In vivo study of three-dimensional printed calcium phosphate scaffolds for bone defect repair in rabbits

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INTRODUCTION

Calcium phosphate materials such as hydroxyapatite (HAP) and β-tricalcium phosphate (β-TCP) are often used as artificial bone in daily clinical practice of orthopaedic surgery. These materials are known to have excellent biocompatibility and osteoconductivity [1,2]. In recent years, three-dimensional (3D) printing technology has advanced in the field of tissue engineering and is used to repair damaged tissues, enabling the production of scaffolds with arbitrary shapes [3] and a 3D structure with complex internal morphology, such as trabecular bone [4]. The structure of biological bone is considered ideal for bone regeneration because it is easily permeable to cells and body fluids. Therefore, it is expected to be possible to reproduce trabecular bone structure at the macro and micro levels with artificial bone using this 3D technology which contribute to more favorable new bone formation. This study aimed to investigate the in vivo bioactivity of 3D printed β-TCP and HAP scaffolds.

METHODS

This study was approved by the animal ethics committee of our hospital.

Materials: In this study, the pure β-TCP and HAP were provided from Tomita Pharmaceutical Co. Ltd. (Tokushima, Japan).

Scaffold design and fabrication: A 3D printed scaffold was designed using medical image-computing software based on micro-computer tomography (CT) (R_mCT2 FX, Rigaku Corp, Tokyo, Japan) images of a 24-week-old rabbit femur (Japan SLC Inc., Hamamatsu, Japan). Based on the data, scaffolds with a diameter of 4 mm and a height of 6 mm were molded using a 3D printer (SZ-1100, SK Fine Co. Ltd, Shiga, Japan) to reproduce the microstructure of the bone (Fig. 1). Because the cortical bone is a dense body, bone pores were created at intervals of 200 μm to ensure connectivity with the interior.

Animal experiments: 24-week-old male New Zealand white rabbits were used in this study. The animals were anesthetized using inhalation anesthesia with isoflurane and O_2 using a mask, as well as intramuscular administration of medetomidine (0.5 mg/kg), midazolam (2 mg/kg), and butorphanol (0.5 mg/kg). A cylindrical bone defect with a diameter of 4.5 mm and a depth of 8 mm was created in the lateral aspect of the distal femur as previously described [4]. 3D printed scaffold (experimental side) was implanted on the right femur, whereas the left femur was kept free of implantation (control side). The animals were euthanized with an overdose of sodium pentobarbital at 4, 8, or 12 weeks after the operation (n = 5 knees in each group).

Micro- CT imaging: To quantify trabecular bone formation, we performed micro-CT on the harvested femurs of five animals in each group. After scanning, 3D reconstruction was performed using the built-in software. The region of interest was set as a selected area of 10 mm. The bone volume/tissue volume (BV/TV), trabecular bone thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp) of the regenerated trabecular bone was calculated in the region of interest using bone microstructure software (TRI/3D-BON-FCS64, Ratoc System Engineering, Tokyo, Japan).

Histological evaluation: Bone formation was assessed using non-decalcified frozen histology. Coronal sections (5 μm thick) were mounted on adhesive film (Cryofilm, Section-Lab Co. Ltd, Hiroshima, Japan) with a cryostat (Leica, Nussloch, Germany) and stained with hematoxylin and eosin (HE), toluidine blue, or alkaline phosphatase (ALP) /tartrate-resistant acid phosphatase (TRAP).

RESULTS

Micro- CT evaluation: In both β-TCP and HAP, newly formed bone tissue on the experimental side was detected in micro-CT, whereas almost no bone formation was observed on the control side. In β-TCP group, the BV/TV, Tb. Th, and Tb. N measured at 4, 8, and 12 weeks postoperatively gradually increased, while the Tb. Sp reduced over time. In HAP group, there were no differences among time points of the BV/TV and Tb.Th. The Tb. N gradually reduced, while the Tb. Sp increased over time (Fig. 2).

Histological evaluation: Histological sections of the experimental side stained with HE staining are shown in Fig. 3a. The 3D printed scaffolds maintained their original form 4 weeks postoperatively. New bone formation was confirmed from the existing bone toward the center of the scaffold. New bone tissue invaded the deep area of the scaffold 8 weeks postoperatively, and the scaffold was partially degraded. In β-TCP, the scaffold was further degraded 12 weeks postoperatively, and cartilage matrix calcification and woven bone formation were observed. In HAP, the 3D printed scaffold retained most of its original shape even 12 weeks postoperatively. Many active osteoblasts surround newly formed trabecular structures in ALP staining. Osteoclasts were observed using TRAP staining at all time points (Fig. 3b).

DISCUSSION

In this experiment, it was observed that cells involved in bone metabolism adhered, spread, and proliferated in our newly designed 3D printed scaffold with a bone microstructure. The results suggest that this scaffold would have sufficient bioactivity and could induce new bone formation when implanted. Therefore, it is expected that this scaffold may be a more useful artificial bone than the existing one. We are going to conduct further experiments to compare it with commercially available artificial bone.

SIGNIFICANCE/CLINICAL RELEVANCE

The study demonstrated new bone formation in a 3D printed calcium phosphate scaffold with bone microstructure.

REFERENCES

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Fig. 1. Structure of 3D printed scaffold

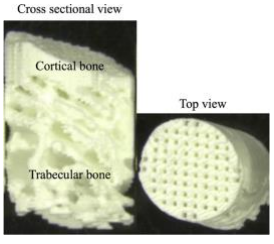

Fig. 2. Micro-CT evaluation. The upper row is β-TCP group, and the lower row is HAP group.

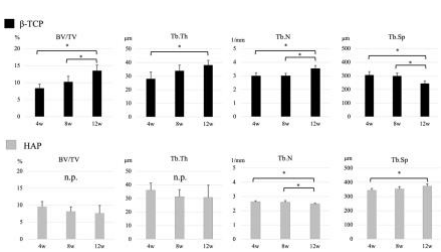

Fig. 3a. HE staining on experimental side. The upper row is β-TCP group, and the lower row is HAP group.

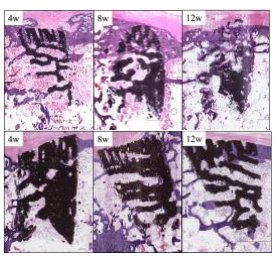

Fig. 3b. ALP (upper row) and TRAP (lower row) staining. The Red arrows :osteoblasts, blue arrows: osteoclasts.