Introduction: Thermal modification of joint capsule and ligament has gained great popularity in the orthopedic community as a treatment method for joint instability. Heating joint capsular ligament to approximately 70 to 80°C by laser or radiofrequency energy produces significant dimensional alterations (shrinkage and thickening) of the tissue treated, resulting in postoperative stabilization of the joint. Early clinical results reported a high success rate of this procedure with no major complications. However, this new surgical modality remains controversial because the mechanisms of its clinical efficacy have not been clarified. We recently reported a unique healing process after thermal modification of joint capsule and ligament, in which thermally treated tissue directly triggered an extensive fibroblastic response with little or no inflammatory phase. The purpose of this study was to understand the regulatory mechanisms of this unique tissue healing after thermal modification of the ligament. Specifically, this study evaluated the factors derived from thermally treated ligament that trigger fibroblast migration.

Materials and Methods: A total of 48 freshly harvested rat lateral collateral ligaments were treated with 80°C physiological saline (thermal treatment), or 37°C physiological saline to serve as a control under aseptic condition.

Cell migration assay: Tissues were incubated in an organ culture system with fresh culture medium for 1 hour (day 0), or 1, 3, and 4 days. After removal of the tissues, chemotactic potency of the resultant medium was evaluated using polycarbonate membrane inserts. Dermal fibroblast suspensions were added to each insert. After 6 hours of incubation, cells that had migrated across the membrane were quantified. Negative and positive controls were included.

Western blot: Tissues were incubated in an organ culture system with fresh culture medium for 4 days. Resultant conditioned media was analyzed by western blot analysis for detection of collagen type I and fibronectin.

Functional assay: Tissues were incubated in an organ culture system with fresh culture medium for 4 days. After removal of the tissues, polyclonal antibodies against PDGF, TGF-β, and fibronectin were added to the resultant media. After 1 hour of incubation, fibroblast migration to the media was evaluated using polycarbonate membrane inserts as described above.

Results: Thermal treatment of ligament resulted in obvious shrinkage.

Cell migration assay: Significantly more cells migrated toward the resultant medium in which the 37°C thermally treated tissue had been incubated when compared with the media from control tissue at each incubation time (Fig. 1). Western blot: Collagen and fibronectin and their fragments of various sizes were strongly detected only in the media derived from thermally treated tissue, whereas these proteins were not detected in the control (Fig. 2).

Discussion: This study revealed that soluble factors released from thermally treated tissue attracted more fibroblasts compared to the control, that large amounts of collagen and fibronectin were released into the media from thermally treated tissue, and that anti-TGF-β antibodies and anti-fibronectin antibodies effectively blocked fibroblast migration in thermal treatment group. The results of this study suggested that intrinsic chemotactic factors released from thermally treated tissue activate fibroblasts in the peripheral area to migrate into the injured site, without progressing through the classical pathway of wound healing (clot formation and inflammation). Particularly, TGF-β and fibronectin appear to play significant roles in recruitment of reparative fibroblasts during the healing process of thermally treated ligament tissue. This specific healing mechanism may be responsible for the unique tissue healing process after thermal modification of joint capsule and ligament.