学 位 論 文 の 要 旨

氏名　DONG NGOC QUANG

学 位 論 文 名	Bone Regeneration Potential of Uncalcined and Unsintered Hydroxyapatite/Poly L-lactide Bioactive/Osteoconductive Sheet Used for Maxillofacial Reconstructive Surgery: An *In Vivo* Study
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著 者 名	Dong Ngoc Quang, Takahiro Kanno, Yunpeng Bai, Jingjing Sha, Katsumi Hideshima

論 文 内 容 の 要 旨

INTRODUCTION

The management of maxillofacial bone fractures, reconstructive surgery, and jaw osteotomy usually requires rigid fixation to optimize the healing of bony tissue so the role of fixation device material cannot be underestimated. Uncalcined and unsintered hydroxyapatite/poly l-lactide (u-HA/PLLA) is a relatively new material that has several favorable characteristics, such as radiopacity, high mechanical strength, biocompatibility, bioresorbability, bone bonding and especially osteoconduction. Regarding osteoconduction, numerous studies using rabbit models have demonstrated the osteoconductive ability and bone bonding features of u-HA/PLLA. However, these animal experiments only tested the osteoconductive ability of u-HA/PLLA using a rod inserted fully into the knee, such that there was full bone contact. The bone regenerative behavior of u-HA/PLLA in the maxillofacial bone under critical size defect conditions has not been investigated.

From an anatomical and functional point of view, the maxillofacial area is unique, having distinctive components such as teeth and paranasal sinuses that interact with the external environment and are also involved in mastication and respiration. Communitive fracture of the craniomaxillofacial area, fracture of the orbital wall, and surgical movement of the tooth-bearing segment can create bony gaps, such that full contact of the bone with the u-HA/PLLA material is not always achieved, as noted in previous animal investigations. Although osteosynthetic materials made from u-HA/PLLA have been utilized successfully for treating craniomaxillofacial trauma, and in jaw correction surgery, no animal research using microscopes has been done to assess the

osteoconductivity of this novel biomaterial when applied specifically to maxillofacial bone defects. Therefore, we conducted this study to evaluate the bone regenerative potential of a u-HA/PLLA sheet covering a critical-size defect in a rat mandibular angle.

MATERIALS AND METHODS

All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University. A total of 21 Sprague-Dawley (SD) male rats (age = 10 weeks; weight = 250–270 g) were assigned to three groups: (1) u-HA/PLLA group (n = 9), (2) PLLA group (n = 9), and (3) sham control group (n = 3). Each group was divided into three subgroups of 2, 4, or 8 weeks of treatment time. A 4-mm-diameter critical-size defect was created at the mandibular angle using a trephine bur to perforate the mandible from the buccal to the lingual side. Then, the defect was covered buccally as follows: rats in the u-HA/PLLA group received the u-HA/PLLA sheet, whereas rats in the PLLA group received the PLLA sheet.

The rats' mandibles were harvested and stained with hematoxylin-eosin (HE), Runx2 antibody and Osteocalcin (OCN) antibody. The amount of new bone formation was assessed using observation and histomorphometry. The expression of Runx2 was assessed using counting method to calculate the percentage of positive cells in the specific areas of the specimen. OCN expression was evaluated by quantifying the intensity of the DAB chromogen stain 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 used to compare the percentage of new bone (histomorphometry), labeling index (Runx2), and digital H-score (OCN) between the u-HA/PLLA and PLLA groups at different time points. An intra-group comparison was also carried out. A p-value of less than or equal to 0.05 was considered to indicate significance.

RESULTS AND DISCUSSION

Since week 4, the amount of new bone formed in the defect area in the test group (using u-HA/PLLA material) was signigicantly higher than the control group (using PLLA material) while no newly formed bone was observed in the sham control. Due to the nature of the surgical procedure, the bone formation could not be explained by the guided bone regeneration mechanism. It was likely to be formed by the characteristics of the test material. This feature showed that the u-HA/PLLA material could be effectively applied for the osteosynthetic purpose in some special areas where the communication with the external environment is unavoidable.

The expression of Runx2 was expressed highly in week 2 but gradually decreased since week 4 to week 8. By contrast, the labeling index values of Runx2 were relatively stable in the PLLA group. This implied that the osteoblastic activity was more intense in the u-HA/PLLA group than in the PLLA group and subsequent bone formation could be expected in the test group as

shown in the histomorphometry evaluation.

The OCN expression was more intense in the new bone of the u-HA/PLLA group than in the PLLA group. This reflected that the new bone formed in the test group was mineralized and reached maturity earlier and was more abundant, than in the PLLA group.

Despite these weaknesses, the results of our evaluations were all in agreement and showed that it was feasible to form new bone in a critical-size defect in a rat mandible with a u-HA/PLLA sheet.

CONCLUSION

The histological and immunohistochemical evaluations in this study have confirmed the bone regeneration capability of u-HA/PLLA material in the rat mandible model. These results may lead to the development of novel applications of u-HA/PLLA osteosynthetic reconstruction materials in the maxillofacial region and could shed light on the clinical feasibility and outcomes of using u-HA/PLLA in maxillofacial reconstructive surgery. Future studies examining the application of u-HA/PLLA in situations simulating specific clinical settings should be considered.