Journal of Osteoporosis & Physical Activity

Kokubun et al., J Osteopor Phys Act 2017, 5:4
DOI: 10.4172/2329-9509.1000208

Review Article Open Access

Key Determinants of Anterior Cruciate Ligament Spontaneous Healing

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Received date: October 03, 2017; **Accepted date:** October 12, 2017; **Published date:** October 19, 2017

Abstract

Anterior cruciate ligament (ACL) injuries are known to have very low rates of spontaneous healing. There have been several studies since the mid-1960s concerning the approaches to accelerate spontaneous healing of ACL injuries. Recent studies have identified similarities in the healing response of ACL and other extra-articular ligaments, in terms of their cellular response and vascularity. Research has demonstrated that mechanical stress has an important influence on the biological response of tissue healing. Novel treatment approaches may exploit the role of mechanical loads on the regulation of gene expression in achieving spontaneous healing of injured ACL. This article reviews the determinants of the ACL healing response and their relationship to mechanical stress and spontaneous healing, and explores novel concepts that are emerging in the management of ACL injuries.

Keywords: Anterior cruciate ligament (ACL); Non-surgical treatment; Spontaneous healing; Gene expression; Mechanotransduction

Current Treatment for ACL injuries

The anterior cruciate ligament (ACL) plays an important role in motion at the knee joint and therefore, in the related physical activities of daily living. The ACL stabilizes the knee and guides the movement of the tibia and femur at the joint. Injuries involving the ACL are well known for hopeless of spontaneous healing. Several studies have investigated the mechanisms of low healing response and the approaches to accelerating spontaneous healing of ACL injuries, since the mid-1960s [1-4]. The reasons for the failure of the ACL to heal spontaneously are related to specific intra-articular features [5,6] as well as owing to various molecular biological characteristics [7,8]. A number of studies have addressed on explaining the reasons for the failure of the ACL to heal spontaneously [1,3,9-13]. Since ACL injuries contribute to an increased risk of knee osteoarthritis [14,15], the current gold standard for the management of complete ACL rupture is reconstruction using a tendon autograft. However, there are no high-quality randomized controlled trials comparing ACL reconstruction with other modalities of treatment [16-19].

However, it is clear that the spontaneous healing capacity of the ACL has been underestimated. Several clinical studies have reported results of ACL healing with non-surgical treatment alone [20-22]. Moreover, an organ culture model of the human ACL showed a high intrinsic healing capacity [23]. Although these studies have demonstrated that the human ACL remnant has functional healing capacities, this was not proved in rigorous scientific methods. Consequently, current non-surgical treatment for ACL injuries aims at rehabilitating patients to modify the patients' physical activities to continue their dairy living, but not to support their spontaneous healing of the injured ACL [24,25]. This comprises muscle strength exercises, range of motion exercises, neuro-muscular training, and bracing. To summarize, although surgical reconstruction with autograft is considered to be the standard of care for restoring knee function following complete ACL injury, there is inadequate evidence supporting it as the comprehensive methods of treatment.

Key player in ACL spontaneous healing?

Extra-articular ligaments of knee, such as the medial collateral ligament (MCL) and the lateral collateral ligament (LCL), are known to heal spontaneously like the other connective tissue [26], unlike the ACL. Many researchers have analyzed the differences in the healing of intra-articular and extra-articular ligaments [27].

Bray and colleagues investigated the differences in the vascular supply following partial disruption of the MCL and ACL in rabbits [28]. They demonstrated a significant increase in blood flow and substantial angiogenic response associated with inflammation and scar formation following MCL hemisection. Conversely, there was no evidence of increased blood flow following the disruption of ACL, and the ligaments underwent atrophy. The authors concluded that the major contributor to the different between MCL and ACL was owing to their different vascular response to injury. Angiogenesis and increased blood flow, which are essential factors for ligament healing, were found to be deficient in ACL.

Studies also focused on the difference of molecular biological response between MCL and ACL. Menetrey et al. [8] investigated the differences in myofibroblasts between MCL and ACL based on the expression of α-smooth muscle actin (α-SMA) and transforming growth factor β receptor I (TGF-βRI). They demonstrated a significant correlation between α-SMA and TGF-β RI expression in injured MCL, and concluded that the combined presence of myofibroblasts and TGF-β RI may be critical for the initiation and evolution of the healing process. However, in injured ACL cells, only t TGF-β RI was identified, without a corresponding increase α-SMA expression, suggesting that

the TGF-β levels were insufficient to induce myofibroblasts differentiation. The authors concluded that this combined difference in the levels of myofibroblasts and TGF-β may also explain the difference in the healing process of ACL and MCL. Lee et al. [10] investigated whether there was any difference in the expression of the growth factors, platelet-derived growth factor (PDGF), TGF-β, and basic fibroblast growth factor (bFGF) in injured rabbit knee MCL and ACL. They identified that PDGF TGF-β and to a lesser extent, bFGF, were active in the early stage of MCL healing. However, these growth factors had a limited presence in injured rabbit ACL.

Collagenase activity may be an additional factor that prevents spontaneous healing in ACL injuries. Amiel et al. [9] confirmed the effect of collagenase activity in ACL based on studies involving hemarthrosis and synovectomy. They demonstrated that while hemarthrosis alone clearly had no effect, hemarthrosis combined with synovectomy appeared to increase the amount of active collagenase present in the ACL. These results suggested that collagenase activity may affect the ACL spontaneous healing of injured ACL.

Murray et al. [29] focused on the lack of scaffolding between the two ends of the injured ACL. They observed that the presence of plasmin in the synovial fluid contributed to the premature dissolution of the functional fibrin clot at the wound site. Treatment of ACL injuries with collagen-PRP hydrogel resulted in increased filling of the wound site with repair tissue that had similar profiles of growth factors and protein expression as in extra-articular ligament injuries [30]. They also attempted to add provisional scaffold by use of surgical suture through the injured ACL [31-34]. These studies have concluded the biomechanical features of healed ACL following the use of "bio-enhanced" ACL repair techniques were equivalent to that of conventional ACL reconstruction [33]. These results confirmed that the lack of scaffold between the two ends of the injured ACL was a key mechanism behind its failure to spontaneously heal. Murray and colleagues, recently, have attempted that the use of the bridge-enhanced ACL repair (BEAR) scaffolds methods (Figure 1) in 10 patients and confirmed a continuous ACL [35].

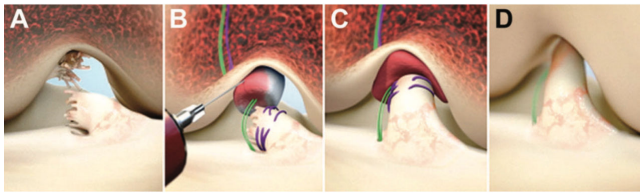

Figure 1: The bridge-enhanced ACL repair (BEAR) technique using the BEAR scaffold. For details, see Murray et al. [35].

Novel Candidate for ACL Healing

Several researchers have attempted to identify a breakthrough solution to achieve spontaneous healing of completely served ACL. However, these studies adopted a one-sided view of healing being impaired owing to intra-articular factors. Although, the ACL exists in an intra-articular milieu that is filled with synovial fluid, the knee joint is an extremely complex and dynamic functional joint, wherein the intra-articular structures, such as ACL, posterior cruciate ligament (PCL) and meniscus are subjected to both external mechanical stress arising from gravity and normal or abnormal joint motion and internal force generated by muscular activity. It is well known that these external and internal mechanical forces are important for tissue homeostasis and healing such as Wolff's law. It is also well known that connective tissue cells modify their extra-cellular matrix (ECM) to adapt to changes in mechanical load, as seen in bone remodeling or wound healing [36,37]. The susceptibility of biological factors to mechanical stress is explained by through the phenomenon of mechanotransduction. This implies that mechanical stress secondary to joint kinematics may influence the effect of biological factors on the spontaneous healing of ACL. Therefore, researchers have to focus on studies concerning the effect of joint motion, especially abnormal tibiofemoral subluxation, on the intra-articular environment, with specific reference to spontaneous healing following complete rupture of ACL.

Joint instability, especially abnormal tibiofemoral subluxation occurs secondary to ACL injuries. Clinically, this results in abnormal "giving-way" during physical activities of daily living, and sports. McDonald and colleagues attempted to determine the mechanism of abnormal tibiofemoral subluxation in knees with and without intact ACL [38]. They identified that among untreated ACL injuries, tibiofemoral subluxation was greater in the chronic phase than in the acute phase. This could be owing to greater estrangement between tibial and femoral remnants in the injured and untreated knee. Further, the "surgical transection of ACL model" is widely used in experimental in vivo osteoarthritis research because it induces permanent instability in the knee joint (stifle joint) [39]. In this model, changes of osteoarthritis are visible in articular cartilage as well as in intra-articular tissues (synovitis) [40]. The results of these studies demonstrate that the abnormal mechanical stress secondary to ACL transection-related joint instability induces a negative effect on the biochemical response of the intra-articular structures. A recent study established a relationship between the spontaneous healing response and abnormal tibiofemoral subluxation by designing an ACL spontaneous healing rodent model. In this model, abnormal tibiofemoral subluxation following surgically transected ACL was prevented by using nylon structures at the extra-articular capsule (Figures 2 and 3) [41]. The authors demonstrated spontaneous healing of the ACL in this model, and observed that the gap between the tibial and femoral remnants of the ligament was bridged by tissue that consisted of important extra-cellular matrix components including collagen type I and III. The histological and biochemical results of this study suggest that control of the abnormal tibiofemoral subluxation may result in upregulating the ACL spontaneous healing response. More importantly, the healed ACL in this model was found to have 50% of the biomechanical features of intact ACL at 8 weeks following the injury. The mechanical features of healing ACL are the most important outcome since they are crucial in serving the ligament function of connecting bones to form joint, and in maintaining their positional relation. The results of spontaneous healing can be compared to that of the reconstructed ACL, which were variously reported as following: 25% of the intact ACL by Viatesu et al. [42]; 44-49% with the use of patellar tendon autograft, by Ng et al. [43]. Although these results do not compare healing at identical timelines, they do indicate that spontaneous healed ACL has comparable biomechanical properties as reconstructed ACL.

To summarize this section, extra-articular factors and abnormal tibiofemoral subluxation are among the key determinants of ACL spontaneous healing. Mechanical stress secondary to multiple factors affects the healing response in completely injured ACL, possibly by influencing biochemical signaling in ACL cells and intra-articular structures. This phenomenon, which has an important role in

controlling the acute phase of injury, is related to as mechanotransduction. The role of mechanotransduction is well established in the development of tissues like bone, tendons, and ligaments, as well as wound healing. However, the detailed mechanisms of mechanotransduction and its role in the spontaneous healing of ACL are still under investigation.

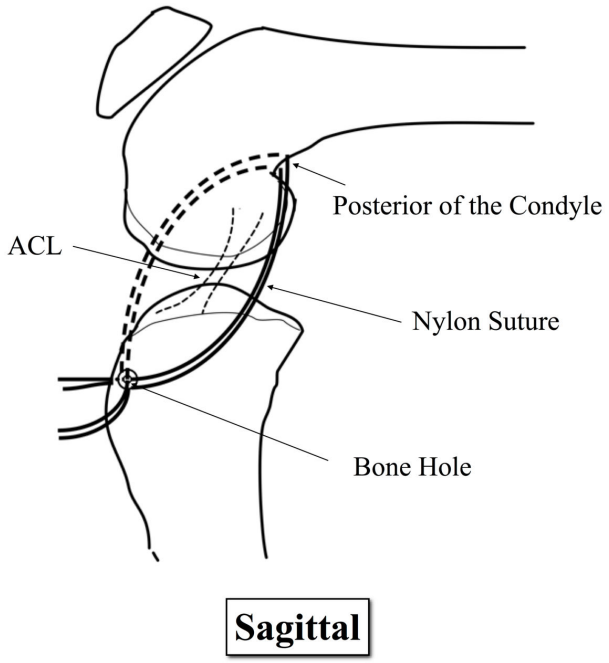

Figure 2: To control anterior tibial translation, a double 3-0 nylon suture was placed through the tibial bone hole posterior to the condyle of the distal end of the femur. For details, see Kokubun et al. [41].

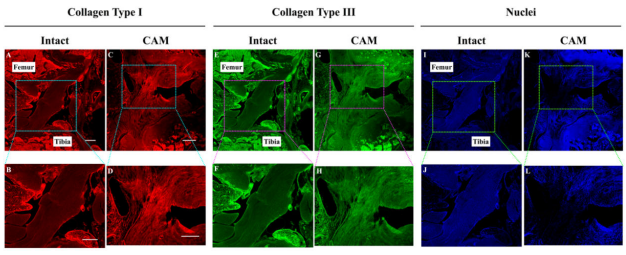

Figure 3: In immunofluorescence staining, both Col I and Col III were expressed in healed ACLs, especially in the healing region, according to immunofluorescence staining results. For details, see Kokubun et al. [41].

Conclusions and Perspectives

In this review, we focused on the novel treatments for ACL complete tear. ACL injuries affect immediate and long-term quality of life, in addition to being a well-known risk factor for post-traumatic osteoarthritis. ACL injury is common in young athlete as well as in middle-age individuals. The prolongation of the average life span has meant that ACL injury is also becoming common in the elderly [44-46]. It may be preferable to avoid surgical reconstruction in middle-age and elderly patients, owing to the diversity of their activity level following ACL injury. A better understanding of the reasons for the failure of ACL to spontaneously heal will help us develop newer treatment methods. Although we are still unaware of the entire mechanism, and the signaling pathways involved in ACL spontaneous healing, recent studies have identified few probable candidates that may be possible key players [41,47-50]. Some studies have reported that injured ACL has the same functional healing response as other extra-articular ligaments in terms of cellularity and vascularity. These biological healing responses are affected by mechanical stress, which in the case of the knee joint are induced by joint kinematics. Understanding mechanotransduction as a depending on multiple intra- and extra- articular factors may improve our ability to appreciate the true spontaneous healing capacity of the completely injured ACL. It is our recommendation that future strategies for the treatment of complete ACL transection should include progressive non-surgical treatment as the first choice, with surgical reconstruction being considered as the next choice only in the absence of gap-bridged healing. This strategy emphasizes the importance of the biomechanical aspects of the ACL-deficient knee, and relies on the mechanotransduction mechanism of ACL healing. It is our opinion that additional research in the mechano-regulation of gene expression in the ACL remnants will aid in developing newer therapeutic strategies and approaches for improving the repair and regeneration of injured ACL.

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