学 位 論 文 の 要 旨

氏名　BAI YUNPENG

学 位 論 文 名　Feasibility of a Three-Dimensional Porous Uncalcined and Unsintered Hydroxyapatite/poly-d/l-lactide Composite as a Regenerative Biomaterial in Maxillofacial Surgery

発 表 雑 誌 名　Materials
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著 者 名　Yunpeng Bai, Takahiro Kanno, Hiroto Tatsumi, Kenichi Miyamoto, Jingjing Sha, Katsumi Hideshima and Yumi Matsuzaki.

論 文 内 容 の 要 旨

INTRODUCTION

The maxillofacial area is a relatively complex part of the human body, consisting of bone, cartilage, and networks of nerves and vessels. Reconstruction of maxillofacial bones is difficult due to the unique aesthetic requirements and functional demands, which include mastication and the expression of emotions.

In this study, we evaluated the feasibility of a novel three-dimensional (3D) porous composite of uncalcined and unsintered hydroxyapatite (u-HA) and poly-D/L-lactide (PDLLA) (3D-HA/PDLLA) for the bony regenerative biomaterial in maxillofacial surgery, focusing on cellular activities and osteoconductivity properties in vitro and in vivo.

We attempted to further clarify the unique properties of this 3D-HA/PDLLA composite as a bone regeneration biomaterial for mandibular bony defects and its influence on cellular responses.

A better understanding of the 3D-HA/PDLLA composite will contribute to the development of a new generation of bioactive/bioresorbable materials, allowing for increased long-term biocompatibility in maxillofacial surgery.

MATERIALS AND METHODS

1. In Vitro Experiments

Porous 3D-HA/PDLLA and dense-HA/PDLLA composite was provided by Teijin (Teijin

Medical Technologies Co., Ltd., Osaka, Japan). Superpore® (Pentax SKM, Tokyo, Japan) was used as the β-TCP biomaterial in the in vivo study. The preosteoblastic cell line MC3T3-E1 was purchased from the RIKEN Cell Bank (Cell Engineering Division, RIKEN BioResource Research Center, Tokyo, Japan).

1.1. Cell Proliferation Assay

MC3T3-E1 cells (2.0 × 104 cells/cube) were injected into the center of the 3D-HA/PDLLA cellular cubes. At 0, 24, and 48 h after cell injection AlamarBlue solution (AbD Serotec Ltd., Oxford, UK) was added to the medium, 1 mL culture samples were transferred to a 12-well plate and the fluorescence intensities were measured using a microplate reader.

1.2. Cell differentiation Assay

3D-HA/PDLLA and dense-HA/PDLLA sheets placed in a 6-well plate at a cell concentration of 4.0×10^5 in 5 mL of α-MEM medium.

Total RNA was extracted from the cells cultured on the sheet samples and 1 μg of total RNA was subjected to reverse transcription. qRT-PCR was performed on a CFX96 PCR System.

2. In Vivo Experiments

All animal experiments in this study were approved by the Animal Care and Use Committee of Shimane University. The Seventy-two male Sprague Dawley rats were created the mandibular critical bone defect, and divided into four groups: a no transplantation group as the sham group, a 3D-HA/PDLLA group, a dense-HA/PDLLA group, and a β-TCP group.

2.1. Micro-Computer Tomography (CT) Evaluation

The samples were fixed in 10% neutral buffered formalin for 7 days and then scanned by micro-CT.

2.2. Histomorphological Examination

Hematoxylin and eosin (H&E) staining was performed for histological analysis.

3. Statistical Analysis

The results of the cell proliferation were compared using an unpaired *t*-test. The qRT-PCR results were analyzed using a one-factor ANOVA test and Tukey's honest significant difference test. The difference in BV/MV% between the 3D-HA/PDLLA and β-TCP groups

was analyzed by the Kruskal–Wallis H test. In all analyses, differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

1. Cell Proliferation of Cubic Composites

The cell proliferation rate in the 3D-HA/PDLLA cellular cubic composites increased over 2 days of culturing. Cell proliferation demonstrated a dramatic increase by 48 h.

2. Gene Expression of Osteogenic Markers

The level of Runx2 expression in the 3D-HA/PDLLA groups increased during osteogenic differentiation and was significantly higher than in the dense-HA/PDLLA groups on days 3, 7, and 14 ($p < 0.05$). The levels of Sp7 were upregulated significantly over time in the 3D-HA/PDLLA group ($p < 0.05$), with the highest levels of expression on day 7 and the lowest on day 14.

3. In Vivo Experiments, Histomorphology, and Micro-CT Evaluation

Histomorphological analysis shown that at low magnification, the 3D-HA/PDLLA and β-TCP groups demonstrated an almost identical level of bone formation ($p > 0.05$). At high magnification, mild regeneration of bone, cell, and tissue was observed in the pores of the 3D-HA/PDLLA and β-TCP biomaterials at 2 weeks. During week 4, an abundance of newly formed bone tissue was seen inside the materials and along the boundary between the composite and mandible. Micro-CT demonstrated that at the four-week follow-up, parts of the borders between the biomaterial and mandible became difficult to discern, indicating slight integration of the 3D-HA/PDLLA and β-TCP biomaterial with host bone.

CONCLUSIONS

In our in vitro and in vivo experiments, the 3D-HA/PDLLA composite showed a good bioactivity, biocompatibility, and osteoconductivity. However, due to the limitations of our study, we could not measure the mature bone formation or the complete degradation of 3D-HA/PDLLA material and β-TCP. The specific mechanism allowing 3D-HA/PDLLA to function as a bone regeneration scaffold in osteogenesis and cell proliferation/differentiation also remains unknown. In future studies, we will further enhance the capability of this material in promoting bone regeneration by loading it with human mesenchymal stem cells and investigate the performance of 3D-HA/PDLLA as a scaffold in maxillofacial surgery.