High intrinsic healing potential of human anterior cruciate ligament

Organ culture experiments

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We studied the intrinsic healing capacity of human semitendinosus tendon specimens, comparing them to human anterior cruciate ligament specimens collected at the time of ligament reconstructions, and using an organ culture model. On the day after cultivation, migration of cells and synthesis of collagen fibers were observed in the ligament group with preserved synovium (PS), the ligament group with resected synovium (RS), and the semitendinosus tendon group (ST). Migrating cells and newly-formed collagen fibers were most prominent in the PS group, followed by the RS, and ST groups. When the area occupied by the migrating cells and newly-formed collagen fibers in the prepared hole was measured, no differences were observed between the groups at week 2. However, differences were noted between the ST and PS or RS groups at weeks 4 and 6. Our findings indicate that the human anterior cruciate ligament possesses a high intrinsic healing capacity. It is also suggested that the synovium plays an important role in enhancing ligament healing capacity.

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Anterior cruciate ligament injuries are unlikely to heal either with closed treatment or primary repair (Arnoczky et al. 1979, McDaniel and Dameron 1980, Fruensgaard et al. 1992). Best results are achieved with reconstructive surgery (Shino et al. 1984, Ochi et al. 1993a, b). The poor results of closed treatment may be due to virtual absence of intrinsic healing potential of the ligament itself or due to inappropriate ligament protection against excessive external forces during convalescence. In vivo, these 2 possible causes cannot be investigated separately, since they are intricately related. We examined the intrinsic healing capacity of the human anterior cruciate ligament in an organ culture system and assessed the effects of the synovium on the healing process.

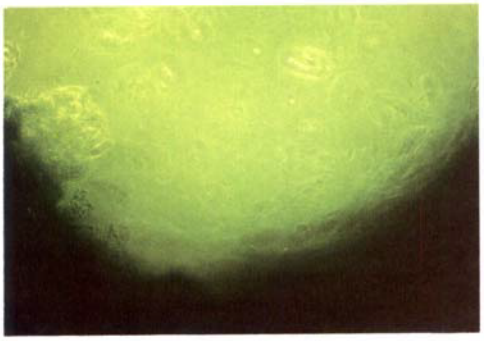
Material and methods

Anterior cruciate ligament remnants were collected from 35 patients who underwent ligament reconstruction between 1992 and 1993. There were 16 men and 19 women, mean age 25 (15–38) years. Our control samples were semitendinosus tendon segments used as ligament substitutes. The interval between injury and specimen collection was 4–7 months. Ligament and tendon specimens were placed in a culture unit under aseptic conditions immediately after surgery.

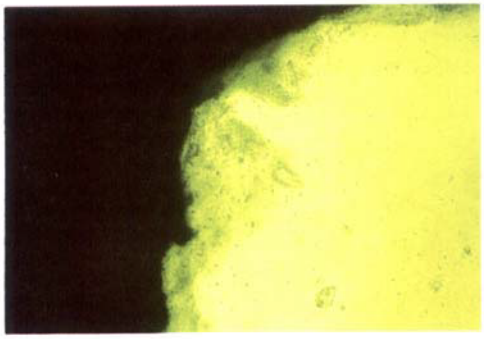
Culture specimens were divided into 3 groups: 1) the ligament group in which the synovium was carefully resected (RS), 2) the ligament group in which the synovium was preserved (PS), and 3) the semitendinosus tendon group (ST). A small piece of each specimen, measuring $10 \times 10 \times 3$ mm, was used, and a 3 mm-diameter hole was prepared therein with a dispopunch (Steefel-Laboratorium Co., Ofenbuch, Germany) (Becker et al. 1981). The tissue specimen was cultured in Eagle's minimum essential medium (MEM) (Nihon Pharmaceutical Co., Ltd., Tokyo, Japan) containing 10% fetal calf serum (FCS) (Nihon Pharmaceutical Co., Ltd., Japan). Incubation was performed at 37 °C with 5% CO_2 at 100% humidity. The medium was changed every 3 days (Frank et al. 1980).

Evaluation was based on time-course morphologic changes in phase-contrast microscopic observations until week 2 of cultivation, and light-microscopic observations of cells and collagen fibers growing in the hole. Specimens for light microscopy were fixed in 10% formalin solution, embedded in paraffin, and stained with hematoxylin and eosin (5 specimens per group were used at week 0 of cultivation, 10 specimens per group were used at weeks 2, 4, and 6). The area in the hole occupied by migrating

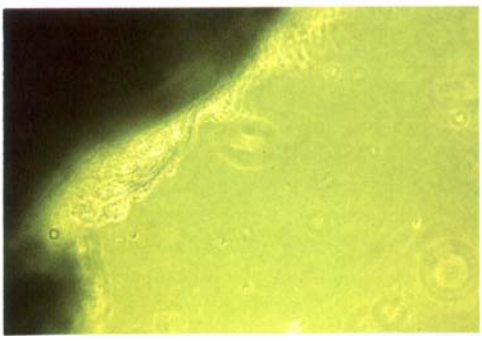
Figure 1. Phase-contrast micrographs (×40) at week 1 of cultivation. Cell migration and collagen fiber hyperplasia are seen.



PS group.



RS group.

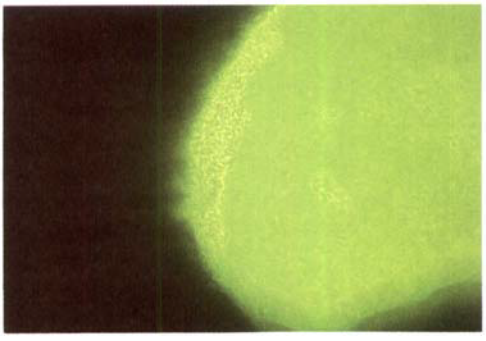


ST group.

Figure 2. At week 2 cell proliferation and collagen fiber hyperplasia are more prominent. Particularly in the PS group, multilayered cell proliferation is conspicuous around the hole.

PS group.

RS group.



ST group.

cells and proliferating collagen fibers was measured using the Cosmo Zone I.S.A. (Nikon Co., Tokyo, Japan).

Statistics

The areas at weeks 2, 4, and 6 were compared using the one-way arrangement variance analysis (ANOVA) and Tukey's multiple comparison test. Significance was determined at $P < 0.05$.

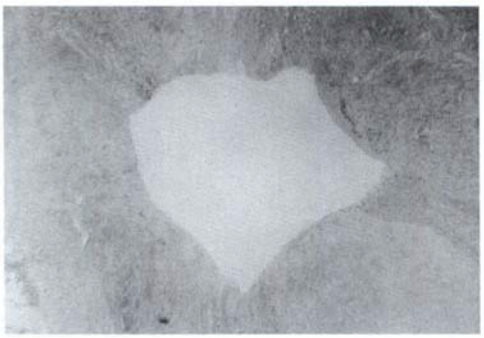
Results

Phase-contrast microscopy

On the day following the start of the culture, cell migration in the hole was observed in the PS, RS and ST groups. At week 1 of culture, the degree of cell migration and new collagen fiber formation were nearly equal in the 3 groups (Figure 1).

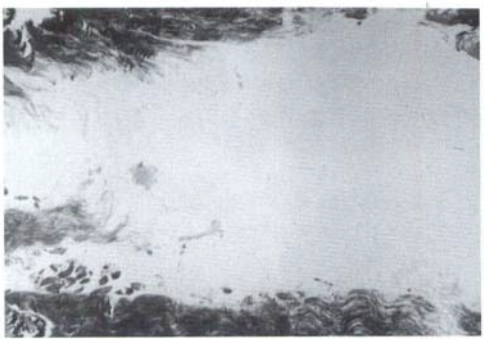
At week 2 of cultivation, multi-layered cell proliferation was prominent around the hole in the PS group. In the RS and ST groups, cell migration was

Figure 3. Light micrographs (HE staining, ×100) at week 2 of cultivation.



PS group.

RS group.



ST group.

Figure 4. Light micrographs (HE staining, ×100) at week 6 of cultivation. Newly-formed collagen fibers and migrating cells are seen.

PS group.

RS group.

observed over time, although it was not so prominent as in the PS group (Figure 2).

Light microscopy

At week 2 of the culture, migration of cells that were flat or oval in shape, and consisted of a slightly red-stained cytoplasm with an eosinophilic nucleus, was found in the RS and ST groups. Synthesis of juvenile collagen fibers was also noted. In the PS group, in contrast with the other 2 groups, fibroblast migration was conspicuous around the hole at week 2 (Figure 3). After 6 weeks of cultivation, proliferation of fibroblasts was observed in all 3 groups. Whereas only partial and random synthesis of collagen fibers was seen in the RS group, the synthesis of collagen fibers in the PS group was marked along the circumference of the hole, as if the hole was confluent with fibers, which indicates a high healing capacity (Figure 4).

Comparisons of newly-formed collagen fibers and migrating cells

The rate of hole occupation by newly-formed collagen fibers and migrating cells in the PS, RS and ST groups increased with time. At week 2 of the culture, no differences were noted in the occupation rate between the groups. At week 4 of the culture, differences were noted between the PS and ST groups, as well as between the RS and ST groups ($P < 0.05$). Differences were also evident at week 6 of cultivation between the PS and ST groups, and between the RS and ST groups ($P < 0.01$). Between groups PS and RS, no differences were observed at weeks 4 or 6 (Figure 5).

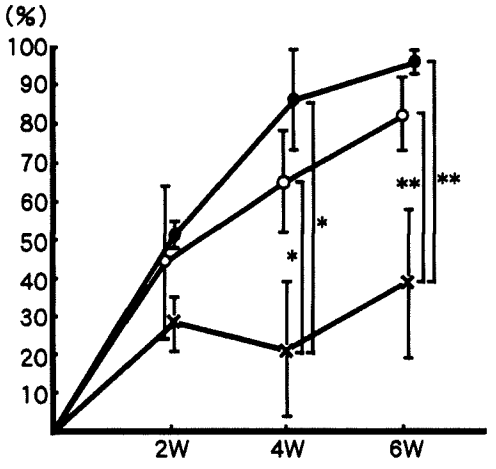


Figure 5. Changes in rate of hole occupation (%) by migrating cells and newly-formed collagen fibers.
Bars indicate 1 SD.
• PS group, ○ RS group, and X ST group,
* $P < 0.05$, ** $P < 0.01$.

Discussion

This is the first study to examine the intrinsic healing capacity of the human anterior cruciate ligament. In a rabbit model, Marshall et al. (1978) completely severed the ligament at its center, and repaired it primarily. They found no ligament repair 6 weeks after surgery.

O'Donoghue et al. (1966) also found no well-healed ligaments after primary repair in dogs. Partially injured ligaments, in which the anteromedial band had been transected, have also been shown to heal poorly after primary repair (Amiel et al. 1990). However, it is possible that the intrinsic healing capacity of the anterior cruciate ligament is difficult to assess in vivo, since several factors, including joint immobilization, are involved. For this reason, we used a culture model to determine the healing capacity of the ligament.

Intrinsic healing of tendons was investigated in rabbits by Frank et al. (1980) and Lundborg et al. (1985) using the culture systems. In our study, intrinsic healing potential was demonstrated in the human semitendinosus tendon.

In an in vivo model using rabbits, Amiel et al. (1984) compared knee ligaments (collateral and cruciate) with tendons (patellar and achilles) and found that ligaments had higher metabolic activity and synthesized a greater amount of type III collagen. Ross et al. (1990) have reported that rabbit cruciate ligaments synthesize more protein than collateral ligaments, suggesting that the former may have greater healing potential than the latter. In our study, we found that the intrinsic healing potential of the injured anterior cruciate ligament was as great as that of the normal tendon. In the joint after ligament injury or reconstructive surgery, it is known that the synovium covers the injured ligament and promote healing by supplying minor circulation (Muller 1983). In the culture system, the cruciate ligament with preserved synovium exhibited a more marked and more orderly proliferation (as if reconstituting the defect) of fibroblasts, as well as a greater synthesis of collagen fibers than did the ligament with resected synovium. Synovium promotes healing of the ligaments by a number of mechanisms.

Our findings indicate that the poor clinical outcome of closed treatment for anterior cruciate ligament injuries is not attributable to a low intrinsic healing potential of the ligament, but to other factors.

Acknowledgement

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References

Amiel D, Frank C, Harwood F, Fronek J, Akeson W. Tendon and ligament: a morphological and biochemical comparison. J Orthop Res 1984; 1 (3): 257–65.

Amiel D, Kuiper S, Akeson W H. Cruciate ligament: response to injury. In: Knee Ligaments (Eds. Daniel D M, Akeson W H, O'Connor J J). Raven Press Co, New York 1990; 19: 365–77.

Arnoczky S P, Rubin R M, Marshall J L. Microvasculature of the cruciate ligaments and its response to injury. J Bone Joint Surg (Am) 1979; 61: 1221–9.

Becker H, Graham M F, Cohe I K, Diegelmann R F. Intrinsic tendon cell proliferation in tissue culture. J Hand Surg 1981; 66: 616–9.

Frank R, Eiken O, Bergenholtz A, Lundborg G, Erkel L J. Flexor tendon specimens in organ culture. Scand J Plast Reconstr Surg 1980; 14: 179–83.

Fruensgaard S, Kroner K, Riis J. Suture of the torn anterior cruciate ligament. Acta Orthop Scand 1992; 63 (3): 323–5.

Lundborg G, Rank F, Heinau B. Intrinsic tendon healing. Scand J Plast Reconstr Surg 1985; 19: 113–7.

Marshall J L, Rubin R M, Wang J B. The anterior cruciate ligament. The diagnosis and treatment of its injuries and their serious prognostic implications. Orthop Rev 1978: 35–46.

McDaniel J W, Dameron J T. Untreated ruptures of the anterior cruciate ligament. J Bone Joint Surg (Am) 1980; 62: 696–705.

Muller W. The knee. Springer-Verlag Co, New York 1983.

Ochi M, Jitsuiki J, Yamanaka T, Sumen Y, Deie M, Ikuta Y. Arthroscopic-assisted anterior crucitate ligament reconstruction – A new substitute composed of autogenous tendons and L-K tape (in Japanese with English abstract) Kansetsukyo 1993a; 18: 171–6.

Ochi M, Yamanaka T, Sumen Y, Ikuta Y. Arthroscopic and histologic evaluation of anterior cruciate ligaments reconstructed with the Leeds-Keio ligament. Arthroscopy 1993b; 9 (4): 387–93.

O'Donoghue D H, Rockwood C A Jr, Frank G R. Repair of the ACL in dogs. J Bone Joint Surg (Am) 1966; 48: 503–19.

Ross S M, Joshi R, Frank C B. Establishment and comparison of fibroblast cell lines from the medial collateral and anterior cruciate ligaments of the rabbit. Dev Biol 1990; 26: 579–84.

Shino K, Kawasaki T, Hirose H, Gotoh T, Inoue M, Ono K. Replacement of the anterior cruciate ligament by an allogeneic tendon graft. J Bone Joint Surg (Br) 1984; 66: 672–81.