Localization of VEGF at the early stage of cartilage repair using bioabsorbable synthetic polymer scaffold


1 Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine, Kobe, Japan,
+2 Department of Orthopaedic Surgery, Hyogo College of Medicine, Nishinomiya, Japan,
3 Department of Tissue Engineering Development, Teijin Technology Innovation Center, TEIJIN Limited, Japan

INTRODUCTION
The repair of articular cartilage defect is challenging due to the limited capacity of cartilage to heal itself. Several methods, including tissue engineering techniques, for articular cartilage repair have shown good results. Bioabsorbable scaffolds have a potential to repair the osteochondral defect without cultured cells [1]. Vascular endothelial growth factor (VEGF) has been necessary for chondrocyte survival during cartilage development [2] and the regulation of VEGF signaling during the chondrogenic differentiation is important. The objective of the present study is to investigate the localization of VEGF at the early stage of cartilage repair using bioabsorbable synthetic scaffold in a rabbit model.

METHODS
Electrospinning PLG scaffold (Fig.1)
To make the electrospinning PLG scaffold, PLG was purchased from Absorbable Polymers International, Inc. (AL, USA). The molar ratio of lactide to glycolide was 50:50. In electrospinning, synthetic ultra-fine polymer fiber ranging from 5 µm to 10 µm in diameter was fabricated in a highvoltage electron field. The porosity of each scaffold was 85±0.8 %.

Surgical procedures
Forty-eight skeletally mature female Japanese white rabbits (Kitayama Labes, Nagano, Japan) were used in this study. Osteochondral defect (5 mm diameter, 5 mm depth) was created on the patellar groove of the right knee under general anesthesia using the Osteochondral Autograft Transfer System (Arthrex, Naples, FL, USA). Then, rabbits were divided into two groups. In the scaffold group, the defect was treated with PLG scaffold and the defect in the control group, the defect was left untreated. At postoperative 1, 3, 5 days and 1, 2, 4 weeks, the femoral condyles were harvested and examined macroscopically and histologically. Each specimen was sliced at the center of each defect and sections were stained with toluidine blue. Immunolocalization of VEGF and type II collagen were analyzed.

RESULTS
Macroscopic Findings (Fig.2)
In both groups, the defect was filled with hematoma at postoperative day 1, and the filling tissue such as hematoma was concaved and the border with the normal articular surface was clearly defined until day 5. In the scaffold group, the surface of the scaffold was covered with fibrous cartilage-like tissue at postoperative week 1, and the border between the scaffold and normal articular surface became unclear. At postoperative week 4, the implanted scaffold was covered with newly formed tissue. However the defect was not healed even at 4 weeks after the surgery.

Histological Findings (Fig.3)
By postoperative week 1, the osteochondral defects of both groups were filled with hematoma. At postoperative week 2, in the scaffold group, fibrous membrane covered the surface, and the border between the defect and surrounding bone became unclear especially at the deep area. At postoperative week 4, bone formation was observed at the deep area and cartilage regeneration with metachromatic matrix was found at the entire articular surface. By contrast, in the control group, the regenerated tissue was concaved and cartilage regeneration with metachromatic matrix was found at the peripheral area of the defect.

Immunolocalization of VEGF and type II collagen (Fig.4)
At postoperative week 2 in both groups, VEGF was detected in the whole defect, however, type II collagen was not detected in the defect. At postoperative week 4, in the scaffold group, localization of VEGF decreased and type II collagen was observed at extracellular matrix in the articular surface of the defect. In the control group, VEGF was still observed at the articular surface of the defect and type II collagen was not detected.

DISCUSSION
The control of angiogenesis during chondrogenic differentiation is one of the most important issues affecting the application of stem cells for cartilage repair. VEGF is necessary for chondrocyte survival during cartilage development in mouse model, [2] however it also inhibits cartilage repair or maturation in OA model. [3, 4]

In the present study, VEGF localization was seen in both groups by postoperative week 2. This finding suggests the induction of angiogenesis at the defect in the both groups. At postoperative week 4, in the scaffold group, VEGF expression decreased instead of starting of type II collagen expression. By contrast, at postoperative week 4, in the control group, VEGF was still observed at the concaved center of the defect. These results suggest that VEGF localization indicating angiogenesis have ceased and type II collagen production have started in the scaffold group; however, that VEGF have continued to localize at the concaved center of untreated defect and cartilage regeneration delayed. Therefore, we have concluded that the optimal scaffold which could hold the ruptured bone marrow cells have a potential to repair the osteochondral defect with preventing collapse of the regenerated tissue and with supporting sequential differentiation of it.

REFERENCES
[1] Toyokawa N, 55th Annual Meeting of the ORS 2008; No 1311