The application of a bioresorbable scaffold composed of hydroxyapatite and polylactide

Summary of Doctoral Thesis

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Fractures are common in small animal orthopedics, with the rate of incidence being approximately 7%. The incidence rate of nonunion fractures in the dog was about 4%. In some nonunion fracture cases with a large bone defect, restoration of the limb length requires reconstruction. Several methods of bone reconstruction using an autograft, allograft, and a bioresorbable scaffold have been reported. Bioresorbable scaffolds have the advantages of being minimally invasive, not requiring any special storage facilities, and ease of intervention; they are therefore needed in veterinary orthopedic surgery.

Bioresorbable scaffolds are divided into two major groups: bioresorbable polymer and calcium phosphate. Several bioresorbable polymers have been used as the bioresorbable osteosynthetic material, particularly poly-L-lactide (PLLA), because PLLA maintains mechanical strength during bone healing and is absorbed without any inflammatory reaction. Furthermore, PLLA also has a piezoelectric effect and promotes local bone formation. Although the use of bioresorbable osteosynthetic implants was not popular in veterinary orthopedics, the bioresorbable scaffold might have a potency to be a usefull implant as an artificial bone for the dog with nonunion. However, it has been pointed out that PLLA caused pain and acute aseptic necrosis five years after implantation in the past report. Then, a bioresorbable osteosynthetic material composed of a combination of a hydroxyapatite(HA) and PLLA was developed. The biocompatibility and osteoinductive potential of HA are equal to those of beta-tricalcium phosphate (β-TCP), and it maintains mechanical strength longer than β-TCP. It has been reported that a HA/PLLA osteosynthetic implant showed good biocompatibility and direct integration with the host bone. However, PLLA needs a long period for its degradation and absorption more than poly-D-lactide (PDLA), which is an structural isomer of PLLA. Then, HA/PDLLA, which is composed of HA and PDLLA, has recently developed as a new bioresorbable material. I considered that HA/PDLLA might be suitable for a bioresorbable scaffold.

In this study, I studied on the application of this bioresorbable scaffold as an artificial bone. In chapter 2, we discussed the application and issues associated with a frozen cortical allografts(FCAs) for a femoral nonunion patient. In chapter 3, the substitution process and inflammatory reaction of the HA/PLLA composite was compared to the PLLA composite in the cortical bone. In chapter 4, the HA/PDLLA scaffold was studied, and the substitution process and mechanical strength were compared to those of the β-TCP scaffold at the unloading site. The HA/PDLLA composite has a characteristics to be transformed with heat and trimmed with a scalpel. We conducted an experiment to analyze the transforming effect of heat on the substitution process. In chapter 5, the substitution process of HA/PDLLA scaffold was studied histologically and compared to the β-TCP scaffold at the loading site using the canine tibial ostectomy model.

2: The application and issues associated with frozen cortical allografts(FCAs) at a femoral nonunion fracture.

We performed reconstructive surgery using a frozen cortical allografts(FCAs) and cancellous autograft for femoral nonunion patients. The remodeling process of the FCAs itself and the interface of proximal and distal portions of the FCAs and host bone were analyzed according to the radiographic score system reported by Weiland et al. The bony union between the proximal or distal host bone and FCAs was observed after 3 months. The remodeling of the FCAs was recognized at six months. The continuousness between the ends of the host bone and FCAs were observed. Moreover, it was observed that remodeling of the FCAs itself were progressed after 12 months. These results indicated that the FCAs showed good mechanical strength and biocompatibility in the grafted site. However, it has been reported that the implanted allograft needs a long period more than 7 years to be completely substituted by the host bone. These findings indicated that a long-term follow-up would be needed. Moreover, FCAs need special storage to maintain the temperature at -80° and normal dogs to retrieve their bone. Therefore, reconstructive surgery using FCAs was conducted in only a few facilities in Japan in the field of veterinary orthopedics. It was concluded that more convenient bioabsorbavle scaffolds would be needed to treat a bone defect of nonunion fractures.

3: Comparative study of PLLA composite and HA/PLLA composite on the substitution process in canine femur

It has been reported that the HA/PLLA osteosynthetic implants achieved direct union with the host bone and demonstrated a superior biocompatibility to the PLLA osteosynthetic implants. We therefore studied the use of HA and PLLA osteosynthetic implants as a bone scaffold. The basis of this chapter was a study of whether the HA/PLLA osteosynthetic material achieved substitution to the host bone. The aim of this study was to analyze the substitution process of the HA/PLLA osteosynthetic implants to cortical bone histologically and compare it to the PLLA osteosynthetic implants. The HA/PLLA screws and PLLA screws were inserted in the femur and a histological analysis was performed at 12, 36, 60, and 84 months.

It was confirmed that the screw hole were closed radiographically at 60 months in the HA/PLLA group, and histological analysis demonstrated that screw holes were substituted by bony tissue. However, in the PLLA group, screw holes were not closed by the bony tissue at 84 months. Additionally, the PLLA screw showed severe histiocyte cell infiltration at 60 months, whereas the HA/PLLA screw did not show severe infiltration during the follow-up period. This study showed that the HA/PLLA composite showed superior biocompatibility compared to the PLLA screw, direct union with the host bone, and substitution to the host bone. These results indicated that a bioresorbable composite composed of HA and PLLA might be available for use as a bioresorbable artificial bone.

4: A comparative study on the substitution process of HA/PDLLA scaffold and β-TCP scaffold, and the heat-transforming difference of HA/PDLLA scaffold at the unloading site.

In this chapter, I studied on the application of the HA/PDLLA scaffold compared to the β-TCP scaffold. Experiment 1 was conducted to analyze the area of new bone formation and the residual HA/PDLLA and β-TCP scaffolds. Experiment 2 was conducted to evaluate the transforming influence by heat. Experiment 3 was conducted to analyze the adherence strength of HA/PDLLA scaffold and host bone compared to that of the β-TCP scaffold. Then, the average molecular weight (Mw) of HA/PDLLA scaffold was measured.

The results of experiment 1 showed that bone formation and scaffold absorption of the HA/PDLLA scaffold was delayed compared to the β-TCP scaffold. Although the remodeling process of the HA/PDLLA scaffold was not stopped, numerous Runt-related transcription factor 2 (Runx2) -positive cells and type I collagen(Col-I)-positive tissue were observed in the HA/PDLLA scaffold compared to that in the β-TCP scaffold. The results of experiment 2 showed that the heat-transforming of the HA/PDLLA scaffold did not affect any substitution process. The results of experiment 3 showed that the HA/PDLLA scaffold and β-TCP scaffold had equal bonding strength, and the Mw of HA/PDLLA scaffold decreased significantly with time. These results indicated that the substitution process of the HA/PDLLA scaffold was slightly delayed compared to the β-TCP scaffold at the unloading site.

5: A comparative study on substitution process of the HA/PDLLA scaffold and β-TCP scaffold at the loading site.

It has been reported that the degradation rate of PDLLA was promoted at the loading site. In this chapter, I evaluated the substitution process of the HA/PDLLA scaffold at the loading site. The tibial diaphysis was ostectomized(15 mm) and the HA/PDLLA scaffold and β-TCP scaffold were inserted on each side, and three experimental groups were prepared (1, 3, and 12 months). After the follow-up period, we conducted a histological analysis. The results of this study showed that the HA/PDLLA scaffold and β-TCP scaffold represented equal bone formation. The HA/PDLLA scaffold showed earlier and better infiltration of the cell and tissue than the β-TCP scaffold. In the HA/PDLLA scaffold, numerous vessel cavities and osteoclast like-cells as well as Runx2-positive cells were present that were responsible for primary bone formation and scaffold substitution, as well as differentiation to osteoblasts, respectively. These results showed that the HA/PDLLA scaffold had induced numerous cells that were essential for bone remodeling and promoted the substitution process. There were many differences in the substitution process between the HA/PDLLA scaffold and β-TCP scaffold. However, both scaffolds did not show complete substitution at 12 months; a long-term follow-up is therefore needed for observation of complete substitution.

This study showed that the HA/PDLLA scaffold, which was developed as a new artificial bone, showed no inflammatory reaction as well as the the β-TCP scaffold and were substituted to the host bone. The HA/PDLLA scaffold was slightly delayed in the substitution process compared to the β-TCP scaffold at the unloading site, although their bonding strength was equal. Furthermore, the HA/PDLLA scaffold showed equal bone formation compared to the β-TCP scaffold, and superior infiltration of tissue and cell compared to the β-TCP scaffold at the loading site. It was concluded that the HA/PDLLA scaffold was expected to be usefull as a new bioresorbable artificial bone. However, neither scaffold achieved complete substitution to the host bone, therefore, it was considered that the follow-up study would be needed to confirm the complete substitution.