Biologic Orthopedic Journal

Original Article
DOI: 10.22374/boj.v3iSP2.45

BIOLOGIC ACL REPAIR AUGMENTATION: STATE-OF-THE-ART

Tiago Lazzaretti Fernandes, MD, MSc, PhD, João Vitor de Castro Fernandes, MD, Sergio Ferreira Barbosa Junior, MD, Teófilo Josué Alecrim da Costa Vieira, MD, Arnaldo José Hernandez, MD, PhD

Sports Division, Institute of Orthopedics and Traumatology, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil

Author for correspondence: João Vitor de Castro Fernandes: joaovcf@gmail.com

Submitted: 2 June 2022. Accepted: 3 February 2023. Published: 6 March 2023

Abstract

The incidence of anterior cruciate ligament (ACL) injuries is on the rise due to increased physical and sports activities. Thus, ACL reconstruction is a common surgical procedure to treat ACL ruptures, however, it has a failure rate of 0.7–21% and only 63% of the patients are able to recover to their pre-injury sport level. Orthobiologics, including platelet-rich plasma (PRP), growth factors, and stem cells, are being explored as alternative treatment methods to maximize the results and reduce surgical morbidity and healing time. PRP is derived from a blood sample with high platelet concentration and containing growth factors and interleukins. Studies have shown that PRP can improve the maturation of ACL reconstruction but more research is required to support its use in ACL surgery. On one hand, growth factors play a crucial role in ligament healing and PRP, which contains high levels of growth factors, has been shown to stimulate angiogenesis and encourage cell proliferation. On the other hand, stem cells have the ability to differentiate into other cell types and limit the inflammatory microenvironment during acute ligament injury. Ligament-derived stem cells show better potential for lineage-specific tendon/ligament differentiation when used with differentiation inducers. The use of stem cells in ACL reconstruction is still in the early stages of investigation. Therefore, cell therapy agents have shown promising results in preclinical models, but more research is required to determine the most effective biological agents for treatment.

Keywords: *ACL tears*; *biologic ACL augmentation*; *PRP*; *growth factors*; *stem cells*

INTRODUCTION

There is a strong link between anterior cruciate ligament (ACL) injuries and physical/sports activities, and also a gradual increase in injuries in the practical orthopedic routine due to the increase in these activities.[1,2] Treating these injuries can be a challenging task, ranging in some cases from conservative treatment to surgery, depending on the patient's symptoms and demands.[3] Traditional epidemiological studies have revealed that about 175,000 patients undergo ACL reconstruction in the United States every year. These numbers are essential to understand the socio-economic impact of these injuries and intensify the search for optimizing treatment strategies.[4]

When surgery is indicated, ACL reconstruction has been routinely performed to treat patients with ligament rupture. Although numerous studies have

reported that contemporary single-bundle ACL reconstruction effectively restores knee stability, many recent studies have reported radiographic osteoarthritis (OA) that are prevalent 10 years after ACL reconstruction.[2] Reports demonstrate that surgery failure rates are around 0.7–21%, which stimulates more scientific efforts to find solutions for better graft maturation, aiming to decrease the risk of failure and allow a faster recovery for patients.[5,6] Regarding returning to sports activities, out of the 82% that were reported, only 63% recovered to the pre-injury sport level. The rate is observed to be lower in elite athletes with less than 50% returning to sports at its highest level. In addition, postponing the return to sports practice to prevent failure is also common, although little has been done to reduce the healing time of the anterior cruciate ligament graft.[7–9]

Aiming to maximize the results of the treatment of ACL injuries, alternative therapeutic procedures are currently discussed parallel to surgery, especially in cases of partial ACL tears. Treating this type of injury besides the habitual reconstruction is still controversial but it may represent an attractive prospect when considering surgical morbidity and faster return to activities.[10]

Currently, there is a significant progress in the field of regenerative medicine, considering cellular therapies and ortho-biological agents, including platelet-rich plasma (PRP), growth factors, and stem cells, acting to positively stimulate tissue healing.[11] The aim of this work is to discuss the use of orthobiologics agents in ACL reconstruction to outline what is in the literature regarding these procedures, their risks, and their benefits in a narrative review.

PRP for ACL Augmentation

PRP is commonly defined as a blood sample with platelet concentrations above baseline values derived from an autologous centrifugation process.[12] This process, together with the centrifuge, contains a platelet concentrate mixed with growth factors and interleukins, and this composition might be responsible for improving the recovery of injured tissue.[13,14]

PRP for conservatively treated injuries is applied after identifying the area of injury based on physical examination and sometimes with ultrasound. During arthroscopic surgery, however, the site for application of platelet-rich therapy can be visualized more directly.[13,14] When undergoing surgery for ACL reconstruction with preservation of remnant and injection of PRP, there is a technique called biological augmentation (Figure 1).

The use of PRP has begun to be linked to orthopedic surgery, mainly in sports medicine. One of its main applications is knee surgery in the case of ligament injuries, especially ACL. There are current demonstrations of increased ACL density and neovascularization in *in vitro* animal studies, resulting in better organization of collagen fibers generating more excellent tissue resistance.[15]

In ACL reconstruction procedures, studies reviewed experimental clinical evidence showing that PRP injection increased the expression of procollagen gene and collagen protein, also contributing to the reduction of apoptosis and stimulating the metabolic activity of fibroblasts in the grafts.[16] In animal experiments, Riediger et al. demonstrated that PRP adoption increased in traction load and linear stiffness of the grafts and clinical data suggest favorable effects in its use. Biological evidence such as osseointegration of the graft-tunnel interface and intra-articular molecular bonding indicates beneficial effects when considering graft maturation.[17]

Ventura et al performed a systematic review of the current literature on the use of PRP in ACL

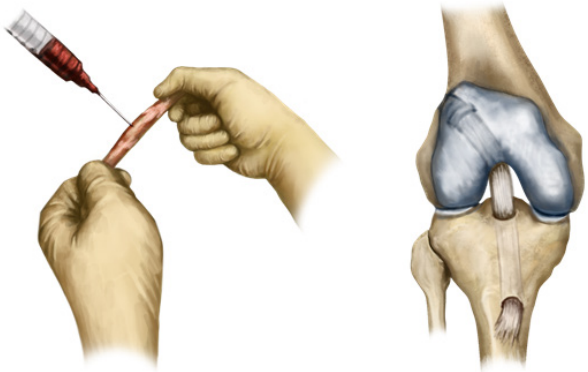

Figure 1. Technique of biologic augmentation injecting platelet-rich plasma at varying depths along the graft and the diagram of the anterior cruciate ligament reconstruction.

reconstruction and found 11 articles initially demonstrating an increase in intra-articular component remodeling of the graft during the first 10 years of follow-up.[18] Among these, one study showed an improvement in the integration.[19] However, the global assessment is poor when it comes to validating the mentioned studies and practical scores to measure the quality of the graft. This issue was due to the technical difference, volume, amount, and concentration of PRP used in each case and not bringing accurate evaluations of the knee after the reported study.

On the other hand, another recent review from Van Dyck et al. with 34 studies, concluded that graft maturity based on magnetic resonance imaging (MRI) could not predict the actual clinical conditions of patients, not being a reference for the evaluation and long-term follow-up. [20] In addition, some literature reviews support the idea that current levels of evidence also do not support the use of PRP for graft healing in humans.[21]

When evaluating the use of PRP with injections in the patellar and tibial bone joint spaces and also directly for filling the patellar tendon, it was noted that studies differ on postoperative pain improvement after 2 months of surgery and maintenance of stages in isokinetic and functional tests such as Lysholm, IKDC, and Tegner after 6 months of surgery.[22,23,24] De Almeida et al. found a significant positive difference in the evolution of MRI after 6 months of surgery, whereas another study by Walters showed that when PRP was mixed with autologous cancellous bone chips did not obtain the same result.[23, 25]

Although PRP in reconstruction of total ACL tear is still controversial, there is some evidence supporting the use of alternative treatments such as ortho-biological injections in augmentation repairs for partial ACL tears.[26] This alternative may preserve the native ligament insertion site, improving the result of proprioception and biomechanical function of the knee in repair.[26] Experimental studies performed on other animals for single partial ACL tears have not been concluded for restoring native knee stability, yet few studies show promising results. However, the study by Seijas et al., reported a high return to sport in 19 professional soccer players with partial ACL tears treated with arthroscopic intraligamentary PRP injections. In addition, radiological improvement was observed on MRI, complete ligamentization at 1-year follow-up, and no complications related to the procedure were observed.[27] However, this study in question lacks technical limitations, and there is no control group and evaluation of functional parameters.

Koch and colleagues performed a study using a technique called intraligament autologous plasma application and healing response in partial ACL ruptures that obtained a failure rate of 9.5%, showing effective results in clinical tests, including stable Lachman test, negative pivot, and a significant reduction in anterior-posterior laxity when considering the preoperative period. Moreover, reports show very similar functional tests compared with the healthy side with a 71% success rate in the group range of the patients returning to activities after an average of 5.8 months.[28]

A recent review by Kon et al. demonstrated favorable results in the analysis of graft maturation over time after ACL reconstruction using PRP.[29] The analysis in most studies was performed by imaging exams, demonstrating a better and faster appearance of the graft after the use of biologicals.[29] Sanchez et al performed a histological evaluation of the grafts with biopsies during arthroscopy 15 months after the primary reconstruction of the ACL.[30] The group using PRP showed significantly better involvement by mature connective tissue with elements of better graft maturation according to the temporal evolution of the surgery.[30]

As per the most recent clinical trials, results are still inadequate to recommend PRP in ACL reconstruction and ACL augmentation to improve graft production and operation results. In addition, although there is encouraging, but still controversial, evidence to support PRP in graft ligamentotaxis, there is no recommendation for its use aiming at faster and safer rehabilitations with lower failure rates.

Biological Augmentation with Growth Factors in the Treatment of Partial ACL Tears

In particular, growth factors are signaling molecules whose function is to regulate cellular activities.[31]

Several growth factors are directly involved in ligament healing, as demonstrated in *in vitro* studies by Deie et al. and Schmidt et al.[31–33] It is known that growth factors such as fibroblast growth factor 2 (FGF-2), platelet derived growth factor (PDGF), and epidermal growth factor (EGF) are present in high concentrations in healing tendons of canines.[34] A classic study by Murray et al. using human anterior cruciate ligament cells found that the transforming growth factor beta (TGF-beta), PDGF, and FGF increases cellularity proliferation while TGF and PDGF also enhance collagen synthesis.[35] In a study by obayashi`s et al. using animals in vivo, FGF has been shown to improve healing and neovascularization of a partially torn ACL.[36] Thus, the addition of these growth factors in the treatment of partial ACL tears has been studied as a strategy to stimulate healing and ligament recovery.[37]

Though the treatment options with growth factors are numerous, it remains to be seen which of the factors is functional in the attempt to treat ligament injuries. Murray et al. used collagen-glycosaminoglycan in the laboratory for cell migration but with slow results.[38] Kobayashi et al. used FGF-2 to treat central ligament defect and reported better orientation and organization as well as better blood supply to the lesion site.[36] Thus, when examining the wound healing process, it was concluded that it is a complex process initiated by platelet activation and from this a mixture of platelets and plasma proteins with a soluble collagen is used.[37] Murray's clinical trials proved that this mixture was fibro-inducing for cells present in ACL with cell proliferation and collagen production.[39]

More recent studies, such as the one by Uchida et al., have shown stimulating activity of extrinsic angiogenesis and the encouragement of cell proliferation by transforming growth factor beta-1 (TGF-β1), PDGF, FGF-2, hepatocyte growth factor (HGF) and vascular endothelium.[40] Platelet-rich plasma are substances with a high content of growth factors and thus have been a part of many current studies on the attempt to regenerate ligament tissues.

Cellular Therapies for Ligament Injuries

Mesenchymal stem cells consist of culture-expanded cells that exhibit plastic adherence and possess specific cell surface markers. These cells have the ability to differentiate into other cells such as adipocytes, chondrocytes, and osteoblasts, originating from the bone marrow but also from other types of tissue, including adipose, skin and synovial fluid, and synovial membrane.[41–44]

In orthopedics, the paracrine immunomodulatory effect of stem cells is used to limit the inflammatory microenvironment characteristic of acute ligament injury and promote regeneration.[45] The collection of this bone marrow is performed mainly in the iliac crest, distal metaphysis of the femur, proximal humerus, and tibia but it is very heterogeneous and with a small part of real stem or progenitor cells.[46] Therefore, it has been thought to expand studies to the culture of laboratories in more purified strains with a higher concentration of cells to promote a higher degree of activity.[47]

Cell therapy agents used in knee ligament injuries have yielded promising results in preclinical in vitro models, however, specific mechanisms of action understanding, quantity, and reproducible preparation of biological agents are currently lacking in the clinical studies and orthopedic literature. Studies by Mishra et al. provided evidence suggesting a relationship between stem cells and PRP, in which chondrogenic differentiation was potentiated with the mutual use of these two agents.[48] This also shows a future perspective of treatment in ligament injuries.

According to a recent review by Baird et al., four studies were found on cell therapies and their relationship with augmented ACL reconstruction, including two randomized clinical trials, a cohort study, and a case series.[49–53] Among the cell therapies, concentrated bone marrow aspirate, collagenase/centrifuge processed adipose, marrow stimulation combined with PRP, and cells cultured from allograft bone marrow aspirate were included.[49,51] Baird's study did not support the use of bone marrow aspirate and adipose tissue.[49] The marrow stimulation technique, combined with repair, has reported promising clinical results. On the other hand, the use of allograft-cultured cells had positive results as reported by the patient and also in the analysis of postoperative radiographic findings.[49]

Table 1. The use of biologic augmentation therapy and their updated evidence.

	Biological Characteristics	Outcomes
Platelet Rich Plasma	Increases the expression of pro-collagen gene and collagen protein. Reduces apoptosis and stimulates the metabolic activity of fibroblasts in the grafts.	Results are inadequate to recommend PRP in ACL reconstruction and ACL augmentation. Although some promising studies have been performed and among the topics studied, it is the one with the best perspective.
Growth Factors	Signaling molecules whose function is to regulate cellular activities, directly involved in ligament healing.	In addition to PRP, growth factors have been a part of many current studies in an attempt to regenerate ligament tissues.
Cellular Therapies	Better capacity to differentiate into ligament, fibrocartilage, and bone, promoting graft regeneration.	Still do not present clinical results, further investigations are required in this area.

Wang et al. concluded that many stem cell lines had good capacity to promote tendon-bone regeneration in animal models.[54] When considering the various types of stem cells, ligament-derived stem cells (LDSC) showed better potential for lineage-specific tendon/ligament differentiation.[55] Stem cells have a better capacity to differentiate into ligament, fibrocartilage, and bone, promoting graft regeneration, especially when they are used with differentiation inducers, such as growth factors, mechanical, and biomaterial stimuli.[56,57] Thus, even though cell therapies still do not present clinically with proven results, further investigations are needed in this area. Table 1 summarizes the biological characteristics and outcomes of each of the techniques described in the work for the use of biologics, which allows a more targeted view of individual factors.

CONCLUSION

There are few studies on the treatment of partial ACL injuries with biological agents. Given the current literature and publications on this issue, a limited number of high-level evidence in the investigation, alongside significant variability between authors and methods used, there is not a single conclusion on the topic.[59,60]

REFERENCES

1. Spindler KP, Wright RW. Clinical practice. Anterior cruciate ligament tear. N Engl J Med. 2008 Nov 13;359(20):2135–42. http://dx.doi.org/10.1056/NEJMcp1805931
2. Wang L, Lin L, Feng Y, Fernandes TL, Asnis P, Hosseini A, et al. Anterior cruciate ligament reconstruction and cartilage contact forces—A 3D computational simulation. Clin Biomech (Bristol, Avon). 2015 Dec;30(10):1175–80. http://dx.doi.org/1016/j.clinbiomech.2015.08.007
3. Sonnery-Cottet B, Colombet P. Partial tears of the anterior cruciate ligament. Orthop Traumatol Surg Res. 2016 Feb;102(1 Suppl):S59–67. http://dx.doi.org/10.1016/j.otsr.2015.06.032
4. Bollen S. Epidemiology of knee injuries: diagnosis and triage. Br J Sports Med 2000; 34: 227–228. http://dx.doi.org/10.1136/bjsm.34.3.227-a
5. Fernandes TL, Moreira HH, Andrade R, Sasaki SU, Bernardo WM, Pedrinelli A, Espregueira-Mendes J, Hernandez AJ. Clinical outcome evaluation of anatomic anterior cruciate ligament reconstruction with tunnel positioning using gold standard techniques: a systematic review and meta-analysis. Orthop J Sports Med. 2021 Jun 28;9(6):23259671211013327. http://dx.doi.org/10.1177/23259671211013327
6. Jaürvelaü T. Double-bundle versus single-bundle anterior cruciate ligament reconstruction: a prospective, randomized clinical study. Knee Surg Sports Traumatol Arthrosc 2007; 15: 500–507. http://dx.doi.org/10.1007/s00167-006-0254-z
7. Ardern CL, Webster KE, Taylor NF, Feller JA. Return to sport following anterior cruciate ligament reconstruction surgery: a systematic review and meta-analysis of the state of play. Br J Sports Med. 2011;45:596–606. http://dx.doi.org/10.1136/bjsm.2010.076364
8. Webster KE, Feller JA. Return to level I sports after anterior cruciate ligament reconstruction: evaluation of age, sex, and readiness to return criteria. Orthop J Sports Med. 2018;6:1–6. http://dx.doi.org/10.1177/2325967118788045

9. Chahla J, Cinque ME, Mandelbaum BR. Biologically augmented quadriceps tendon autograft with platelet-rich plasma for anterior cruciate ligament reconstruction. Arthrosc Tech. 2018 Oct 1;7(11): e1063–e1069. http://dx.doi.org/10.1016/j.eats.2018.06.011
10. Chahla J, Kennedy MI, Aman ZS, RF LP. Orthobiologics for ligament repair and reconstruction. Clin Sports Med. 2019:97–107. http://dx.doi.org/10.1016/j.csm.2018.08.003
11. Di Matteo B, Filardo G, Kon E, Marcacci M. Platelet-rich plasma: evidence for the treatment of patellar and Achilles tendinopathy—a systematic review. Musculoskelet Surg 2015; 99: 1–9. http://dx.doi.org/10.1055/s-0041-1735475
12. Hall MP, Band PA, Meislin RT, Jazrawi LM, Cardone DA. Platelet-rich plasma: current concepts and application in sports medicine. J Am Acad Orthop Surg. 2009;17:602–8. http://dx.doi.org/10.5435/00124635-200910000-00002
13. Moraes VY, Lenza M, Tamaoki MJ, Faloppa F, Belloti JC. Platelet-rich therapies for musculoskeletal soft tissue injuries. Cochrane Database Syst Rev. 2014. http://dx.doi.org/1002/14651858.CD010071.pub2
14. Zhang JY, Fabricant PD, Ishmael CR, Wang JC, Petrigliano FA, Jones KJ. Utilization of platelet-rich plasma for musculoskeletal injuries: an analysis of current treatment trends in the United States. Orthop J Sports Med. 2016;4:24–6. http://dx.doi.org/10.1177/2325967116676241
15. Ardern CL, Webster KE, Taylor NF, Feller JA. Return to sport following anterior cruciate ligament reconstruction surgery: a systematic review and meta-analysis of the state of play. Br J Sports Med. 2011;45:596–606. http://dx.doi.org/10.1136/bjsm.2010.076364
16. Andriolo L, Di Matteo B, Kon E, Filardo G, Venieri G, Marcacci M. PRP augmentation for ACL reconstruction. Biomed Res Int. 2015. http://dx.doi.org/10.1155/2015/371746
17. Riediger MD, Stride D, Coke SE, Kurz AZ, Duong A, Ayeni OR. ACL reconstruction with augmentation: a scoping review. Curr Rev Musculoskelet Med. 2019;12:166–72. http://dx.doi.org/10.1007/s12178-019-09548-4
18. Ventura A, Terzaghi C, Borgo E, Verdoia C, Gallazzi M, Failoni S. Use of growth factors in ACL surgery: preliminary study. J Orthop Traumatol. 2005;6: 76–9. http://dx.doi.org/10.1007/s10195-005-0085-6
19. Orrego M, Larrain C, Rosales J, Valenzuela L, Matas J, Durruty J, et al. Effects of platelet concentrate and a bone plug on the healing of hamstring tendons in a bone tunnel. Arthroscopy. 2008;24:1373–80 http://dx.doi.org/10.1016/j.arthro.2008.07.016
20. Van Dyck P, Zazulia K, Smekens C, Heusdens CHW, Janssens T, Sijbers J. Assessment of anterior cruciate ligament graft maturity with conventional magnetic resonance imaging: a systematic literature review. Orthop J Sports Med. 2019;7:1–9. http://dx.doi.org/10.1177/232596711984901
21. Davey MS, Hurley ET, Withers D, Moran R, Moran CJ. Anterior cruciate ligament reconstruction with platelet-rich plasma: a systematic review of randomized control trials. Art Ther. 2020:1–7. http://dx.doi.org/10.1016/j.arthro.2019.11.004
22. Seijas R, Cuscó X, Sallent A, Serra I, Ares O, Cugat R. Pain in donor site after BTB-ACL reconstruction with PRGF: a randomized trial. Arch Orthop Trauma Surg. 2016;136:829–35. http://dx.doi.org/10.1007/s00402-016-2458-0
23. De Almeida AM, Demange MK, Sobrado MF, Rodrigues MB, Pedrinelli A, Hernandez AJ. Patellar tendon healing with platelet-rich plasma: a prospective randomized controlled trial. Am J Sports Med. 2012;40:1282–8. http://dx.doi.org/10.1177/0363546512441344
24. Cervellin M, de Girolamo L, Bait C, Denti M, Volpi P. Autologous platelet-rich plasma gel to reduce donor-site morbidity after patellar tendon graft har-vesting for anterior cruciate ligament reconstruction: a randomized, controlled clinical study. Knee Surg Sport Traumatol Arthrosc. 2012;20:114–20. http://dx.doi.org/10.1007/s00167-011-1570-5
25. Walters BL, Porter DA, Hobart SJ, Bedford BB, Hogan DE, McHugh MM, et al. Effect of intraoperative platelet-rich plasma treatment on post-operative donor site knee pain in patellar tendon autograft anterior cruciate ligament reconstruction: a double-blind randomized controlled trial. Am J Sports Med. 2018;46:1827–35. http://dx.doi.org/10.1177/0363546518769295. Epub 2018 May 9
26. Dallo I, Chahla J, Mitchell JJ, Pascual-Garrido C, Feagin JA, LaPrade RF. Biologic approaches for the treatment of partial tears of the anterior cruciate ligament: a current concepts review. Orthop J Sports Med. 2017;5:1–9. http://dx.doi.org/10.1177/2325967116681724
27. Seijas R, Ares O, Cuscó X, Álvarez P, Steinbacher G, Cugat R. Partial anterior cruciate ligament tears treated with intraligamentary plasma rich in growth factors. World J Orthop. 2014;5:373–8. http://dx.doi.org/10.5312/wjo.v5.i3.373

28. Koch M, Mayr F, Achenbach L, Krutsch W, Lang S, Hilber F, Weber J, Pfeifer CG, Woehl R, Eichhorn J, Zellner J, Nerlich M, Angele P. Partial Anterior Cruciate Ligament Ruptures: Advantages by Intraligament Autologous Conditioned Plasma Injection and Healing Response Technique-Midterm Outcome Evaluation. Biomed Res Int. 2018 Jul 25;2018:3204869. http://dx.doi.org/10.1155/2018/3204869
29. Kon E, Di Matteo B, Altomare D, Iacono F, Kurpyakov A, Lychagin A, et al. Biologic agents to optimize outcomes following ACL repair and reconstruction: A systematic review of clinical evidence. J Orthop Res. 2022 Jan;40(1):10–28. http://dx.doi.org/10.1002/jor.25011
30. Sánchez M, Anitua E, Azofra J, Prado R, Muruzabal F, Andia I. Ligamentization of tendon grafts treated with an endogenous preparation rich in growth factors: gross morphology and histology. Arthroscopy. 2010 Apr;26(4):470–80. http://dx.doi.org/10.1016/j.arthro.2009.08.019
31. Deie M, Marui T, Allen CR, Hildebrand KA, Georgescu HI, Niyibizi C, et al. The effects of age on rabbit MCL fibroblast matrix synthesis in response to TGF-beta 1 or EGF. Mech Ageing Dev. 1997;97(2):121–30. http://dx.doi.org/10.1016/s0047-6374(97)00049-3
32. DesRosiers EA, Yahia L, Rivard CH. Proliferative and matrix synthesis response of canine anterior cruciate ligament fibroblasts submitted to combined growth factors. J Orthop Res. 1996;14(2):200–8. http://dx.doi.org/10.1002/jor.1100140206
33. Schmidt CC, Georgescu HI, Kwoh CK, Blomstrom GL, Engle CP, Larkin LA, et al. Effect of growth factors on the proliferation of fibroblasts from the medial collateral and anterior cruciate ligaments. J Orthop Res. 1995; 13(2):184–90. http://dx.doi.org/10.1002/jor.1100130206
34. Tsubone, T, Moran SL, Amadio, PC, Zhao C, An KN. Expression of growth factors in canine flexor tendon after laceration in vivo. Ann Plastic Surg. 2004;53(4):393–7. http://dx.doi.org/10.1097/01.sap.0000125501.72773.01
35. 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(17):2393–402. http://dx.doi.org/10.1016/s0142-9612(00)00426-9
36. 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;5(3):189–94. http://dx.doi.org/10.1007/s001670050049
37. Murray, M., Vavken, P. and Fleming, B., 2013. The ACL Handbook. New York, NY: Springer, pp. 203–224.
38. Murray MM, Martin SD, Spector M. Migration of cells from human anterior cruciate ligament explants into collagen-glycosaminoglycan scaffolds. J Orthop Res. 2000;18(4):557–64. http://dx.doi.org/10.1002/jor.1100180407
39. Murray MM, Forsythe B, Chen F, Lee SJ, Yoo JJ, Atala A, et al. The effect of thrombin on ACL fibroblast interactions with collagen hydrogels. J Orthop Res. 2006;24(3):508–15. http://dx.doi.org/10.1002/jor.20054
40. Uchida R, Jacob G, Shimomura K, Horibe S, Nakamura N. Biological Augmentation of ACL Repair and Reconstruction: Current Status and Future Perspective. Sports Med Arthrosc Rev. 2020 Jun;28(2):49–55. http://dx.doi.org/10.1097/JSA.0000000000000266
41. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8:315–7. http://dx.doi.org/10.1080/14653240600855905
42. Riekstina U, Muceniece R, Cakstina I, Muiznieks I, Ancans J. Characterization of human skin-derived mesenchymal stem cell proliferation rate in different growth conditions. Cytotechnology. 2008;58:153–62. http://dx.doi.org/10.1007/s10616-009-9183-2
43. Wagner W, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, et al. Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol. 2005;33:1402–16. http://dx.doi.org/10.1016/j.exphem.2005.07.003
44. Zhang X, Yang M, Lin L, Chen P, Ma KT, Zhou CY, et al. Runx2 overexpression enhances osteoblastic differentiation and mineralization in adipose - Derived stem cells in vitro and in vivo. Calcif Tissue Int. 2006;79:169–78. http://dx.doi.org/10.1007/s00223-006-0083-6
45. Saether EE, Chamberlain CS, Aktas E, Leiferman EM, Brickson SL, Vanderby R. Primed mesenchymal stem cells alter and improve rat medial collateral ligament healing. Stem Cell Rev Reports. 2016;12:42–53. http://dx.doi.org/10.1007/s12015-015-9633-5
46. Narbona-Carceles J, Vaquero J, Su.rez-Sancho SBS, Forriol F, Fern.ndez-Santos ME. Bone marrow

mesenchymal stem cell aspirates from alternative sources: is the knee as good as the iliac crest? Injury. 2014;45:S42–7. http://dx.doi.org/10.1016/S0020-1383(14)70009-9
47. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther. 2019;10:68. http://dx.doi.org/10.1186/s13287-019-1165-5
48. Mishra A, Tummala P, King A, Lee B, Kraus M, Tse V, et al. Buffered platelet-rich plasma enhances mesenchymal stem cell proliferation and chondrogenic differentiation. Tissue Eng Part C Methods. 2009;15(3):431–5. http://dx.doi.org/10.1089/ten.tec.2008.0534
49. Baird JPE, Anz A, Andrews J, Plummer HA, McGowan B, Gonzalez M, et al. Cellular Augmentation of Anterior Cruciate Ligament Surgery Is Not Currently Evidence Based: A Systematic Review of Clinical Studies. Arthroscopy. 2021 Dec 15:S0749-8063(21)01102-6. http://dx.doi.org/10.1016/j.arthro.2021.11.056
50. Alentorn-Geli E, Seijas R, Martínez-De la Torre A, Cuscó X, Steinbacher G, Álvarez-Díaz P, et al. Effects of autologous adipose-derived regenerative stem cells administered at the time of anterior cruciate ligament reconstruction on knee function and graft healing. J Orthop Surg (Hong Kong). 2019 Sep-Dec;27(3):2309499019867580. http://dx.doi.org/10.1177/2309499019867580
51. Gobbi A, Karnatzikos G, Sankineani SR, Petrera M. Biological augmentation of ACL refixation in partial lesions in a group of athletes: results at the 5-year follow-up. Tech Orthop. 2013;28:180–184. http://dx.doi.org/10.1097/BTO.0b013e318294ce44
52. Silva A, Sampaio R, Fernandes R, Pinto E. Is there a role for adult non-cultivated bone marrow stem cells in ACL reconstruction? Knee Surg Sports Traumatol Arthrosc. 2014 Jan;22(1):66–71. http://dx.doi.org/10.1007/s00167-012-2279-9
53. Wang Y, Shimmin A, Ghosh P, Marks P, Linklater J, Connell D, et al. Safety, tolerability, clinical, and joint structural outcomes of a single intra-articular injection of allogeneic mesenchymal precursor cells in patients following anterior cruciate ligament reconstruction: a controlled double-blind randomized trial. Arthritis Res Ther. 2017 Aug 2;19(1):180. http://dx.doi.org/10.1186/s13075-017-1391-0
54. Wang C, Hu Y, Zhang S, Ruan D, Huang Z, He P, Cai H, Heng BC, Chen X, Shen W. Application of Stem Cell Therapy for ACL Graft Regeneration. Stem Cells Int. 2021 Aug 2;2021:6641818. http://dx.doi.org/10.1155/2021/6641818
55. Bi Y, Ehirchiou D, Kilts TM, Inkson CA, Embree MC, Sonoyama W, et al. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nature Medicine. 2007;13(10):1219–1227. http://dx.doi.org/10.1038/nm1630
56. Shen H., Yoneda S., Abu-Amer Y., Guilak F., Gelberman R. H. Stem cell-derived extracellular vesicles attenuate the early inflammatory response after tendon injury and repair. Journal of Orthopedic Res. 2019;38:117–127. http://dx.doi.org/10.1002/jor.24406
57. Wang YJ, He G, Guo Y, Tang H, Shi Y, Bian X, et al. Exosomes from tendon stem cells promote injury tendon healing through balancing synthesis and degradation of the tendon extracellular matrix. J Cell Mol Med. 2019;23(8):5475–5485. http://dx.doi.org/10.1111/jcmm.14430
58. Chamberlain CS, Clements AEB, Kink JA, Choi U, Baer GS, Halanski MA, et al. Extracellular vesicle-educated macrophages promote early Achilles tendon healing. Stem Cells. 2019;37(5):652–662. http://dx.doi.org/10.1002/stem.2988
59. Centeno C, Lucas M, Stemper I, Dodson E. Image Guided Injection of Anterior Cruciate Ligament Tears with Autologous Bone Marrow Concentrate and Platelets: Midterm Analysis from A Randomized Controlled Trial. Bio Orthop J [Internet]. 2022 Jan. 2 [cited 2023 Jan. 28];3(SP2):e7–e20. Available from: https://www.biologicortho.com/index.php/BiologicOrtho/article/view/24
60. Murrell WD, Anz AW, Badsha H, Bennett WF, Boykin RE, Caplan AI. Regenerative treatments to enhance orthopedic surgical outcome. PM R. 2015 Apr;7(4 Suppl):S41–S52. http://dx.doi.org/10.1016/j.pmrj.2015.01.015