Regeneration of ligamentous tissue by using a stent like shaped poly-L-lactide scaffold

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Introduction
Severe ligament injuries result in a loss of stability of joint. Due to their limited healing ability, ligament injuries with defects remain the challenges for surgeons. Numerous attempts have been tried to improve the healing response. Surgical reconstruction with autograft have been performed, however these techniques accompany with damage of the normal tissue. We have reported that the optimal scaffold can induce stem cells from surrounding tissues and repair tendon defects without cultured cells1. In the present study, we prepared a stent like shaped scaffold made from PLLA (poly l-lactic acid) fibers and evaluated its ability in reconstructing ligament defect in a rabbit model.

Methods
Preparation of a stent like shaped scaffold
16 PLLA fibers (0.2 mm diameter) were knitted by the special device and a stent like shaped scaffold was fabricated (Figure 1). The diameter of scaffold is 3 mm and the length is 34 mm.

Implantation into rabbit medial collateral ligament (MCL) defect
General anesthesia was administered to female Japanese white rabbits (2.7–3.5 kg), then 3cm-length incision was made in the medial portion of the knee. MCL was exposed and resected from its femur and tibia insertion. In the femoral side, a bone tunnel was created by 2.0 mm drill from insertion site through a contralateral cortical bone, and in the tibial side 1.2 mm hole was drilled perpendicular to the cortex bone. Then the scaffold was threaded into bone tunnels, and the femoral end of the scaffold was fixed with a button (Meira, Nagoya, Japan). The tibial end of the scaffold or graft was fixed with an interference screw (Meira, Nagoya, Japan). As a control, a flexor digiti longs (FDL) tendon was implanted into the other side limb using the same manner. The FDL tendon was harvested from another normal rabbits. At each time period (4, 8, and 16 postoperative weeks), twelve rabbits were euthanized with intravenous dose of sodium pentobarbital and femur-ligament-tibia complex was excised. Four rabbits were examined histologically by Hematoxylin-Osin (H-E) staining and immunostaining of type I and III collagen. The other eight rabbits were examined biomechanically.

Results
Histological examination
Macroscopically, neither infection nor inflammatory reaction was found in any rabbits. At microscopic examinations in the scaffold group, spindle shaped cells, arranged along with newly formed fibrous tissues which were associated with vascular formation, were seen at postoperative 4 weeks (Figure 2a). At postoperative 8 and 16 weeks, vascularity was decreased and the fibers seemed to be parallel in regenerated tissues (Figure 2b, c). In the control group, the rupture of the ligament fiber was observed at the inside of the allograft at postoperative 4 weeks (Figure 2d), then the fibers were remodeled like a normal ligament at 8 and 16 weeks (Figure 2e, f). Immunostaining showed both type I and III collagen were detected in the scaffold group at postoperative 16 weeks (Figure 3), same as the control group.

Mechanical examination
In the scaffold group, the mean ultimate failure load was 47 N at 4 weeks postoperatively, 66 N at 8 weeks and 73 N at 16 weeks. In contrast, that in the control group was 62 N at 4 weeks, 51 N at 8 weeks and 74 N at 16 weeks. In both groups mechanical property increased in a time dependent manner, and statistical differences were not seen between the two groups at each time points. The ultimate failure load of normal MCL was 82 N and there was no significant difference compared to scaffold group at postoperative 16 weeks.

Discussion
Most of the scaffolds used in previous researches of ligamentous regeneration have solid form1. In this study, we have developed a stent like shaped scaffold which is a sparse structure and has a hollow at the center of the scaffold. Because of these structural features, it seemed that the cells could easily come into the scaffold as observed in our histological examinations. Moreover, our stent shaped scaffold has less volume of PLLA fibers compared with a solid form, therefore inflammatory reactions are supposed to be less in the stent shaped scaffold. Thus, we assume that this stent like shape is to be advantageous in ligament regeneration.

The results of immunostaining for type I and III collagen showed that the regenerated tissue in the hollow of the scaffold have a property as a ligament tissue. Furthermore, the biomechanical assessment demonstrated that PLLA scaffold alone can have a similar mechanical strength compared to the allograft until 16 postoperative weeks. These results indicated that this PLLA scaffold can be useful for the treatment of ligamentous defect without cell transplantation.

In conclusion, the novel stent shaped scaffold has a potential to repair a large ligament defect which could not be repaired by primary suture.

Significances
The stent like shaped PLLA scaffold without cell transplantation showed a similar mechanical strength at 16 postoperative weeks when compared to the allograft, therefore our new PLLA scaffold can be the possible option for the treatment of large ligamentous defects.

References
2) Chen X. et al. (2008): Biomaterial