**Angiogenesis Activity of Cartilage**

The activity of cartilage had no changes during the study period. This suggested that angiogenesis at the osteochondral junction might facilitate the progression of OA. Despite the presence of vascular invasion into the cartilage from the subchondral bone, it is largely unknown when vascular invasion occurs. Since vascular invasion depends on the angiogenic activities of tissue, we hypothesized that there might be a specific period with elevated angiogenic activities during the development of OA.

The purpose of this study was to investigate the change of angiogenic activities during the development of OA by biochemically and histochemically in rabbit OA model.

**Methods:** OA was surgically induced by anterior cruciate ligament transection (ACL-T) in left knee of 12 months old female New Zealand white rabbits. Contra lateral knees were sham operated. Animals were necropsied at 2, 4, 6, 8, and 12 weeks postsurgery. Six animals were allocated each time point. All the knees were examined macroscopically and three rabbits for histological evaluation and three were for angiogenic activity analysis. Histologic evaluation was performed with haematoxylin and eosin, safranin-O staining. OA changes were evaluated by the grading score of OARSI.

Angiogenic activity analysis subchondral bone and cartilage of the medial femoral condyle (MFC) and those of lateral femoral condyle (LFC) were obtained from each knee at each time point. The specimens were analyzed automatically by Angiogenesis Image Analyzer (KURABO, Osaka, Japan). The adhesion and proliferation of HUVECs were examined. The adhesion, proliferation and tube formation activity of HUVECs were examined. Then, the CD-ECM and HAM powders were mixed individually in Matrigel and injected subcutaneously into nude mice in vivo. The vessel invasion into the Matrigel was examined after 1 week.

**Results:** The adhesion and proliferation of HUVECs were more efficient on the HAM membrane than on the CD-ECM membrane. The adhesion and proliferation of HUVECs were more efficient on the HAM membrane than on the CD-ECM membrane.

**Conclusions:** Several previous studies reported that invasion of vascular tissue from the subchondral bone into the cartilage was related with degeneration of articular cartilage. This study indicated that the peak of the angiogenic activities of subchondral bone was in the early stage of OA and presumably followed by the invasion of vascular tissue into the cartilage. This result might implicate new OA treatment in terms of angiogenesis.

**Poster Presentations**

**Angiogenesis & Synovial Tissue Biology**

*100 ANGIOGENIC ACTIVITY OF SUBCHONDRAL BONE INCREASES IN THE EARLY STAGE OF OSTEOARTHRITIC KNEE*

**M. Saito,** T. Sasho, S. Yamaguchi, N. Ikegawa, R. Akagi, Y. Muramatsu, S. Mukoyama, K. Takahashi. *Dept. of Orthopaedic Surgery, Graduate Sch. of Med., Chiba University, Chiba, Japan*

**Purpose:** Angiogenesis is implicated as a cause of many diseases as well as an inevitable repair process. In osteoarthritic (OA) knees, pathological changes in the subchondral bone are considered to be related to disease initiation, progression, and a potent source of knee pain. Previous studies indicated that angiogenesis at the osteochondral junction might facilitate the progression of OA. Despite the presence of vascular invasion into the cartilage from the subchondral bone in end-stage OA knees, it is largely unknown when vascular invasion occurs. Since vascular invasion depends on the angiogenic activities of tissue, we hypothesized that there might be a specific period with elevated angiogenic activities during the development of OA.

The purpose of this study was to investigate the change of angiogenic activities during the development of OA by biochemically and histochemically in rabbit OA model.

**Methods:** OA was surgically induced by anterior cruciate ligament transection (ACL-T) in left knee of 12 months old female New Zealand white rabbits. Contra lateral knees were sham operated. Animals were necropsied at 2, 4, 6, 8, and 12 weeks postsurgery. Six animals were allocated each time point. All the knees were examined macroscopically and three rabbits for histological evaluation and three were for angiogenic activity analysis. Histologic evaluation was performed with haematoxylin and eosin, safranin-O staining. OA changes were evaluated by the grading score of OARSI.

Angiogenic activity analysis subchondral bone and cartilage of the medial femoral condyle (MFC) and those of lateral femoral condyle (LFC) were obtained from each knee at each time point. The specimens were analyzed automatically by Angiogenesis Image Analyzer (KURABO, Osaka, Japan) for 11 days. The lumens were identified, the vessels were immunostained with anti-CD31 antibodies and four parameters concerning angiogenic activities, i.e. cell adhesion, proliferation and tube-forming ability of human umbilical vein endothelial cell (HUVEC) and fibroblasts using Angiogenesis Kit (KURABO, Osaka, Japan) for 11 days according to the manufacturer’s instruction. After formation of the vessel lumens were identified, the vessels were immunostained with anti-CD31 antibodies and four parameters concerning angiogenic activities, i.e. the length, area, number of joints and paths of newly created vessels, were analyzed automatically by Angiogenesis Image Analyzer (KURABO, Osaka, Japan).

To correct age and tissue specific angiogenic activities, each parameter of OA knees was divided by corresponding sham operated knees.

**Results:** The characteristic OA features were detected from 4 weeks in MFC, and from 6 weeks in LFC macroscopically and microscopically and the grading score of OARSI increased time dependently.

The calculated parameters of the angiogenesis at subchondral bone of MFC and LFC increased time dependently in the early period, however it decreased at the later period. Angiogenic activities of MFC started to increase at 4 weeks and increased to the peak at 6 weeks. After the peak, it took downward turn at 8 weeks and decreased until 12 weeks. Those of LFC took the same tendency and started to increase at 6 weeks, but started to decrease at 8 weeks. On the other hand, the angiogenic activity of cartilage had no changes during study period.

This suggested that angiogenic activities of subchondral bone increased in the early to moderate phase of OA when cartilage had been degenerated, and activity decreased in the severe OA when cartilage had been lost.

**Conclusions:** Several previous studies reported that invasion of vascular tissue from the subchondral bone into the cartilage was related with degeneration of articular cartilage. This study indicated that the peak of the angiogenic activities of subchondral bone was in the early stage of OA and presumably followed by the invasion of vascular tissue into the cartilage. This result might implicate new OA treatment in terms of angiogenesis.

*101 THE CHONDROCYTE-DERIVED EXTRACELLULAR MATRIX INHIBITS VESSEL FORMATION IN VITRO AND IN VIVO*


**Purpose:** To examine the effect of the chondrocyte-derived extracellular matrix (CD-ECM) on the vessel formation in vitro and in a nude mice model.

**Method:** Human umbilical vein endothelial cells (HUVECs) were plated on a bio-membrane made of CD-ECM or human amniotic membrane (HAM) in vitro. The adhesion, proliferation and tube formation activity of HUVECs were examined. Then, the CD-ECM and HAM powders were mixed individually in Matrigel and injected subcutaneously into nude mice in vivo. The vessel invasion into the Matrigel was examined after 1 week.

**Results:** The adhesion and proliferation of HUVECs were more efficient on the HAM membrane than on the CD-ECM membrane. The endothelial cells exposed to HAM membrane had significant increase in growth compared to the CD-ECM membrane at all experiment period (Fig. 1a).

**Fig. 1. Influence of the chondrocyte-derived ECM (CD-ECM) on the cell adhesion, proliferation and tube-forming ability of human umbilical vein endothelial cells (HUVECs). (a) Cell adhesion and proliferation on membranes made of CD-ECM and human amniotic membrane (HAM). (b) Tube formation assay on basement membrane matrix/CD-ECM and HAM powder.**