore, despite of smaller proportion of u-HA particles, u-HA/PLLA/PGA appears to stimulate bone formation more rapidly than u-HA/PLLA in the early stage.

There were 4 potential sources of osteoblastic cells in our mandible rat model, including endosteum, periosteum, circulating blood cells, and bone marrow. In our IHC analyses, the presence of Double positive cells of Runx2 and LepR as multilayered cells concentrated in the same position on the edge and bone marrow area of the host bone implied that bone marrow-derived mesenchymal stem cells might be the primary source of osteoblastic cells for bone regeneration in our mandibular bone defect model.

The fibrous tissue was observed surrounding the material sheets in both groups without the signs of inflammation and foreign body giant cells at the early time points. At the late time points, the newly formed bone was seen in the thin fibrous tissue near the parent bone and the material sheets. This confirmed that u-HA/PLLA/PGA material might possess high biocompatibility.

CONCLUSION

The results obtained in this study showed that newly developed u-HA/PLLA/PGA bioresorbable nanomaterial exhibited an equal bone regeneration ability, biocompatibility, and accelerated bone regeneration with a shorter degradation time than u-HA/PLLA material in the rat model. These findings show that this new nanomaterial, u-HA/PLLA/PGA, has excellent potential for clinical application as a fourth-generation bioresorbable material.