Differences among mechanoreceptors in healthy and injured anterior cruciate ligaments and their clinical importance

Mandeep Sing Dhillon
Kamal Bali
Sharad Prabhakar

Deptt of Orthopaedics PGIMER Chandigarh, India

Corresponding authors:
Mandeep Sing Dhillon
House No 92, Sector 24, Chandigarh, India
e-mail: drdhillon@gmail.com

Sharad Prabhakar
House No 110, Sector 16/A, Chandigarh, India
e-mail: sharad.ortho@gmail.com

Summary

Mechanoreceptors in an intact Anterior Cruciate Ligament (ACL) contribute towards functional stability of the knee joint. Injury to the ACL not only causes mechanical instability, but also leads to a disturbance in the neuromuscular control of the injured knee due to loss or damage to mechanoreceptors. ACL reconstruction restores proprioceptive potential of the knee to some extent, but the results vary. Although the remnant ACL contains residual mechanoreceptors, the number and functionality of these receptors is dependent, to some extent, on the physical characteristics of the remnant and duration of injury. Remnants, especially that adherent to the PCL, may actually act as a possible source of reinnervation of the graft. These remnants are worth preserving during ACL reconstruction and can play an important role in restoration of proprioception of knee following ACL reconstruction.

Key words: mechanoreceptors, ACL, proprioception.

Introduction

Proprioception is the specialized variation of the sensory modality of touch that encompasses the sensation of joint movement and joint position. It has three components: a static awareness of joint position, awareness/detection of movement and acceleration, and a closed loop efferent activity, which starts reflex response and regulates muscles. As our understanding of this complex sensory mechanism evolved, it was made clear that it contributed significantly to stability and controlled motion of the various joints of the body. Despite the complexity of the knee joint ligaments, they were previously thought to be passive structures that had a mechanical function only, and resisted abnormal stretching. It was left to Abbott et al. (1) to attribute more to these structures, as they first described the knee ligaments as having rich sensory innervation, which allowed them to act as the first link in the kinetic chain. Ever since, our understanding of this complex functioning has evolved, and it is now widely accepted that movement or change in knee position stimulates receptors in knee ligaments that allow the conscious appreciation of limb position in space (2).

Proprioception is receptor and neural arc mediated. The stimulation of mechanoreceptors in the knee ligaments initiates different types of reflex muscle contractions through the neural arc involving the dorsal root ganglion sensory neurons. Many studies over the last 30 years have demonstrated significant presence of mechanoreceptors in the fibers of an intact ACL (3-7). These were first described by Schultz et al. (3) in 1984, and it was subsequently established that the receptors included not just Paccinian, Ruffini or Golgi tendon type bodies, but also numerous nerve endings distributed all over the ACL. These receptors (along with the mechanoreceptors located in the PCL, the collateral ligaments and capsular fibers), play an important role in the complicated neural network of proprioception (8,9). They are capable of detecting changes not only in tension, speed, acceleration, and direction of movement, but also allow a subconscious determination of the position of knee joint in space (3-7,10). It becomes a corollary that damaged mechanoreceptors would alter neuromuscular functions secondary to diminished somato-sensory information (proprioception and kinesthesia). In modern orthopaedics this has become a key factor in understanding functional instability after ACL injuries (11,12), and methods to treat it.

Subsequent to an ACL injury, it has been often observed that the relationship between passive stability and the functional stability of the knee joint is often ambiguous (10,13). Borsa (14) proposed that the functional instability that occurs after an injury to the ACL is due to the combined effects of excessive tibial translation and a lack of "coordinated muscle activity" to stabilize the knee joint. This lack of coordinated muscle stabilization of the knee joint is thought to be due to diminished or absent sensory feedback from the ACL to the neuromuscular system.

Mechanoreceptors in intact ACL

The first histological demonstration of mechanoreceptors in the human ACL was done by Schultz et al. (3). The cruciate ligaments were obtained at the time of total knee replacement and from autopsy and amputation specimens. The histological sections of the ligaments were examined for the presence of mechanoreceptors using the Bodian, Bielschowsky, and Ranvier gold-chloride stains for axons and nerve-endings. Although the cruciate ligaments obtained at the time of total knee replacements were found to be too distorted by disease processes to provide sufficient evidence, the autopsy and amputation specimens contained fusiform mechanoreceptor structures measuring 200 by 75 µm, with a single axon exiting from the capsule of the receptor. One to three receptors were found at the surface of each ligament beneath the synovial membrane, but were absent from the joint capsules and menisci. Morphologically the receptors resembled Golgi tendon organs, and it seemed likely that they provided proprioceptive information and contributed to reflexes inhibiting injurious movements of the knee.

The ultrastructure of nerve endings in a human knee joint capsule was subsequently described by Halata et al. (4). These authors found three types of nerve endings: free nerve endings, Ruffini corpuscles and Pacini corpuscles. In the joint capsule, free nerve endings were located below the synovial layer and within the fibrous layer near blood vessels. These nerve terminals derived from myelinated A delta-fibres or from unmyelinated C-fibres. Ruffini corpuscles were present within the fibrous layer and the ligaments of the capsule in three variations: small Ruffini corpuscles without a capsule, small corpuscles with a connective tissue capsule, and large Ruffini corpuscles with an incomplete perineural capsule. Their afferent axons were myelinated and measured 3-5 micron in diameter. Inside the corpuscle nerve terminals were anchored in the connective tissue belonging to the fibrous layer or to the ligaments respectively. The presence of an incomplete perineural capsule depended on the structure of the surrounding connective tissue. In ligaments with collagenous fibrils oriented in a parallel fashion, the perineural capsule was well-developed and the Ruffini corpuscle resembled a Golgi tendon organ; in areas where the fibrils showed no predominant orientation, Ruffini corpuscles lacked a capsule. Small Pacini corpuscles were situated within the fibrous layer near the capsular insertion at the meniscus articularis or at the periosteum. They consisted of one or several inner cores and a perineural capsule of 1-2 layers. Larger Pacini corpuscles with one or several inner cores and a perineural capsule consisting of 20-30 layers were found on the outer surface of the fibrous layer.

Zimny et al. (15) subsequently tried to identify and quantitate mechanoreceptors in the human ACL using light microscope. Ligaments from six human subjects were obtained at autopsy, cut into cross-sectional segments 1.0-1.5 cm thick, and kept oriented as to the femoral and tibial attachments. The segments were stained in bulk by using a modified gold chloride method, frozen, and sectioned on a sliding microtome at 100 microns. With cross sectional maps of every tenth section, a computerized, morphometric analysis of the ACL was done, thus obtaining the percentage of receptors in each section and in each ACL. In addition to free nerve endings, the authors identified two morphologically distinct mechanoreceptors, namely Ruffini end organs and Pacinian corpuscles. The concentration of these mechanoreceptors were found to be greater at the femoral and tibial ends of the ligament and constituted approximately 2.5% of the ligament.

Haus et al. (15) have also studied proprioception in the anterior cruciate ligament of the human knee joint using light, scanning and transmission electron microscopy. In 21 human ACLs removed with their synovial sheaths during autopsy and operation, nerves and nerve endings were demonstrated. Ultra-structural examination allowed a classification of nerve endings into three types: Ruffinian corpuscles, Pacinian corpuscles and free (afferent and efferent) nerve endings. The nerve endings corresponded to those characteristic of articular capsules.

In 1994, Fromm and Kummer (7) investigated the nerve supply of ACLs and cryopreserved bone-ACL-bone allografts in a rabbit model with immune-histochemical methods. The ACL was found to be innervated by three different classes of nerve fibres: 1) fibres of large diameter, characterized by Neurofilament P immunoreactivity, which are fast-conducting mechanoreceptive sensory afferents; 2) fibres of small diameter, characterized by Substance P immunoreactivity, which are slow-conducting nociceptive sensory afferents; and 3) sympathetic efferent vasomotor fibres, characterized by their immunoreactivity to the rate limiting enzyme of noradrenaline synthesis, tyrosine hydroxylase. The ACLs showed numerous fibres of all three nerve classes, and specialized sensory nerve endings only Ruffini corpuscles were observed. All nerve fibres were located subsynovially, and none within the collagen core of the ligament itself. No nerve fibres were detected in the ACL allografts implanted in these rabbits at 3 and 6 weeks. Sparse fibres were detected at 12 weeks, while the 24, 36 and 52-week specimens showed plenty of all three fibre types. However, no mechanoreceptors could be demonstrated in the ACL allografts.

Using monoclonal antibody to neurofilament protein, Kruaspe et al. (16) then showed the distribution of mechanoreceptors in the entire ACL of uninjured knee joints. Numerous neurofilament-positive fibres were found in bundles. These bundles were mostly located near blood vessels in the subsynovial layer and in interfascicular gaps. Only a few single nerve fibres were found independent of blood vessels in interfascicular gaps and between collagen bundles. Neurofilament-containing nerve fibres were preferentially located near the bony attachments of the anterior cruciate ligament. Two types of corpuscular-like endings were found, i.e. spiral-like (type I) and spray-like (type II) endings. Similar to nerve fibres, both types of corpuscular-like endings were found mainly near the tibial and femoral attachment sites (15 of 17), whereas only two were found in the middle third of the ligament. Most likely, the type I and type II corpuscular-like endings served a mechanoreceptive function involved in the sensory control of normal movements and in stress protection.

Parsch et al. (17) in 1996 studied the sensory innervation of the rabbit anterior cruciate ligament by retrograde tracing technique using wheat-germ-agglutinin-horseradish-peroxidase (WGA-HRP) and Fast Blue as neuronal tracers. Injection of the tracer into the ligament was followed by histo- and immunohistochemical investigation of labelled nerve cell bodies located in the dorsal root ganglia. In 4 animals the tracer was injected into the joint cavity to label general joint afferents. The segmental distribution of retrogradely labelled neurons following injection into the ACL was significantly different from the distribution pattern after

injection into the knee joint. Retrogradely labelled nerve cells innervating the ACL were further investigated using immuno-histochemical and morphometric analysis. The authors concluded that the sensory innervation of the ACL was comprised of at least 2 different qualities of sensory afferent nerves: smaller neurons (immune-reactive to the inflammatory peptide substance P) most likely transmitting nociceptive information centrally (44%); and larger, presumably fast conducting A-fibre-afferents (characterized by neurofilament proteins) transmitting proprioceptive information from corpuscular mechanoreceptors (43%).
By the turn of the century, it was becoming clear that the mechanoreceptors located in the ACL constitute an afferent source of information toward the central nervous system. Kapreli et al. (18) proposed that ACL deficiency causes a disturbance in neuromuscular control, affects central programs and consequently the motor response resulting in serious dysfunction of the injured limb. The authors examined brain activation by using functional magnetic resonance imaging technique (1.5-T scanner) and concluded that ACL deficiency can cause reorganization of the central nervous system, suggesting that such an injury might be regarded as a neurophysiologic dysfunction, not a simple peripheral musculoskeletal injury. This evidence could explain the variation of clinical symptoms that accompany this type of injury, and the degrees of dysfunction in different individuals with an ACL deficient knee.

Mechanoreceptors in the stump of an injured ACL

A few authors (10,19-23) have tried to categorize the proprioceptive potential that may exist in the residual stump of an injured ACL. Denti et al. (10) used Ruffini gold chloride staining to look for mechanoreceptors in the injured ACL stumps. They found that in untreated ACL lesions in humans (n=20), morphologically normal mechanoreceptors persisted in the ACL remnant for about 3 months after injury. Beyond that time, the number of receptors gradually decreased. By the ninth month after injury, only a few nerve endings were found, and they were totally absent after 1 year. Their results indicate that the proprioceptive potential of the stump may diminish with the passage of time, and this may have a potential bearing on surgical outcomes in cases where reconstruction is delayed.
Ochi et al. (19) also demonstrated reproducible cortical somatosensory evoked potentials induced by electrical stimulation in 15 of 32 ACL remnants. They hypothesized that the original sensory neurons are preserved in the ACL remnants to some extent.
Georgoulis et al. (20) studied the presence of proprioceptive mechanoreceptors in the remnants of the ruptured ACL as a possible source of re-innervation of the ACL autograft. They identified two types of ACL remnants; in 15 patients the ACL was found adhered to the PCL, and in all these ligaments mechanoreceptors were observed. In five patients mushroom-like remnants were found which revealed either none or small numbers of mechanoreceptors; however, free nerve endings were found in both patient groups. The authors concluded that in patients with an ACL remnant adherent to the PCL, the mechanoreceptors existing in the residual stump might actually act as a possible source of reinnervation of the graft.
Lee et al. (21) identified mechanoreceptors using immunohistological methods in the 37 tibial remnants of the ruptured human ACL and in two normal ACL specimens taken from healthy knee amputated at thigh level due to trauma. Nineteen (8 Ruffini, 11 Golgi) mechanoreceptors (evenly distributed at both tibial and femoral attachments) were identified in the two normal ACLs. In the remnant group, mechanoreceptors were observed in 12 out of 36 cases (33%) with a total of 17 (6 Ruffini and 11 Golgi) mechanoreceptors observed. No significant differences in the harvest volume, number of sections, age, or time between injury to surgery was observed between the 12 mechanoreceptor-present and the 24 mechanoreceptor-absent ones. Although the mechanoreceptors were detected relatively less frequently than expected, the authors considered that it did not negate the necessity of remnant-preserving ACL reconstruction.
Dhillon et al. (22) also evaluated the proprioceptive potential in residual ACL remnants using immunohistological methods. The authors harvested the remnants of ruptured ACLs in 63 consecutive patients undergoing arthroscopic ACL reconstruction. These were then examined for evidence of residual proprioceptive fibers using H and E stains, and monoclonal antibodies to S-100 and NFP (neurofilament protein). Histological findings included good sub-synovial and intra-fascicular vascularity, with free nerve endings in the majority of the residual stumps. Morphologically normal mechanoreceptors (H and E) and proprioceptive fibers (positivity with monoclonal antibody for NFP) were found in 46% and 52.4% of stumps, respectively (Figs. 1-3). A statistically significant relationship was found between injury duration and persistence of mechanoreceptors and proprioceptive fibers. The proprioceptive potential was also higher in stumps in which ACL remnant was adherent to PCL. Their study showed persistent residual proprioceptive fibers in an injured ACL, (especially early cases with PCL adherence). They thus concluded that preserving the ACL remnants might improve functional outcome after ACL reconstruction as some re-innervation and recovery of proprioception is likely in such cases.
One of the issues that still sparks debate is the time period of persistence of mechanoreceptors in the stump of an injured ACL; the duration of persistence of viable mechanoreceptors is differently reported. Denti et al. (10) could not demonstrate any mechanoreceptors in stumps after one year of injury, whereas Dhillon et al. (22) reported some mechanoreceptors even as late as 42 months after injury in one patient. Georgoulis et al. (21) have reported persistence of mechanoreceptors upto 3 years in the ACL stump.
Shimuzu et al. (24) performed reconstructive surgery on the ACL of rabbits using a free bone-patellar tendon-bone (BTB) graft and evaluated nerve regeneration in the graft. They reported the appearance of mechanoreceptors in the graft between 2 and 4 weeks postoperatively with an increase to control levels some weeks postoperatively, suggesting that regeneration of mechanoreceptors occurred during this time period. At 4 and 8 weeks postoperatively, there was no significant difference in the number of mechanorecep-

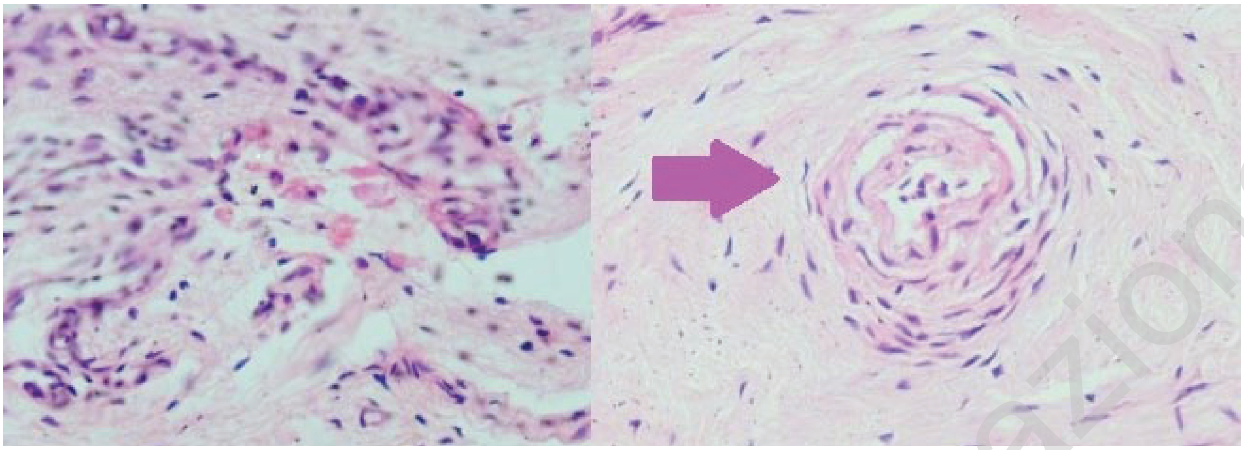

Figure 1 (a,b). Intra-ligamentous Paccinian like Mechanoreceptors, both low and high power. H & E

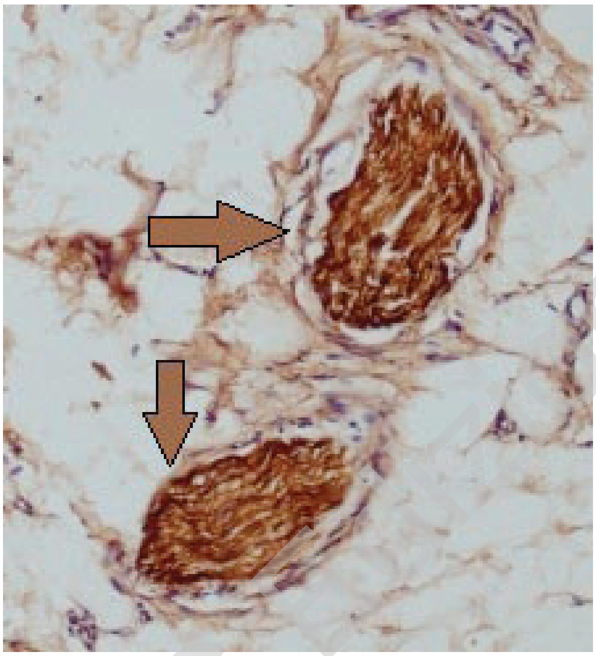

Figure 2. S-100 positive nerve endings.

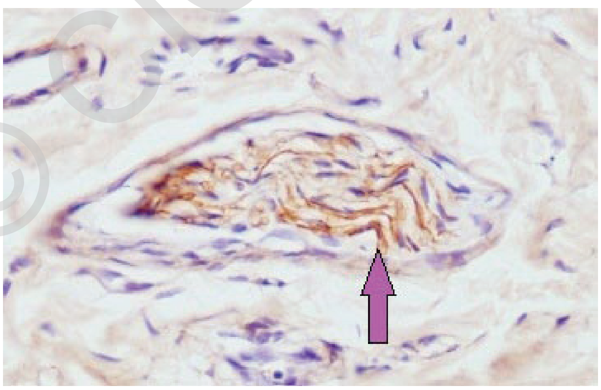

Figure 3. NFP positive slender axons.

tors between the intra-articular portion of the grafted tendon and the contralateral ACL. The authors suggested that this regeneration may restore mechanoreceptors in BTB grafts to normal levels in due course after ACL reconstruction.

Clinical importance

ACL reconstruction involves using bone-patellar tendon-bone autograft or hamstrings to replace the torn cruciate ligament. Conventionally the torn ligament remnants are shaved off from the knee before the graft is inserted. Shaving optimizes visualization and improves technical performance of the procedure (20). It is well documented that this removal of the remnant ACL stumps helps to reduce the incidence of arthrofibrosis (25,26) and also decreases the chances of developing a subsequent cyclops lesion.

Previous studies of ACL anatomy and histology have demonstrated that the maximum concentration of the nerve endings is at the attachment sites of the ligament to the bone. This serves as this main tract for proprioceptive feedback (10). These are the stumps that are seen at arthroscopy and are routinely removed, thereby aggravating the sensory damage to the knee joint.

Arthroscopic ACL reconstruction with a tibial remnant preserving technique using a hamstring graft was first described by Lee et al. (27). The authors used a hamstring graft and looped sutures according to the amount of the tibial ACL remnant. Lee et al. (28) subsequently analyzed the clinical results of ACL reconstruction with the remnant-preserving technique. They divided the patients into two groups on the basis of extent of tibial remnant: group 1 with more than 20% and group 2 with less than 20% of tibial remnant. Though evaluation of the functional outcomes did not reveal any significant differences in terms of mechanical stability between the two groups, a significant difference was detected in functional outcome and proprioception with group one (>20% remnant) showing better results. The authors thus postulated that the more the tibial remnant was kept intact, the better would be the preservation of proprioceptive function and the functional outcome for the patient.

A remnant preserving double-bundle ACL reconstruction technique using autogenous quadriceps tendon graft was developed by Kim et al. (29). The authors suggested that the remnant-preserving technique could be an effective alternative to traditional remnant shaving techniques, as such a technique provides superior proprioceptive and vascular recovery over the traditional shaving techniques.

Li et al. (30) stated that the preserved remnant provided synovium for the reconstructed ACL, and it could accelerate revascularization of the graft. The remnants not only improved proprioception but also provided mechanical stability in certain cases. The preserved remnant could prevent the enlargement of the tibial tunnel by avoiding the washing effect of the joint fluid. However, there was always a risk of developing a cyclops lesion if the remnant was preserved and this could lead to impingement. The authors concluded that remnant preservation in ACL reconstruction, although technically demanding, can provide better clinical results as compared to remnant sacrificing techniques.

The re-adaptation of the ACL remnant as it becomes adherent to the PCL may contribute to some degree of functional stability of the knee. The PCL adherent stumps were also found to be longer (21,22); this can probably be explained by the fact that an increased length allows an ACL stump to fall onto the PCL, and subsequent adhesions develop with time.

Treatment delays are a significant issue in countries of the Asian and African continents. It is presumed that degeneration of tissues and neural elements would be more in cases seen with delay as compared to those with a shorter duration of injury. Denti et al. (10) and Georgoulis et al. (21) emphasized that the proprioceptive potential of the ACL stump decreases with time. Dhillon et al. (22) found that with presence of degenerative changes the proprioceptive potential of the injured stump decreased more, further substantiating the fact that delays in treatment negatively affect the mechanoreceptor and proprioceptive fibres in the stump. The authors postulated that in ACL deficient knees of long duration, repeated episodes of giving way and re-injury due to instability may render the stump prone to degeneration and decrease its proprioceptive potential.

Conclusion

Mechanoreceptors in intact ACL contribute towards functional stability of the knee joint. Injury to ACL not only causes mechanical instability, but also leads to a disturbance in the neuromuscular control of the injured knee due to loss/damage to mechanoreceptors. ACL reconstruction restores proprioceptive potential of the knee to some extent, but the results vary. Although the remnant ACL contains residual mechanoreceptors, the number and functionality of these receptors is dependent, to some extent, on the physical characteristics of the remnant and duration of injury. Nevertheless, these remnants are worth preserving during ACL reconstruction and can play an important role in restoration of proprioception of knee following ACL reconstruction.

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