Regeneration of tendon tissue by using a cell free knitted poly-L-lactide scaffold in a rabbit model

Hanako Nishimoto, Atsuyuki Inui, Takeshi Kokubu, Takeshi Makino, Issei Nagura, Ryosuke Sakata, Masahiro Kuroskak
*Hiroyuki Fujioka  **Kumiko Yokota  **Chihi Hiro
Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine
7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan.
*Department of Orthopaedic Surgery, Hyogo college of Medicine, Nishinomiya Japan  **Department of Mechanical Engineering, Kobe University Graduate School of Engineering

Introduction
Treatment of chronic tendon ruptures with tendon defects presents challenges for surgeons. Surgical reconstruction with tendon graft or tendontransfer is performed; however these technique accompanies with damage of the normal tissue. We have described that optimal scaffold can induce stem cells from surrounding tissues and repair tendon defects without cultured cells1. In the present study, we prepared a tubular knitted scaffold made from PLLA (poly l-lactic acid) and evaluated its ability in repairing large tendon defect, which could not be repaired by primary suture, in a rabbit model.

Methods
Preparation of knitted scaffold
Tubular scaffold was knitted with 16 strands of PLLA fiber, 0.2mm in diameter, by a special device and tubular scaffold was fabricated (Figure 1a). This scaffold has a diameter of 5mm and a length of 30mm.

Transplantation to rabbit Achilles tendon defects
General anesthesia was administered to female Japanese white rabbits (2.7–3.5 kg). A skin incision was made in the right hind leg of each rabbit and the Achilles tendon was exposed. To create an Achilles tendon defect, the tendon was cut at the site of 1 cm and 2 cm proximal to the Achilles tendon attachment to the calcaneus and a segment of the tendon (1 cm in length) was removed. After tendon excision, the rabbits were divided into two groups, the scaffold group and the control group. To repair this defect, the scaffold was transplanted into the defect and the scaffold was sutured to the Achilles tendon at both of the proximal and distal ends with 4-0 nylon (Fig. 1b). As control, an allograft of the flexor digitorum superficialis tendon was transplanted. At postoperative 4, 8 and 16 weeks, six rabbits from each group were euthanized with an intravenous fatal dose of sodium pentobarbital and the Achilles tendon and calcaneus complex was excised. Two rabbits were examined macroscopically and histologically, and four rabbits were examined mechanically at each time point in both group. Immunostaining using anti-type I and type III collagen antibody was also performed.

Results
Histologically examination
Macroscopically, neither infection nor inflammatory reaction was found in any rabbits. At microscopic examinations in scaffold group, spindle shaped cells arranged along with the newly formed fibrous tissue which was associated with vascular formation were seen at postoperative 4 weeks (Fig. 2a). At postoperative 8 and 16 weeks, vascularity was decreased and the regenerated tissue seemed to be matured (Fig. 2b, c). In control group, rupture of the tendon fiber was observed at the inside of the allograft at postoperative 4 weeks (Fig. 2d), and the fibers remodeled as a normal tendon at 8 and 16 weeks (Fig. 2e, f). In immunostaining of the regenerated tissue in the scaffold, type I collagen was detected, however, type III collagen was not observed in the scaffold at postoperative 16 weeks (Fig. 3).

Mechanical examination
In scaffold group, the mean ultimate failure load of the repaired tendon was 105N at 4 weeks postoperatively, 92N at 8 weeks and 47N at 16 weeks. In contrast, that in control group was 112N at 4 weeks, 51N at 8 weeks and 56N at 16 weeks. Mechanical property tended to decrease in both groups, however statistical differences were not seen between the two groups at each time points.

Discussion
A combination of scaffolds, cells and growth factors has been successfully used in tissue regeneration1. Most of scaffolds used in tendon repair have high density of fibers to maintain the mechanical strength during maturation of the migrated cells into the scaffold1). However, these fibers inhibit maturation of the regenerated tissue because of its slow degradation rate of the fiber. In order to keep mechanical property of the scaffold, we produced a tubular knitted scaffold made from 16 strands of fine PLLA fiber. The tubular knitted scaffold has less influence to surrounding tissue and similar mechanical property in its longitudinal axis of the allograft tendon during the entire postoperative period. When the scaffold was transplanted into a 1cm Achilles tendon defect, the defect was filled with fibrous tissue at postoperative 4 weeks. The spindle shaped cells lined to longitudinal axis with newly formed fiber and became matured at 8 weeks postoperatively. Tensile force might be effective the cells to line in longitudinal axis. Immunostaining showed the expression of type I collagen, which is main constituent of normal tendon tissue, in the regenerated tendon. Mechanically, the scaffold group had similar mechanical property as control group.

Conclusion
The novel type of the tubular knitted scaffold has a potential to repair large tendon defect which could not be repaired by primary suture.

References
1) Inui A. et al. (2009) Regeneration of rotator cuff tear by using poly-L-lactide(PLLA) cell free scaffold in a rabbit model: 55th Orthopaedic Research Society