学 位 論 文 の 要 旨

氏名　　都田　絵梨奈

学 位 論 文 名　Feasibility of Application of the Newly Developed Nano-Biomaterial, β-TCP/PDLLA, in Maxillofacial Reconstructive Surgery: A Pilot Rat Study

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著 者 名　Erina Toda, Yunpeng Bai, Jingjing Sha, Quang Ngoc Dong, Huy Xuan Ngo, Takashi Suyama, Kenichi Miyamoto, Yumi Matsuzaki, and Takahiro Kanno

論 文 内 容 の 要 旨

INTRODUCTION

Managing maxillofacial bone defects caused by malignant tumors or trauma is challenging due to the complex three-dimensional shape, aesthetic considerations, and importance of mastication and speech. The conventional method, autogenous bone graft, does not meet the demands of bone restoration and has a number of disadvantages, such as donor site morbidity and the requirement for secondary surgery. To achieve the complete restoration of function in the area of the bone defect, various types of artificial bone graft substitutes have been developed to provide a suitable microenvironment for the growth and orientation of osteoblasts. However, because of insufficient intensity, foreign body reactions, and lack of osteoconductivity, the previous biomaterials have very limited clinical utility.

Recently, a novel nano-biocomposite of β-TCP particles and poly-D-L-lactide (PDLLA) (β-TCP/PDLLA) was developed in an attempt to combine the advantages of both components with better properties for the promotion of bone regeneration. β-TCP can undergo ion exchange with the original bone and accelerate the calcification of new bone, and directly benefit fibrovascular invasion and bone replacement due to its vast network of interconnected pores. However, it cannot be trimmed to the shape of the defect, and the β-TCP implants with low-stress levels show brittle fracture behavior in clinical practice. Blending PDLLA with β-TCP confers greater plasticity to overcome the shortcoming above.

In this study, we evaluated the feasibility of β-TCP/PDLLA material for the biofeatures and bony regenerative capacity in a rodent mandibular critical bone defect model. To our knowledge, this is the first animal study to assess the bone regeneration ability of this novel

regenerative nanomaterial.

MATERIALS AND METHODS

Three different types of biocomposite were used in this study, including β-TCP/PDLLA, pure β-TCP, and pure PDLLA, which were manufactured as cylinders with 4 mm in diameter and 2 mm in thickness. A total of 28 Sprague Dawley (SD) male rats (age = 10 weeks, weight = 306-320 g) were divided into four groups: (1) β-TCP/PDLLA group (n = 8), (2) β-TCP group (n = 8), (3) PDLLA group (n = 8), and (4) sham group (n = 4). After anesthesia using the 3-mixed anesthetic solution (a combination of 0.15 mg/kg medetomidine, 2 mg/kg midazolam, and 2.5 mg/kg butorphanol), a 1cm-length sagittal incision was made through the full thickness of the mandible skin and muscle layers, and the mandibular bone surfaces were exposed. A critical bone defect was created in each rat using a 4 mm-diameter trephine bur. Except for the sham group (the defects were filled with no material), in the other 3 groups, the defects were filled with a cylinder of the corresponding biocomposite. The wounds were closed, and the rats were maintained in a warm environment until recovery.

The rats' mandibles were harvested at 2 and 4 weeks and then soaked in 10% neutral buffered formalin before conducting micro-CT scanning, hematoxylin-eosin (HE) staining, and immunohistochemical (IHC) stainings using Runx2 antibody, Leptin Receptor (LepR) antibody, and Osteocalcin (OCN) antibody. For micro-CT data, the bone mineral density (BMD) and bone volume to total volume (BV/TV) ratio were calculated using Fiji software. For IHC staining, expressions of Runx2, LepR, and OCN were evaluated by quantifying IHC optical density (OD) score using Fiji software.

Statistical analyses were performed using SPSS software for Mac OS (version 27.0; IBM Corporation, Armonk, NY, USA). The differences in BMD and BV/TV were compared using the Mann-Whitney U test. The results of IHC staining for Runx2, LepR, and OCN at 2 and 4 weeks were compared using the independent-samples Kruskal-Wallis test, and the pairwise comparisons were used as the post hoc test. The significance was adjusted using the Bonferroni method; $p < 0.05$ was taken 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: 31-39).

RESULTS AND DISCUSSION

For micro-CT data, due to the radiolucent of PDLLA, comparisons were only carried out in the β-TCP/PDLLA and β-TCP groups. At both 2 and 4 weeks, the β-TCP group showed higher

BMD and BV/TV values that show to have superior capacity for bone integration. However, these differences were not significant statistically. In β-TCP groups, newly formed bone was detected in all three groups at 2 weeks, and new bone extended from the host mandibular bone tissue and fused with β-TCP. At 4 weeks, the degree of fusion between host bone and β-TCP was increased, and new bone was also found in the material's pores. However, in β-TCP/PDLLA group, the new bone formation was distinct in material, the fusion between bone and the material was rare, and most newly formed bone was only detected inside the pores.

The IHC results revealed more details about osteoblast differentiation and maturation. Runx2 and LepR showed very similar expression trends and identical distributions in all three groups at 2 and 4 weeks, with positive cells detected predominantly adjacent to the newly formed bone. Runx2 is the main regulator of osteoblast differentiation and is expressed in mature osteoblasts. And LepR is an excellent biomarker for identifying bone marrow mesenchymal stem cells. Therefore, the observation in our study indicated that bone marrow is the main source of osteoblastic cells for bone regeneration in our study. In the PDLLA group, the levels of Runx2 and LepR expressions were highest at 2 weeks, but the levels decreased at 4 weeks. In the β-TCP/PDLLA group, because of the degradation, the pore size increased and more cells infiltrated into these areas making the levels of Runx2 and LepR expression in the β-TCP/PDLLA surpass those in the PDLLA group at 4 weeks. The β-TCP group also showed the same findings, although the LepR expression was insignificantly lower than that in the PDLLA group at 4 weeks. This indicated that the novel nano-biocomposite β-TCP/PDLLA showed good bioactive/conductivity.

OCN is a major component of bone extracellular matrix and a marker of osteogenesis and is mainly secreted by mature osteoblasts. The level of OCN expression was higher in the β-TCP/PDLLA group than the other two groups at 2 weeks, but was similar among all three groups at 4 weeks, suggesting that this biomaterial may provide favorable conditions for osteoblast cell differentiation and maturation in the initial period.

CONCLUSION

Based on our pilot in vivo study, the novel β-TCP/PDLLA nano-biocomposite showed good biocompatibility, bioresorbability, and bioactive/osteoconductivity. In addition to these unique biological features, β-TCP/PDLLA can easily be reshaped during surgery. Moreover, its compressive strength is similar to natural human bone tissue, and its degradation rate can be regulated by adjusting the ratio of its internal particles. The mechanisms underlying how this nanomaterial affects osteoblastic cell differentiation and osteogenesis warrant further study and investigation.