Published in final edited form as:

Clin Sports Med. 2009 January ; 28(1): 51–61. doi:10.1016/j.csm.2008.08.005.

CURRENT STATUS AND POTENTIAL FOR PRIMARY ACL REPAIR

Martha M. Murray, MD

Department of Orthopaedic Surgery, Children's Hospital of Boston, Boston, MA

Abstract

ACL rupture occurs in hundreds of thousands active adolescents and young adults each year. Despite current treatment, post-traumatic osteoarthritis following these injuries is commonplace within a decade of injury in these young patients. Thus, there is widespread clinical and scientific interest in improving patient outcomes and preventing osteoarthritis. The current emphasis on the removal of the torn ACL and subsequent replacement with a tendon graft (ACL reconstruction) stems from adherence to a long held and widely accepted doctrine that the ACL has only a limited healing response and therefore cannot heal or regenerate with suture repair. Recent work has shown that the premature loss of the provisional scaffold in the wound site after ACL rupture with or without repair prevents healing. Additional studies have detailed findings after placement of a substitute provisional scaffold in the wound site of the ACL injury to initiate healing of the ruptured ligament after primary repair. This technique, called enhanced primary repair, has significant potential advantages over current ACL reconstruction techniques, including the preservation of the complex attachment sites and innervation of these structures, thus retaining much of the biomechanical and proprioceptive function of these tissues. This manuscript summarizes the recent in vitro and in vivo studies in the area of enhancing ACL healing using biologic supplementation. Subsequent work in this area may lead to the development of a novel approach to treatment of this important injury.

CLINICAL SIGNIFICANCE OF ACL INJURY

Ruptures of the anterior cruciate ligament of the knee affect over 175,000 patients every year, including an estimated 38,000 high school students¹. These injuries are devastating, not only at the time of the acute injury, but also as patients who sustain an ACL tear have a 78% risk of radiographic osteoarthritis within only 14 yrs following their injury (Figure 2), whether they undergo surgical reconstruction or not². For a high school or college student with an ACL tear, that is a striking and troubling statistic.

Current Treatment of ACL tears

The anterior cruciate ligament has long been thought to have poor capacity for healing, even with suture repair. Rates of failure of healing (non-union) and structural laxity of the ACL, even with surgical repair, range from 40 to 100%^{3–5}. This is in contrast to other ligaments, such as the medial collateral ligament (MCL), where successful healing is essentially universally achievable with only six weeks of brace treatment. The lack of functional healing seen in the ACL after suture repair has been previously attributed to the “hostile” environment of synovial fluid^{6; 7}, to alterations in the cellular metabolism after injury^{8; 9}, and to intrinsic

Corresponding author: Martha M. Murray, Hunnewell 2, 300 Longwood Ave, Boston, MA 02115, Phone: 617 355 7132, Fax: 617 730-0321, Email: martha.murray@childrens.harvard.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

cell deficiencies^{10–16}. This has led to abandonment of suture repair, and almost universal adoption of ACL reconstruction for treatment. In ACL reconstruction, the torn ACL tissue is removed from the knee surgically and replaced with a tendon graft harvested either from the medial hamstrings, the middle third of the patellar tendon, or allograft tissue.

However, although ACL reconstruction is an excellent operation for restoring the sagittal plane stability of the knee, significant problems remain. In the short term, ACL reconstruction requires harvesting of other tissues from the knee, a procedure with its own associated morbidities. Allograft tissue carries the risk of disease transmission and delayed biologic incorporation, and also presents issues related to cost and availability. It also removes the native ACL tissue and the sensory nerve fibers (and thus neuromuscular function) of the ligament. Lastly, it replaces a complex, fan-shaped bundle of 17 different ligament fascicles with a single or double bundle of tendon fibers. The point-to-point graft is unable to restore the normal rotational kinematics of the knee. Some of these deficiencies may contribute to the premature degeneration of the joint which occurs after ACL injury, even with ACL reconstruction². Perhaps the most concerning problem, however, is the fact that even with ACL reconstruction, patients have a high risk for premature post-injury osteoarthritis. Thus, even with our gold standard of treatment for ACL injury as many as 78% of patients will have radiographic signs of arthritis at only 14 years after surgery².

DEFINING THE ACL RESPONSE TO INJURY

Response in the human ACL

The histologic response to injury in the human ACL has been found to be significantly different from that previously reported in the medial collateral ligament (MCL). One study examined human ACL tissue before and after rupture^{17; 18} and compared the cellular responses in the ACL with those previously reported for the MCL^{6; 19}. Using histology and immunohistochemistry techniques, it was found that like MCL cells, the ACL cells within the ligament proliferate and the ligament revascularizes after rupture^{6; 18; 19} (FIGURE 3). Collagen production was also noted to continue within the ligament up to one year out from injury²⁰. Cells in both the intact and ruptured ACL were found to migrate easily into a simulated wound site^{21; 22}.

However, the provisional scaffold which reconnected the torn collagen fascicles in the wound site of the MCL was missing in the ACL, where the two ends of the ruptured ligament were simply churning around in synovial fluid and never able to reconnect¹⁸. Thus, it appeared that while the cells and vascularity of the ACL were capable of mounting a histologic healing response, there was no structural evidence of a filling in at the wound site in the ACL (FIGURE 4).

Response in animal studies comparing ACL and MCL

The observation that there was essentially no provisional scaffold formation or wound site filling between the ends of the ruptured human ACL led to additional *in vivo* studies comparing the ACL and MCL histologic response to injury in a mechanically stable and contained defect²⁴. Central defects were created in extra-articular ligaments (MCL and/or patellar ligament) and an intra-articular ligament (ACL) in canine knees and the histologic response to injury evaluated at 3 days (n=3), 7 days (n=4), 21 days (n=5) and 42 days (n=5).

The findings in these studies were that MCL and patellar ligament defects exhibited far greater filling of the wound site and increased presence in the wound site of fibrinogen, fibronectin, platelet-derived growth factor-A, transforming growth factor-beta1, fibroblast growth factor and vonWillebrand's Factor and vWF when compared to ACL defects at all time points²⁵ (FIGURE 5). Thus, this study supported the hypothesis that there is a lack of provisional

scaffold found in the intra-articular wound site of the ACL, and this loss is also associated with a decreased presence of important extracellular matrix proteins and cytokines within the wound site of the ACL.

NOVEL HYPOTHESIS FOR ETIOLOGY OF ACL NON-UNION

These findings in humans^{17; 18; 20–22} and in the experimental large animal model^{24–26}, led to a unique hypothesis – namely, that perhaps it was this lack of provisional scaffold between the two ends of the torn ACL that was a key mechanism behind the failure of the ACL to heal. In the MCL and other comparable tissues which heal successfully outside of joints, the very first phase of the healing response is filling of the defect with a fibrin-platelet plug which bridges the wound edges¹⁹. This plug, or scaffold, is then subsequently invaded by reparative cells which remodel the scaffold into a healing fibrovascular scar (FIGURE 4). The formation of the early provisional scaffold is the first critical step in the wound healing process.

However, inside the joint, even though bleeding occurs after ACL injury, no fibrin-platelet plug is observed to form, even at the injury site²⁷ (FIGURE 4). One possible explanation for this finding is that circulating intra-articular plasmin breaks down the fibrin plug as fast as it can form. Recent work has shown that after trauma to the joint, the production of plasmin is upregulated via the increased secretion of urokinase plasminogen activator²⁸ (FIGURE 6). With the additional circulating plasmin, the fibrin network is quickly destabilized within the joint environment and no fibrin-platelet plug forms.

This premature loss of the fibrin-platelet plug would have the significant clinical benefit of preventing overall joint scarring and stiffness (arthrofibrosis) and thus maintenance of joint mobility after injury. However, the degradation of the fibrin-platelet plug in the overall joint also would remove it prematurely from any wound sites. As formation of the fibrin-platelet plug is the essential first step for wound healing of musculoskeletal tissue outside the joint¹⁹, we believe that the loss of this fibrin-platelet plug inside the joint is the key mechanism behind the failure of tissues in the joint (intra-articular tissues) to heal (FIGURE 7)

The hypothesis that the premature failure of the provisional scaffold as the impediment to ACL healing is an important change from previous mechanisms proposed for ACL non-union, which focused predominantly on the intrinsic cell and vascular responses^{11–13; 15; 29–34}. Past research based on the cell-deficiency hypotheses has focused predominantly on stimulation of cells in vitro with growth factors, or on cell transplantation into wound sites (including the use of stem cells and genetically modified cells). However, if it is failure of the provisional scaffold, an additional line of inquiry into placement of a substitute scaffold itself would be a logical step (FIGURE 1).

IN VITRO DEVELOPMENT OF A SUBSTITUTE SCAFFOLD

Based on the early studies on the provisional scaffold failure in the ACL wound site in humans and animals, materials that might be useful in the ACL wound site were evaluated. The substitute scaffold would need to withstand the physical and mechanical conditions of the intra-articular environment. The mechanical boundary conditions require the scaffold be able to withstand the relatively high physiologic strains seen by the connective tissues of interest, strains which typically approach 3% during normal knee motion³⁵. Enzymatically, the bridge would need to be resistant to degradation by circulating intra-articular inflammatory metabolites and proteolytic enzymes that are present after injury or surgery, including plasmin²⁸, matrix metallo-proteinases³⁶ and glycosidases³⁷. It is also likely that biologic stimuli would be required to successfully replicate the successful wound healing environment, either in the form of a single cytokine^{38; 39} (sufficiently upstream in the wound healing

cascade) or in a group of growth factors, including the cohort released by platelets in other wound sites.

BIOLOGIC AUGMENTATION OF PRIMARY ACL REPAIR

Supplementation with Growth Factors

In vitro studies have demonstrated improved cellular proliferation and migration, as well as increased collagen production rates with the addition of growth factors including PDGF^{16; 40}, TGF- β ⁴⁰, and FGF^{29; 40}. In follow-up studies of these in vitro results, healing of the ACL using growth factors in animal models has also been studied^{39; 41}. Kobayashi et al noted improved filling and vascularity surrounding a central defect in a canine ACL model with implantation of a bFGF pellet; however, no biomechanical testing was performed in that study⁴¹. In a study of MCL transection in a rabbit model, Spindler et al reported that the addition of TGF- β 2 to the wound site resulted in an increase in scar size, but not scar strength³⁹.

Supplementing Structure using Scaffolds

Healing of intra-articular defects using substitute provisional scaffolds has been a recent area of interest. Defects in the ACL have been treated with synthetic scaffolds loaded with growth factors including TGF- β ⁴¹ and also with hyaluronic acid⁴². These techniques have had limited success, but unfortunately continue to have problems with implant-host integration, cell survival after transplantation, and degradation with time. Better results have been obtained with the use of collagen scaffolds loaded with platelets.

Hyaluronic Acid As a Scaffold Treatment—In a central defect rabbit model, Wiig et al reported improved covering of a central defect in the ACL with intra-articular injection of hyaluronic acid in a rabbit model⁴². In that study, a greater angiogenic response was seen in the group of HA treated ligaments. More Type III collagen was produced in the ligaments treated with HA than in the group of ligaments treated with saline. No biomechanical testing was reported for that study.

Collagen-Platelet Composites—Recent studies have detailed the outcomes of treating a complete ACL transection with a suture repair augmented with a substitute scaffold. The complete transection model in the pig results in instability of the knee joint itself and provides a harsher healing environment that closely mimics the condition of the human knee after ACL rupture. In this study, bilateral ACL transections were performed in seven 30 kg Yorkshire pigs and repaired with a four stranded, absorbable suture repair⁴³(FIGURE 1). In five animals, one of the repairs was augmented with placement of a collagen-platelet composite at the ACL transection site, while the contralateral knee had suture repair alone. No postoperative immobilization was used. After a four week healing period the animals underwent *in vivo* magnetic resonance imaging followed by euthanasia and immediate biomechanical testing. Six control knees with intact ACLs from three additional animals were used as an intact ACL control group. Supplementation of suture repair with a collagen-platelet composite resulted in formation of a large scar mass in the region of the ACL. Load at yield, maximum load and ACL tangent modulus were all significantly higher in the suture repairs augmented with collagen-platelet composite than in repairs performed with suture alone. This paper concluded that biomechanical healing of the porcine ACL after complete transection and immediate suture repair can be enhanced at an early time point with use of a collagen-platelet composite placed in the wound site at the time of primary repair⁴³.

The results of this study demonstrated that primary repair using the collagen-platelet composite resulted in healing ligament strength which was over 50% of the intact ligament strength at 4

weeks after repair. This strength compares favorably with the current gold standard of ACL reconstruction, where strengths at 3 to 4 weeks are under 25% of the intact ligament strength (Figure 8, Hunt et al, 2005⁴⁴).

FUTURE DIRECTIONS

This first demonstration of histologic and biomechanical healing of the ACL will likely lead to entirely new fields of inquiry surrounding treatment of this important structure. Hypotheses regarding the influence of patient age or gender on repair outcome become interesting and important, as do questions regarding the ability to maintain “mechanical homeostasis” by stimulating sufficient anabolic cellular responses to generate wound strength, while at the same time, encouraging gradual catabolism of the provisional scaffold. It is also likely that the concentration of cellular components of the platelet-rich plasma (specifically platelets and leukocytes) significantly effect the cellular proliferation and migration into the wound site, as might the concentration of the extracellular matrix molecules within the substitute scaffold. One might hypothesize that changes in these fundamental cellular behaviors may result in significant changes in the mechanical, and functional, properties of this ligament. These are just a few of the questions that will likely be investigated over the next few years.

CONCLUSION

The last decade of research into the potential of primary ACL repair has resulted in discovery of a novel mechanism to explain the failure of the ACL to heal and the validation of several large animal models to test new techniques of repair in vivo. Further optimization of these techniques into a successful surgical procedure has the potential to alter the clinical treatment of ACL injuries.

Acknowledgements

Funding was received from NIH grants AR054099, AR052772 and AR049346.

References

1. Myer, GD.; Ford, KR.; Hewett, TE. Rationale and Clinical Techniques for Anterior Cruciate Ligament Injury Prevention Among Female Athletes; J Athl Train. 2004. p. 352-364.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15592608
2. Von Porat, A.; Roos, EM.; Roos, H. High prevalence of osteoarthritis 14 years after an anterior cruciate ligament tear in male soccer players: a study of radiographic and patient relevant outcomes; Br J Sports Med. 2004. p. 263
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15155422
3. Sherman MF, Bonamo JR. Primary repair of the anterior cruciate ligament. Clin Sports Med 1988;7:739–750. [PubMed: 3052880]
4. Kaplan N, Wickiewicz TL, Warren RF. Primary surgical treatment of anterior cruciate ligament ruptures: a long-term follow-up study. Am J Sports Med 1990;18:254–358. [PubMed: 2372074]
5. Feagin JA Jr, Curl WW. Isolated tear of the anterior cruciate ligament: 5-year follow-up study. Am J Sports Med 1976;4:95–100. [PubMed: 984289]
6. Woo SL, Vogrin TM, Abramowitch SD. Healing and repair of ligament injuries in the knee. Journal of the American Academy of Orthopaedic Surgeons 2000;8:364–372. [PubMed: 11104400]
7. Andrish, J.; Holmes, R. Effects of synovial fluid on fibroblasts in tissue culture; Clin Orthop. 1979. p. 279-283.
8. Amiel, D.; Ishizue, KK.; Harwood, FL.; Kitabayashi, L.; Akeson, WH. Injury of the anterior cruciate ligament: the role of collagenase in ligament degeneration; J Orthop Res. 1989. p.

- 486-493.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2544709
9. Saris, DB.; Dhert, WJ.; Verbout, AJ. Joint homeostasis. The discrepancy between old and fresh defects in cartilage repair; *J Bone Joint Surg Br.* 2003. p. 1067-1076.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14516049
 10. Wiig M, Amiel D, Ivarsson M, Nagineni C, Wallace C, Arfors K. Type I procollagen gene expression in normal and early healing of the medial collateral and anterior cruciate ligaments in rabbits: an in situ hybridization study. *J Orthop Res* 1991;9:374-382. [PubMed: 2010841]
 11. Lyon, RM.; Akeson, WH.; Amiel, D.; Kitabayashi, LR.; Woo, SL. Ultrastructural differences between the cells of the medial collateral and the anterior cruciate ligaments; *Clin Orthop.* 1991. p. 279-286.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1934745
 12. Nagineni, CN.; Amiel, D.; Green, MH.; Berchuck, M.; Akeson, WH. Characterization of the intrinsic properties of the anterior cruciate and medial collateral ligament cells: an in vitro cell culture study; *J Orthop Res.* 1992. p. 465-475.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1613622
 13. Gesink, DS.; Pacheco, HO.; Kuiper, SD.; Schreck, PJ.; Amiel, D.; Akeson, WH.; Woods, VL, Jr. Immunohistochemical localization of beta 1-integrins in anterior cruciate and medial collateral ligaments of human and rabbit; *J Orthop Res.* 1992. p. 596-599.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1377240
 14. Schreck, PJ.; Kitabayashi, LR.; Amiel, D.; Akeson, WH.; Woods, VL, Jr. Integrin display increases in the wounded rabbit medial collateral ligament but not the wounded anterior cruciate ligament; *J Orthop Res.* 1995. p. 174-183.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7722754
 15. Lee, J.; Harwood, FL.; Akeson, WH.; Amiel, D. Growth factor expression in healing rabbit medial collateral and anterior cruciate ligaments; *Iowa Orthop J.* 1998. p. 19-25.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9807704
 16. Kobayashi, K.; Healey, RM.; Sah, RL.; Clark, JJ.; Tu, BP.; Goomer, RS.; Akeson, WH.; Moriya, H.; Amiel, D. Novel method for the quantitative assessment of cell migration: a study on the motility of rabbit anterior cruciate (ACL) and medial collateral ligament (MCL) cells; *Tissue Eng.* 2000. p. 29-38.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10941198
 17. Murray MM, Spector M. Fibroblast distribution in the anteromedial bundle of the human anterior cruciate ligament: the presence of alpha-smooth muscle actin- positive cells. *J Orthop Res* 1999;17:18-27. [PubMed: 10073643]
 18. Murray MM, Martin SD, Martin TL, Spector M. Histological changes in the human anterior cruciate ligament after rupture. *J Bone Joint Surg Am* 2000;82-A:1387-1397. [PubMed: 11057466]
 19. Frank C, Amiel D, Akeson WH. Healing of the medial collateral ligament of the knee. A morphological and biochemical assessment in rabbits. *Acta Orthop Scand* 1983;54:917-923. [PubMed: 6670520]
 20. Spindler KP, Clark SW, Nanney LB, Davidson JM. Expression of collagen and matrix metalloproteinases in ruptured human anterior cruciate ligament: an in situ hybridization study. *J Orthop Res* 1996;14:857-861. [PubMed: 8982126]
 21. Murray MM, Martin SD, Spector M. Migration of cells from human anterior cruciate ligament explants into collagen-glycosaminoglycan scaffolds. *J Orthop Res* 2000;18:557-564. [PubMed: 11052491]
 22. Murray MM, Spector M. The migration of cells from the ruptured human anterior cruciate ligament into collagen-glycosaminoglycan regeneration templates in vitro. *Biomaterials* 2001;22:2393-2402. [PubMed: 11511036]

23. Steiner, ME.; Murray, MM.; Rodeo, SA. Strategies to improve anterior cruciate ligament healing and graft placement; *Am J Sports Med.* 2008. p. 176-189.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18166680
24. Spindler, KP.; Murray, MM.; Devin, C.; Nanney, LB.; Davidson, JM. The central ACL defect as a model for failure of intra-articular healing; *J Orthop Res.* 2006. p. 401-406.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16479574
25. Murray, MM.; Spindler, KP.; Ballard, P.; Welch, TP.; Zurakowski, D.; Nanney, LB. Enhanced histologic repair in a central wound in the anterior cruciate ligament with a collagen-platelet-rich plasma scaffold. *J Orthop Res.* 2007.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17415785
26. Murray, MM.; Spindler, KP.; Devin, C.; Snyder, BS.; Muller, J.; Takahashi, M.; Ballard, P.; Nanney, LB.; Zurakowski, D. Use of a collagen-platelet rich plasma scaffold to stimulate healing of a central defect in the canine ACL; *J Orthop Res.* 2006. p. 820-830.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16555312
27. Harrold AJ. The defect of blood coagulation in joints. *J Clin Path* 1961;14:305–308. [PubMed: 13711857]
28. Rosc D, Powierza W, Zastawna E, Drewniak W, Michalski A, Kotschy M. Post-traumatic plasminogenesis in intraarticular exudate in the knee joint. *Medical Science Monitor* 2002;8:CR371–378. [PubMed: 12011780]
29. Amiel D, Nagineni CN, Choi SH, Lee J. Intrinsic properties of ACL and MCL cells and their responses to growth factors. *Med Sci Sports Exerc* 1995;27:844–851. [PubMed: 7658945]
30. Chen H, Tang Y, Li S, Shen Y, Liu X, Zhong C. Biologic characteristics of fibroblast cells cultured from the knee ligaments. *Chinese Journal of Traumatology* 2002;5:92–96. [PubMed: 11904070]
31. Geiger MH, Amiel D, Green MH, Most D, Berchuck M, Akeson WH. Rates of migration of ACL and MCL derived fibroblasts. *Orthop Trans* 1992;17:75.
32. Geiger MH, Green MH, Monosov A, Akeson WH, Amiel D. An in vitro assay of anterior cruciate ligament (ACL) and medial collateral ligament (MCL) cell migration. *Connect Tissue Res* 1994;30:215–224. [PubMed: 8039388]
33. Spindler KP, Imro AK, Mayes CE, Davidson JM. Patellar tendon and anterior cruciate ligament have different mitogenic responses to platelet-derived growth factor and transforming growth factor beta. *J Orthop Res* 1996;14:542–546. [PubMed: 8764862]
34. Spindler KP, Andrich JT, Miller RR, Tsujimoto K, Diz DI. Distribution of cellular repopulation and collagen synthesis in a canine anterior cruciate ligament autograft. *J Orthop Res* 1996;14:384–389. [PubMed: 8676250]
35. Heijne, A.; Fleming, BC.; Renstrom, PA.; Peura, GD.; Beynon, BD.; Werner, S. Strain on the anterior cruciate ligament during closed kinetic chain exercises; *Med Sci Sports Exerc.* 2004. p. 935-941.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15179161
36. Fernandes, JC.; Martel-Pelletier, J.; Pelletier, JP. The role of cytokines in osteoarthritis pathophysiology; *Biorheology.* 2002. p. 237-246.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12082286
37. Shikhman, AR.; Brinson, DC.; Lotz, M. Profile of glycosaminoglycan-degrading glycosidases and glycoside sulfatases secreted by human articular chondrocytes in homeostasis and inflammation; *Arthritis Rheum.* 2000. p. 1307-1314.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10857789
38. Spindler KP, Dawson JM, Stahlman GC, Davidson JM, Nanney LB. Collagen expression and biomechanical response to human recombinant transforming growth factor beta (rhTGF-beta2) in the healing rabbit MCL. *Journal of Orthopaedic Research* 2002;20:318–324. [PubMed: 11918312]

39. Spindler KP, Murray MM, Detwiler KB, Tarter JT, Dawson JM, Nanney LB, Davidson JM. The biomechanical response to doses of TGF-beta 2 in the healing rabbit medial collateral ligament. *Journal of Orthopaedic Research* 2003;21:245–249. [PubMed: 12568955]
40. Meaney Murray M, Rice K, Wright RJ, Spector M. The effect of selected growth factors on human anterior cruciate ligament cell interactions with a three-dimensional collagen-GAG scaffold. *Journal of Orthopaedic Research* 2003;21:238–244. [PubMed: 12568954]
41. Kobayashi, D.; Kurosaka, M.; Yoshiya, S.; Mizuno, K. Effect of basic fibroblast growth factor on the healing of defects in the canine anterior cruciate ligament; *Knee Surg Sports Traumatol Arthrosc.* 1997. p. 189-194.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9335032
42. Wiig, ME.; Amiel, D.; VandeBerg, J.; Kitabayashi, L.; Harwood, FL.; Arfors, KE. The early effect of high molecular weight hyaluronan (hyaluronic acid) on anterior cruciate ligament healing: an experimental study in rabbits; *J Orthop Res.* 1990. p. 425-434.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2324860
43. Murray, MM.; Spindler, KP.; Abreu, E.; Muller, JA.; Nedder, A.; Kelly, M.; Frino, J.; Zurakowski, D.; Valenza, M.; Snyder, BD.; Connolly, SA. Collagen-platelet rich plasma hydrogel enhances primary repair of the porcine anterior cruciate ligament; *J Orthop Res.* 2007. p. 81-91.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17031861
44. Hunt, P.; Scheffler, SU.; Unterhauser, FN.; Weiler, A. A model of soft-tissue graft anterior cruciate ligament reconstruction in sheep; *Arch Orthop Trauma Surg.* 2005. p. 238-248.
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15024579

A. View of the ACL position within the knee

B. Schematic of suture repair supplemented with a growth factor gel.

FIGURE 1A and B.

Schematic of the enhanced suture repair technique. In 1A, the location of the ACL between femur and tibia is illustrated. The enlarged view on the right (1B) shows a transected ACL treated with suture repair where the sutures are attached to an anchor in the bone at the femoral insertion site and then are passed through the longer distal ACL segment and tied to reapproximate the proximal and distal ACL segments. The growth factor hydrogel is introduced into the wound site to stimulate biologic repair. (*Reproduced with permission from Murray MM, Spindler KP, Abreu E, et al. Collagen-platelet rich plasma hydrogel enhances primary repair of the porcine anterior cruciate ligament. J Orthop Res. 2007;25:81–91*).

Figure 2. Premature osteoarthritis after anterior cruciate ligament (ACL) injury. Radiographs of a 40 year old man who had sustained an ACL rupture 20 years earlier. Note the joint space narrowing and osteophyte formation consistent with premature osteoarthritis (arrow) on the medial aspect of the left knee (knee with ACL tear) and the preservation of medial joint space in the knee with the intact ACL on the right (arrow).

Figure 3.

Histologic response of the human ACL to rupture. A. Histologic appearance of the normal ACL showing fibroblasts (blue nuclei; 40X). B. Histologic appearance of ACL tissue 3 months after rupture showing increased cell density in the ligament ends (40X). C. Area of increased vessel density in ruptured ACL fragments at 3 months after injury (20X). D) Synovial layer which has reformed over the ligament ends at 8 weeks after rupture (BV = blood vessel, 40X). Sections are immunohistochemistry for alpha-smooth muscle actin (SMA) where red demonstrates a positive stain for SMA, with a blue counter-stain for cell nuclei18.

Figure 4.

Representative micrographs of slit wounds made with a modified Beaver blade in the center of the MCL and ACL seven days earlier in a canine knee. Note that the MCL wound is filled with a provisional scaffolding material containing high amounts of multiple growth factors important in tissue healing (here, immunohistochemistry for FGF-2 where red is a positive stain). In the ACL wound, however, the defect remains unfilled, even after seven days.

(Adapted with permission from Steiner, ME, Murray, M.M. and Rodeo, S.A. Strategies to Improve Anterior Cruciate Ligament Healing and Graft Placement, American Journal of Sports Medicine, 2008, 36(10), pages 176–8923.)

Figure 5.

Representative micrographs of the wound site in the extra-articular patellar ligament (EA row) and the intra-articular ACL (IA row) after three weeks in vivo. Note the filling of the wound site in the EA ligament with an active repair process occurring within the provisional scaffold (top row). In contrast, the ACL wounds remain unfilled (bottom row) 25. (*Reproduced with permission from Murray, MM., Spindler, K.P., Ballard, P., Welch, T., Nanney, LB. Enhanced Histologic Repair in a Central Defect in the ACL with a Collagen-PRP Scaffold. J Orthop Res, 2007, 25(8):1007–1017.*)

Figure 6.

Proposed pathway for accelerated fibrinolysis after joint injury. The increased secretion of urokinase plasminogen activator (u-PA) results in high levels of plasmin in the inflammatory synovial fluid. This is a likely mechanism for the accelerated fibrinolysis noted in the joint after injury. (t-PA: tissue plasminogen activator. PAI-1 and 2: Plasminogen activator inhibitor 1 and 2. uPAR: urokinase-type plasminogen activator receptor.

Figure 7.

Novel hypothesis of the failure of ACL healing. For the medial collateral ligament (MCL) which is outside the joint, injury is followed by formation of a provisional scaffold in the form of a fibrin clot. The scaffold is gradually remodeled as the tissue heals (top row). No fibrin clot is found at the injury site of the anterior cruciate ligament (ACL), which is inside the knee joint. Without a provisional scaffold, the wound site remains empty and healing cannot proceed.

Figure 1

Figure 8.

Load at yield and maximum load for the three groups: suture repair alone (Suture), suture repair plus collagen-platelet composite (Suture/PRP), and intact ACLs (Intact ACL). Differences were observed between intact ACLs and each of the other two groups (denoted by #). Load at yield and maximum load were both significantly higher for suture repair plus collagen-platelet composite compared to suture repair alone as denoted by asterisks. Error bars represent standard deviations. Arrow designates yield strength of ACL reconstruction at a similar time point⁴⁴. (Adapted with permission from Steiner, ME, Murray, M.M. and Rodeo, S.A. *Strategies to Improve Anterior Cruciate Ligament Healing and Graft Placement*, American Journal of Sports Medicine, 2008, 36(10), pages 176–8923.).