学 位 論 文 の 要 旨

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学 位 論 文 名	In Vivo Evaluation of Bone Regeneration Capacity of the Novel Nanobiomaterial: β-Tricalcium Phosphate Polylactic Acid-co-glycolide (β-TCP/PLLA/PGA) for Use in Maxillofacial Bone Defects
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論 文 内 容 の 要 旨

INTRODUCTION

The maxillofacial skeleton is unique and dynamic owing to complexity of shape, presence of a microbial environment and its role in mastication and speech. Replacement of acquired or iatrogenic bone defects in the maxillofacial region is important to maintain esthetics and functionality. Usage of autologous bone grafts is considered to be the standard method to substitute bone defects, due to the property of osteoinduction. However, autologous grafting has disadvantages of surgery at an additional site, donor site morbidity and may be prone to resorption or failure. In order to obtain maximal bone formation in the defect region, synthetic bone substitutes such as Hydroxyapatite (HA) and β-Tricalcium Phosphate (β-TCP) have been developed and utilized. Though considered popular because of osteoinductivity and osteoconductivity, these materials have limited application in the maxillofacial region due to lack of plasticity and poor handling characteristics. Other types of biomaterials with malleability do not induce osteogenicity.

To improve material handling characteristics while retaining desirable properties of either material, β-TCP has been combined with second generation copolymer polylactic acid-co-glycolide (PLLA/PGA) and a novel new electrospun nanobiomaterial with a cotton fiber-like structure has been generated. This change in material shape allows for increase in fiber volume upon addition of blood and confers easy packing into bone defects of any shape or size, while exploiting the osteoinductive/osteoconductive properties of β-TCP.

In this research study, we assessed the bone regeneration ability of electrospun

β-TCP/PLLA/PGA nanobiomaterial in comparison to that of conventional pure β-TCP bone substitute block. To the best of our knowledge, this is the first animal research to evaluate the bone regeneration capability of β-TCP/PLLA/PGA nanobiomaterial in critical defects.

MATERIALS AND METHODS

We compared two kinds of biomaterials in this study: β-TCP/PLLA/PGA nanobiomaterial (70:30 ratio) and pure β-TCP bone substitute block. Sprague-Dawley (SD) male rats, n=21 (10 weeks old, average weight of 305g) were subdivided into two study groups: β-TCP/PLLA/PGA (n=9), β-TCP (n=9) and a sham group (n=3). A 3-mixture anesthetic solution consisting of 0.15 mg/kg medetomidine, 2.5 mg/kg butorphanol and 2 mg/kg midazolam were administered through intra-peritoneal injection. Following anesthesia, a full thickness sagittal incision about 1-cm in length was made in the skin overlying the right mandible and muscle layers and were dissected to expose bone after a periosteal incision. Using a trephine bur, a critical size defect 4-mm in diameter was created in the right posterior mandible of all the rats. The study group rats received either β-TCP/PLLA/PGA or β-TCP biomaterials; animals in sham group did not receive any material. Wound closure was done, and the health condition of the rats was monitored regularly.

The animals were sacrificed at three time points: week 2, week 4, and week 12; the right hemi mandibular specimens were extracted and stored in 10% neutral buffered formalin. For assessment of new bone formation, micro-computed tomography (CT) scanning and hematoxylin-eosin (HE) staining were performed. Immunohistochemical staining (IHC) was done to detect antibodies pertaining to runt related transcription factor 2 (Runx2), leptin receptor (LepR), osteocalcin (OCN) and periostin. From the micro-CT data, bone volume to total volume (BV/TV), bone mineral density (BMD), and reduction in diameter of defect was calculated to assess volume of new bone regenerated. Runx2, LepR, OCN and Periostin expressions were quantified using the optical density (OD) measurement. All analyses were carried out using ImageJ (Fiji) software.

SPSS version 27.0 (IBM Corporation, Armonk, NY, USA) was utilized to conduct statistical tests. Non-parametric Kruskal-Wallis and Mann-Whitney U tests were employed to identify significant differences between β-TCP/PLLA/PGA and β-TCP groups at weeks 2, 4, and 12. To identify significance between the same group at different time points, Wilcoxon-Signed rank test was applied. A p-value of < 0.05 indicated statistical significance. Bonferroni post hoc test decreased error probability due to multiple comparisons.

All experiments with animals in this study was approved by the Animal Care and Use Committee of Shimane University (Approval number: IZ4-38).

RESULTS AND DISCUSSION

Both biomaterials were visible in micro-CT scans due to their calcium-phosphate content.

New bone formation was elicited in both groups at all time points; however, β-TCP group had higher bone volume than β-TCP/PLLA/PGA at all time points, as per BV/TV and BMD results. Volume of newly formed bone was found to be comparable between β-TCP/PLLA/PGA and β-TCP groups. Defect diameter reduction was significantly marked in β-TCP/PLLA/PGA than in β-TCP group by week 4 ($p<0.05$), suggesting that β-TCP/PLLA/PGA induced bone growth from the margins, whereas β-TCP scaffold encouraged osteoconductivity. HE stained images revealed absence of inflammation in either group; especially the lack of inflammation in β-TCP/PLLA/PGA group could be attributed to neutralization of PLLA/PGA degradation byproducts by β-TCP.

IHC analyses shed more light on the osteogenic cell activity relative to the biomaterials. Runx2 is a marker for osteoblastic cell regulation and differentiation, whereas LepR is a direct indicator of bone marrow mesenchymal cell activity. Runx2 and LepR expressions followed a similar trend in both β-TCP/PLLA/PGA and β-TCP groups, with accumulation seen around the areas of active bone regeneration. Though less at initial stages, Runx2 and LepR expression increased significantly by weeks 4 and 12 ($p<0.01$) in β-TCP/PLLA/PGA group to surpass β-TCP group. This shows higher bioactive potential of β-TCP/PLLA/PGA at stages even when bone formation reaches saturation. OCN is an extracellular matrix component and osteogenesis indicator. OCN expression was slightly decreased in β-TCP/PLLA/PGA group during initial weeks, but showed increase at week 12, denoting active bone regeneration. Periostin is secreted by periosteal cells and reflects skeletal stem cell activity. Periostin expression was markedly increased in β-TCP/PLLA/PGA group by weeks 2 and 4 ($p<0.01$) and was found to follow the fiber pattern of β-TCP/PLLA/PGA with a gradual decline by week 12, demonstrating increased recruitment of skeletal stem cells at vicinity of the nanobiomaterial. Through our IHC results, it was clear that β-TCP/PLLA/PGA nanobiomaterial exhibited good bioactive/osteoconductivity.

CONCLUSION

Our research study indicated that the novel β-TCP/PLLA/PGA nanobiomaterial had comparable bone formation to the standard β-TCP bone substitute block, even with a lesser content of β-TCP. β-TCP/PLLA/PGA showed greater expression of osteogenic cell accumulation, activity with periostin accumulation even at later stages by week 12. Therefore, β-TCP/PLLA/PGA nanobiomaterial has easy handling characteristics and is an ideal replacement for conventional β-TCP bone substitute in the maxillofacial region.