Regeneration of the anterior cruciate ligament: Current strategies in tissue engineering

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Abstract
Recent advancements in the field of musculoskeletal tissue engineering have raised an increasing interest in the regeneration of the anterior cruciate ligament (ACL). It is the aim of this article to review the current research efforts and highlight promising tissue engineering strategies. The four main components of tissue engineering also apply in several ACL regeneration research efforts. Scaffolds from biological materials, biodegradable polymers and composite materials are used. The main cell sources are mesenchymal stem cells and ACL fibroblasts. In addition, growth factors and mechanical stimuli are applied. So far, the regenerated ACL constructs have been tested in a few animal studies and the results are encouraging. The different strategies, from in vitro ACL regeneration in bioreactor systems to bio-enhanced repair and regeneration, are under constant development. We expect considerable progress in the near future that will result in a realistic option for ACL surgery soon.

Key words: Anterior cruciate ligament; Tissue engineering; Orthopedic; Ligament regeneration; Stem cell

INTRODUCTION
Knee injuries frequently result in ruptured ligaments, typically through high-pivoting sporting activities such as skiing, football and basketball. In 2005, around 400000 physician office visits in the United States were related...
to knee injuries\(^{[1]}\). The worldwide estimation of young sports players that require surgery following a knee injury lies between 17%-61\(^{[2]}\). The anterior cruciate ligament (ACL), a main stabilizing structure of the knee, is one of the most commonly injured ligaments. In the United States alone, around 350,000 reconstructive surgeries of the ACL are performed annually. According to the National Center for Health Statistics, the annual costs for the acute care of these injuries are around $6 billion\(^{[3]}\).

Historically, the treatment of ACL injuries involved different strategies, from non-operative care to several surgical procedures\(^{[4,5]}\). Simple primary suturing in the 1970s was abandoned due to bad clinical results. Augmented ACL repair using natural as well as synthetic grafts leads to somewhat improved results. Synthetic grafts were popular in the 1980s but resulted in serious complications and bad clinical results. From the early 1990s onwards, ACL reconstruction with autograft or allograft material has become the method of choice for most surgeons (Figure 1). Despite the ongoing success of autografts, problems mostly associated with donor site morbidity remain, such as anterior knee pain, infrapatellar contracture, tendinitis, patellar fracture, muscle weakness and limited graft availability\(^{[6]}\). In terms of allograft material, the risk for transmissions of blood-borne diseases and the delayed biological incorporation were mentioned as the main disadvantages\(^{[7]}\). In addition, relatively high failure rates of ACL reconstruction, especially in young and active patients, have been reported for allografts\(^{[8]}\). An incidence of osteoarthritis as high as 50% within 7-14 years after injury and reconstruction of the ACL is still the main drawback of this surgical strategy. The development of osteoarthritis following ACL injury is not fully understood and may be caused not only by the limitation of the current grafts, but also by the initial joint trauma and the trauma caused by the surgeon. However, this has resulted in enormous ongoing research interest in that topic\(^{[9]}\).

The regeneration of musculoskeletal tissues has become increasingly popular in the field of orthopedic research. Typically, structures that are injured or lost due to trauma and disease are the ideal candidates to be engineered. Tissue engineering as a multidisciplinary field includes strategies of engineering, material science and biology, with the aim of regenerating tissues that not only recreate the morphology, but also restore the normal function. In the late 1980s, Langer and Vacanti\(^{[10]}\) first described the classic four basic components that are needed in tissue engineering: a structural scaffold, a cell source, biological modulators and mechanical modulators\(^{[11]}\).

The ACL, with its limited healing capacity and the consequent need for reconstructive surgery, certainly is an appealing but also challenging structure for tissue engineering. In contrast to extra-articular ligaments, such as the medial collateral ligament, the intra-articular location of the ACL apparently prevents its primary healing. The disruption of the synovial sheath does not allow local hematoma formation crucial for the onset of the inflammatory response that would stimulate primary healing\(^{[12]}\). In addition, the complex three-dimensional structure of the ACL, with different tensioning patterns throughout the knee path of motion, contributes to the difficulty of regenerating this ligament in terms of form and function.

It is the purpose of this article to review the current approaches in tissue-engineering of the ACL, to provide an overview of the current problems and limitations, and to present future directions of this evolving research technology.

### SCAFFOLDS FOR ACL REGENERATION

Many different biomaterials have been introduced as a potential scaffold for ACL tissue engineering. Ideally, the scaffold has to be biocompatible and its mechanical properties should mimic the natural ACL as closely as possible. It also needs to be biodegradable to enable tissue ingrowth, which is crucial for the new ligament to form. Biological materials, biodegradable polymers and composite materials have all been or still are under evaluation for ligament regeneration\(^{[13]}\).

Dunn et al\(^{[14]}\) and Bellincampi et al\(^{[15]}\) developed scaffolds made of collagen fibrils. They showed that ACL fibroblasts adhered to these scaffolds and remained viable, in vitro as well as in vivo. Unfortunately, after 6 wk the constructs were completely resorbed. Goulet et al\(^{[16]}\) reported on the decreasing mechanical strength of collagen scaffolds seeded with ACL fibroblasts. Murray et al\(^{[17]}\) demonstrated that a collagen-glycosaminoglycan composite scaffold supported cell growth and the expression of fibroblast markers. Several techniques have been explored to improve the mechanical properties of collagen-based scaffolds, including cross linking the collagen or a special braid-twist design\(^{[18]}\). However, despite considerable improvements of the mechanical properties, collagen-based scaffolds thus far have not been able to mimic the strength of the natural ACL.
Similar challenges regarding the mechanical strength have also been reported for other biological materials, such as alginate, chitosan and hyaluronic acid. Many different composites of these materials have been explored and it has been shown that some of them may be an interesting option in terms of cell attachment and cell proliferation. However, the mechanical insufficiency of these biological materials remains a considerable problem for their routine practical use in ligament regeneration. To overcome the mechanical weakness, Panas-Perez et al. developed a collagen-silk composite and concluded that a scaffold with > 25% silk provides sufficient mechanical support very close to the properties of the native ACL.

The use of silk in ligament scaffolds is not restricted to combinations with other biomaterials. In various studies, its functionality in diverse tissue engineering approaches, especially in the musculoskeletal field, has been proven. The properties that make silk an attractive candidate as biomaterial are its remarkable strength and toughness compared to other natural as well as synthetic biomaterials. The majority of studies dealing with silk as raw material for scaffold production use fibers from cocoons of the mulberry silkworm Bombyx mori. Due to biocompatibility issues, silkworm silk requires removal of the surface protein layer sericin, which can elicit adverse immune responses. Once sericin is removed, the remaining silk fibroin fibers are non-immunogenic, biocompatible and capable of promoting cell adhesion, growth and, in the case of progenitor cells such as mesenchymal stromal cells (MSCs), differentiation. The classical way to remove this protein layer is to boil raw silk fibers in alkaline solutions such as sodium carbonate. Recently, Teuschl et al. successfully removed sericin from a compact and highly-ordered raw Bombyx mori silk fiber scaffold using borate buffer based solutions. The possibility of removing sericin after the textile engineering process eases the production of complex 3D structures in TE applications because the gliding properties of the silk fiber due to the gum-like sericin assist during textile engineering steps (e.g., braiding and weaving). The pioneers in using silk fibers as raw material for ACL scaffolds are Altman and Kaplan, who demonstrated that the mechanical properties of their twisted fiber scaffolds match that of the native human ACL. Moreover, Horan et al. demonstrated the processability of silk fibers with a huge number of different textile engineering techniques, enabling the generation of complex hierarchical structures with defined properties. Another characteristic that makes silk an attractive candidate for ACL tissue engineering is its slow rate of biodegradation (proteolytic degradation). Thus, ACL scaffolds made out of silk fibroin can provide the primary stability over an extended period of time, allowing ingrowing cells to rebuild neoligamentous tissue without exposing the knee joint to periods of instability. Moreover, the gradual transfer of stabilizing properties from the silk scaffold to the new forming tissue should allow a neotissue formation similar to the initial native tissue regarding collagen alignment, vascularization, etc.

In the literature, silk-based ligament grafts have been tested in animal models in only a few studies. Historically, former ACL studies with synthetic materials have shown that the extrapolation of findings from animal data to humans needs large animal studies, like goat, sheep or pig models. To the best of our knowledge, only two studies have already tested silk-based ACL grafts in large animal studies with encouraging results. In a pig model, Fan et al. demonstrated that their woven silk ligament scaffold in conjunction with seeded MSCs supported ligament regeneration after the 24 wk post implantation period. In conclusion, these very promising in vivo studies suggest that ACL scaffolds fabricated from silk fibroin have great potential for the translation into clinical applications. Moreover, clinical trials of silk-based ACL grafts proving functionality and safety in human knees have already been documented.

Apart from biological materials, synthetic biodegradable polymers have been introduced in ligament tissue engineering. Petriglano et al. mentioned the advantages of synthetic polymers as proper selection and different manufacturing techniques allow for exact adaptation of the mechanical properties, cellular response and degradation rate. Lin et al. used a scaffold composed of polyglycolic acid coated with polycaprolactone. Buma et al. worked with a braided polydioxanone scaffold in an in vivo animal study but reported an early loss of mechanical properties. Lu et al. compared different synthetic braided materials and concluded that poly L-lactic acid (PLLA) scaffolds had the best results in terms of mechanical properties as well as fibroblast proliferation. Laurencin et al. also developed a PLLA scaffold in a 3D dimensional braided fashion with distinct regions for the bony portions and the intra-articular portion of the construct. The same group consequently compared a different PLLA scaffold with different manufacturing techniques and demonstrated that a braid-twist scaffold had the most favorable viscoelastic properties. In another study, a polyethylene glycol hydrogel was added to the PLLA scaffold which resulted in even better viscoelastic performance of the construct, but on the other hand, this also led to a decreased pore size of the scaffold which may negatively influence cell proliferation.

More recently, electrospinning has been used for the development of scaffolds for ligament tissue engineering. This technique can be used to produce very thin fibers in the nanometer to micron range. This allows for a more exact adaptation of the mechanical properties. Some of the studies using this technique reported better cell proliferation and extracellular matrix production. However, these techniques are under constant investigation and while early in vitro studies show interesting results, the overall biological and mechanical performance still has to be examined further to draw any conclusions for a later clinical use of these materials.
capacity is too restricted to be capable of healing ruptured ligaments. As ACL tissue can only be harvested reasonably in diagnostic arthroscopic procedures after ACL rupture, other ligament fibroblast sources have been discussed, such as the medial collateral ligament [65]. Nevertheless, the majority of studies involving cell therapy approaches in ACL tissue engineering uses mesenchymal stromal cells as a cell source since they can be obtained much more easily in higher numbers and, moreover, MSCs show higher proliferation and collagen productions rates compared to ligament fibroblasts [66, 67].

From a cellular view, the knee joint comprises different sources of cells [68] (ligament tissue, synovium, etc.) that have been shown to participate during the ligament regeneration process, such as the above described ACL fibroblasts. Nevertheless, due to their origin, ACL fibroblasts would be the accurate cell type to build up neoligamentous tissue. ASCs: Adipose derived stem cells.

**CELL SOURCES FOR ACL REGENERATION**

Two different types of cells are mainly regarded as the primary choice for ACL regeneration: mesenchymal stem cells (MSC) and ACL fibroblasts [55]. MSCs are present in almost all tissue types of the body [56, 57]. However, for cell therapeutic purposes, bone marrow and adipose tissue are regarded as the main feasible sources to isolate MSCs [58, 59]. The potential of MSCs to differentiate into various mesenchymal lineages, including fibroblastic, osteogenic, chondrogenic and myogenic, was proven in numerous studies. Furthermore, MSCs have already been effectively applied to enhance repair in different musculoskeletal tissues, in particular in bone and ligaments (Figure 2) [49, 50, 62].

The use of ACL fibroblasts involves the risk of local infection in the knee during biopsy harvesting. From the view that the seeded cells should rebuild the ligament tissue by deposition of extracellular matrix, the appropriate cell type would be ACL fibroblasts since they are the native cell type in intact ligament tissue. Therefore, they are used as control cells for cell behavior such as protein expression, especially in in vitro studies. Interestingly, different studies have demonstrated that the ACL tissue contains populations of cells sharing MSC characteristics, such as clusters of differentiation markers or multipotency [63, 64]. Although stem cells are present in the ACL tissue, their regenerative capacity is too restricted to be capable of healing ruptured ligaments. As ACL tissue can only be harvested reasonably in diagnostic arthroscopic procedures after ACL rupture, other ligament fibroblast sources have been discussed, such as the medial collateral ligament [65]. Nevertheless, the majority of studies involving cell therapy approaches in ACL tissue engineering uses mesenchymal stromal cells as a cell source since they can be obtained much more easily in higher numbers and, moreover, MSCs show higher proliferation and collagen productions rates compared to ligament fibroblasts [66, 67].

From a cellular view, the knee joint comprises different sources of cells [66] (ligament tissue, synovium, etc.) that have been shown to participate during the ligament regeneration process, such as the above described ACL fibroblasts or MSCs that are natively recruited after ligament ruptures or tears. The activation and recruitment of regenerating cells can be augmented mechanically, for instance by the surgical procedure (e.g., drilling of bone holes for the graft which gives access to the vasculature of bone tissue) or biochemically, by the use of growth factors or gene-based therapeutic approaches.

**GENE-BASED THERAPEUTIC APPROACHES AND GROWTH FACTORS**

Growth factors can either be directly applied via inserted cells (producing these biochemical signal molecules in
MECHANICAL STIMULATION IN ACL REGENERATION

Mechanical stimuli and dynamic loading are necessary for ligaments to maintain their strength. In a number of studies, Woo et al. demonstrated that immobilization leads to weakened mechanical properties of ligaments. From a mechanistic point of view, it is known that cells react to mechanical stimuli via integrin-mediated focal adhesions and cytoskeleton deformation. Altman et al. demonstrated that mechanical stimuli are able to influence stem cell differentiation as well as the production of extracellular matrix (ECM). Mechanical strain resulted in the differentiation of MSCs into fibroblast-like cells, as seen by the upregulation of ligament markers tenasin-C, collagen types I and III, and the formation of collagen fibers. Petrigliano et al. showed that uniaxial cyclic strain of a three-dimensional polymer scaffold seeded with MSCs resulted in upregulated tenasin-C, collagen type I and III. Berry et al. reported on the proliferative effect of uniaxial strain on young fibroblasts. Park et al. demonstrated that 8% cyclical strain in ligament fibroblasts leads to higher cell proliferation and collagen production compared to a 4% strain and unloaded controls. In their review, Leong et al. mentioned that despite the known fact that mechanical stimuli play an important role in ligament tissue engineering, the timing, direction and magnitude of the stimuli as well as the cell type can all be of significant influence on the cellular response. As an example, they discussed a study by Moreau et al. in which MSCs were stimulated immediately after seeding and showed an inhibited expression of collagen I and II. In contrast, the opposite effect was observed when the mechanical loading was applied at the peak of MSC proliferation. Leong et al. mentioned that in case of ACL tissue engineering, additional investigation is required to elucidate the mechanotransduction pathways that are necessary for tissue formation and maintenance. They also stated that, to date, it is not known if any mechanical stimulation is required prior to implantation of tissue engineered ACL constructs.

FUTURE DIRECTIONS IN ACL REGENERATION

In a recent questionnaire study by Rathbone et al., 300 orthopedic surgeons were asked if they would consider a tissue engineered ACL if it were an available option.
Eight-six percent answered positively if the construct demonstrates biological and mechanical success. For 63%, improved patient satisfaction was important and 76% of the participants mentioned that a tissue engineered ACL would be superior to any of the currently used autograft materials. It was also clearly stated that a fully load-bearing construct for implantation is needed and that several ACL tissue engineering strategies should address this need for mechanical integrity. This seems to be of crucial importance as the presently used ACL reconstruction techniques with autograft or allograft material provide an immediate load bearing environment. It seems obvious that, until the results of any regenerated ACL can compare with the current relatively successful autograft methods, patients are likely to prefer the autograft. As most surgeons do not require immobilization after reconstructive surgery, immobilization is likely to be unacceptable.

Another important aspect that will need consideration is the timing of the tissue engineering process and consequent implantation. In recent studies, our group focused on the mechanical stimulation of silk grafts with a custom-made bioreactor system in order to increase the maturity of cell-loaded grafts prior to implantation (Figure 3). In accordance with a study by Altman et al., we triggered MSCs to produce layers of ECM on silk-based grafts. Our hypothesis is that the applied mechanical stimulation triggers the MSCs into ligamentous cells which in conjunction with the cells’ own secreted ECM leads to more functionality of the cell/scaffold construct and therefore will superiorly fulfill its tasks once it is implanted.

Future studies using a combination of in vitro bioreactor engineering with consequent in vivo implantation are certainly needed to get a clearer picture of this complex topic. On the other hand, engineering mechanically appropriate scaffolds that are implantable at any time also seems to be a good option. Future research efforts may also demonstrate which cell type seeded on these scaffolds is the ideal candidate for direct in vivo implantation in this case. Furthermore, there is also some interest in exploring the regenerative potential of solely implanted scaffolds that would recruit in vivo cells, provided there is the appropriate mechanical and physiological environment. Just recently, Murray et al. proposed the strategy of repair and regeneration. Here, tissue engineering efforts are undertaken to overcome the obstacles to native ACL healing. This group proposed a bio-enhanced ACL repair technique that uses a collagen scaffold saturated with platelet-rich plasma. In a number of animal studies, they demonstrated improved mechanical and biological healing of the ACL [84,105-107]. In a recent randomized large animal trial, bio-enhanced ACL repair had equal results compared with ACL reconstruction. It was also shown that the knees treated with enhanced ACL repair had a lower rate of osteoarthritis in contrast to those treated with ACL reconstruction which developed osteoarthritis in 80% after one year [108]. Despite these interesting findings, it may be problematic to draw direct conclusions as osteoarthritis is not common a year after ACL injury in humans.

CONCLUSION

There is a growing research interest in the tissue engineering of the ACL and the clinical need seems obvious. Different strategies from in vitro engineering of ACL grafts to bio-enhanced repair and regeneration are followed. For the
surgical community, any type of engineered ACL may be a future option provided that it is easy to implant, does allow for at least the same aggressive rehabilitation protocol as currently used and will lead to better patient satisfaction and outcome.

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REFERENCES

1. Goodwin E. The American Orthopaedic Society for Sports Medicine Conference on Allografts in Orthopaedic, Sports Medicine. Keystone, CO, July 14-17; 2005
2. Louw QA, Maniail J, Gummer KA. Epidemiology of knee injuries among adolescents: a systematic review. Br J Sports Med 2008; 42: 2-10 [PMID: 17550921 DOI: 10.1136/ bmj.2007.053560]
3. Hing E, Cherry DK, Woodwell DA. National Ambulatory Medical Care Survey: 2004 summary. Adv Data 2006; (374): 1-33 [PMID: 1684616]

4. Seitz H, Pichl W, Matzi V, Nau T. Biomechanical evaluation of augmented and nonaugmented primary repair of the anterior cruciate ligament: an in vivo animal study. Int Orthop 2013; 37: 2305-2311 [PMID: 24045909 DOI: 10.1007/s00264-012-1908-8]
5. Ma J, Smietana MJ, Kostrominova TY, Wojtys EM, Larkin LM, Arruda EM. Three-dimensional engineered bone-ligament-bone constructs for anterior cruciate ligament replacement. Tissue Eng Part A 2012; 18: 103-116 [PMID: 21902608 DOI: 10.1089/ten.TEA.2011.0251]

6. Jackson DW, Grood ES, Goldstein JD, Rosen MA, Kurzweil H, Borchard G. In vitro evaluation of an RGD-functionalized chitosan derivative for enhanced cell adhesion. Carbohydr Polym 2012; 90: 1494-1500 [PMID: 22944607 DOI: 10.1016/j.carbpol.2012.07.020]

7. Kaeding CC, Aros B, Pedraza A, Pifel E, Amendola A, Andrich JT, Dunn WR, Marx RG, McCarty EC, Parker RD, Wright RW, Spindler KP. Allograft Versus Autograft Anterior Cruciate Ligament Reconstruction: Predictors of Failure From a MONO Prospective Longitudinal Cohort. Sports Health 2011; 3: 73-81 [PMID: 23015994 DOI: 10.1177/1941738110386185]

8. Roos EM. Joint injury causes knee osteoarthrosis in young adults. Curr Opin Rheumatol 2005; 17: 195-200 [PMID: 15711235 DOI: 10.1097/01.rhe.0000151406.64393.00]

9. Lohmander LS, Ostenberg A, Englund M, Roos H. High prevalence of knee osteoarthrosis, pain, and functional limitations in female soccer players twelve years after anterior cruciate ligament injury. Arthritis Rheum 2004; 50: 3145-3152 [PMID: 15476248 DOI: 10.1002/art.20589]

10. Langer R. Vacanti JP. Tissue engineering. Science 1993; 260: 920-926 [PMID: 8493529 DOI: 10.1126/science.8493529]

11. Leong NL, Petriglano FA, McAllister DR. Current tissue engineering strategies in anterior cruciate ligament reconstruction. J Biomed Mater Res A 2014; 102: 1614-1624 [PMID: 25737190 DOI: 10.1002/jbm.a.34821]

12. Dunn MG, Liesch JB, Tik T, Rauschning W, Zawadsky J. Development of fibroblast-seeded ligament analogs for ACL reconstruction. J Biomed Mater Res A 1995; 29: 1363-1371 [PMID: 8582904 DOI: 10.1002/jbm.a.820921107]

13. Bellincampi LD, Clostre D, Germain L, Poole AR, Auger FA. Tendons and ligaments. In: Lanza R, Langer R, Vacanti J. Principles of Tissue Engineering. London: Elsevier Academic Press, 2011; 911-914

15. Murray MM, Specter M. The migration of cells from the ruptured human anterior cruciate ligament into collagen-glycosaminoglycan regeneration templates in vitro. Biomaterials 2001; 22: 2393-2402 [PMID: 11511036 DOI: 10.1016/ S0142-9612(00)00426-9]

16. Koob TJ, Willis TA, Qiu YS, Hernandez DJ. Biocompatibility of NDGA-polymerized collagen fibers. II. Attachment, proliferation, and migration of tendon fibroblasts in vitro. J Biomed Mater Res 2001; 56: 40-48 [PMID: 11309789 DOI: 10.1002/ jbm.10017056]

17. Caruso AB, Dunn MG. Changes in mechanical properties and cellularity during long-term culture of collagen fiber ACL reconstruction scaffolds. J Biomed Mater Res A 2005; 73: 388-397 [PMID: 15880693 DOI: 10.1002/jbm.a.30233]

18. Walters VL, Kwansa AL, Freeman JW. Design and analysis of braided-tissue collagen scaffolds. Connect Tissue Res 2012; 53: 255-266 [PMID: 22149930 DOI: 10.1080/10709663.2011.634552]

19. Cristino S, Grassi F, Toneruzzo S, Piacentini A, Grigolo B, Santi S, Riccio M, Tognana E, Facchini A, Lisiogoli G. Analysis of mesenchymal stem cells grown on a three-dimensional HYAFF 11-based prototype ligament scaffold. J Biomed Mater Res A 2005; 73: 275-283 [PMID: 15789422 DOI: 10.1002/jbm.a.30261]

20. Hasson A, Hashom N, Felson F, Rousselle P, Jordan O, Borchard G. In vitro evaluation of an RGD-functionalized chitosan derivative for enhanced cell adhesion. J Biomed Mater Res A 2012; 90: 1494-1500 [PMID: 22944607 DOI: 10.1016/j.carbpol.2012.07.020]

21. Shao HJ, Lee YT, Chen CS, Wang YH, Young TH. Modulation of gene expression and collagen production of anterior cruciate ligament cells through cell shape changes on polycaprolactone/chitosan blends. Biomaterials 2010; 31: 4695-4705 [PMID: 2004482 DOI: 10.1016/j.biomaterials.2010.02.037]

22. Shao HJ, Chen CS, Lee YT, Wang YH, Young TH. The phenotypic responses of human anterior cruciate ligament cells cultured on poly(epsilon-caprolactone) and chitosan. J Biomed Mater Res A 2010; 93: 1297-1305 [PMID: 19827133 DOI: 10.1002/jbm.a.32629]

23. Masuko T, Iwasaki N, Yamane S, Funakoshi T, Majima T, Minami A, Ohsga N, Ohta T, Nishimura S. Chitosan-RGDS/GCC conjugate as a scaffold material for musculoskeletal tissue engineering. Biomaterials 2005; 26: 5339-5347 [PMID: 15814132 DOI: 10.1016/j.biomaterials.2005.01.062]

24. Yamanaka S, Iwasaki N, Majima T, Funakoshi T, Masuko T, Harada K, Minami A, Monde K, Nishimura S. Feasibility of chitosan-based hyaluronic acid hybrid biomaterial for a novel scaffold in cartilage tissue engineering. Biomaterials 2005; 26: 611-619 [PMID: 15282139 DOI: 10.1016/j.biomaterials.2004.03.013]

25. Majima T, Funakoshi T, Iwasaki N, Yamane ST, Harada K, Nonaka S, Minami A, Nishimura S. Alginante and chitosan polymer complex hybrid fibers for scaffolds in ligament and tendon tissue engineering. J Orthop Sci 2010; 15: 302-307 [PMID: 19599749 DOI: 10.1016/j.jos.2010.02.002]

26. Panes-Perez E, Gatt CJ, Dunn MG. Development of a silk and collagen fiber scaffold for anterior cruciate ligament reconstruction. J Mater Sci Mater Med 2013; 24: 257-265 [PMID: 23053810 DOI: 10.1007/s10856-012-4781-5]

27. Wang Y, Blasioli DJ, Kim HJ, Kim HS, Kaplan DL. Cartilage tissue engineering with silk scaffolds and human articular chondrocytes. Biomaterials 2006; 27: 4434-4442 [PMID: 16767707 DOI: 10.1016/j.biomaterials.2006.03.050]

28. Hofmann S, Knecht S, Langer R, Kaplan DL, Vunjak-Novakovic G, Merkle HP, Meinel L. Cartilage-like tissue engineering using silk scaffolds and mesenchymal stem cells. Tissue Eng 2006; 12: 2729-2738 [PMID: 17588642 DOI: 10.1089/ten.2006.12.2729]

29. Meinel L, Betz O, Fajardo R, Hofmann S, Nazarian A, Cory E, Hilbe M, McCool J, Langer R, Vunjak-Novakovic G, Merkle
Nau T et al. Regeneration of the anterior cruciate ligament

HP, Rechenberg B, Kaplan DL, Kirker-Head C. Silk based biomaterials to heal critical sized femur defects. Bone 2006; 39: 922-931 [PMID: 16757219 DOI: 10.1016/j.bone.2006.04.019]

Park SY, Ki CS, Park VH, Jung HM, Woo KM, Kim HJ. Electrospun silk fibroin scaffolds with macropores for bone regeneration: an in vitro and in vivo study. Tissue Eng Part A 2010; 16: 1271-1279 [PMID: 19905876 DOI: 10.1089/ten.TEA.2009.0328]

MacIntosh AC, Kearns VR, Crawford A, Hatton PV. Skeletal tissue engineering using silk biomaterials. J Tissue Eng Regen Med 2008; 2: 71-80 [PMID: 18385453 DOI: 10.1002/tcr.2008.03.008]

Wang Y, Kim HJ, Vujak-Novakovic G, Kaplan DL. Stem cell-based tissue engineering with silk biomaterials. Biomaterials 2006; 27: 6064-6082 [PMID: 16899088]

Jin HJ. Kaplan DL. Mechanism of silk processing in insects and spiders. Nature 2003; 424: 1057-1061 [PMID: 12944986 DOI: 10.1038/nature01809]

Rockwood DN, Prada RC, Yiel Y, Wang X, Lovett ML, Kaplan DL. Materials fabrication from Bombyx mori silk fibroin. Nat Protoc 2011; 6: 1612-1631 [PMID: 21959241 DOI: 10.1038/nprot.2011.379]

Vepari C, Kaplan DL. Silk as a Biomaterial. Prog Polym Sci 2007; 32: 991-1007 [PMID: 19543442 DOI: 10.1016/j.progpolymsci.2007.05.013]

Teuschi AH, van Griensven M, de Vries-Vatemeijer A, Toponarski I, Boepple HE, Weitzel PP, Richmond JC, Kaplan DL. Human bone marrow stromal cell and ligament fibroblasts responses on RGD-modified silk fibers. J Biomed Mater Res A 2005; 77: 559-570 [PMID: 15467698 DOI: 10.1002/jbm.a.10120]

James R, Toti US, Laurencin CT, Kumbar SG. Electrospun nanofibrous scaffolds for engineering soft connective tissues. Methods Mol Biol 2011; 726: 243-258 [PMID: 21424454 DOI: 10.1007/978-1-61779-052-2_16]

Chen J, Altman GH, Kategeorgiou V, Horan R, Collette A, Volloch V, Colabro T, Kaplan DL. Human bone marrow stromal cell and ligament fibroblasts responses on RGD-modified silk fibers. J Biomed Mater Res A 2005; 77: 559-570 [PMID: 15467698 DOI: 10.1002/jbm.a.10120]

Kaplan DL. Mechanism of silk processing in insects and spiders. Nature 2003; 424: 1057-1061 [PMID: 12944986 DOI: 10.1038/nature01809]

Freeman JW, Qi YY, Wang LL, Yin Z, Yin GL, Zou XH, Ouyang H. Ligament regeneration using a knitted silk scaffold: an in vitro and in vivo study. J Tissue Eng Regen Med 2014; 8: 937-945 [PMID: 23083413 DOI: 10.1002/term.1589]

Kaplan DL. Material fabrication from Bombyx mori silk fibroin. Nat Protoc 2011; 6: 1612-1631 [PMID: 21959241 DOI: 10.1038/nprot.2011.379]

Cardwell RD, Dahlgren LA, Goldstein AS. Electrospun fibre diameter, not alignment, affects mesenchymal stem cell differentiation into the tendon/ligament lineage. J Tissue Eng Regen Med 2014; 8: 937-945 [PMID: 23083413 DOI: 10.1002/term.1589]

Fan H, Liu H, Song J, Li Q, Chesler DA, Yuan K, Guerrero-Cazares H, Bamba K, Megges M, Prigione A, Adjaye J, Kassem M, Aldahmash A. Human bone marrow stromal cell and ligament fibroblasts responses on RGD-modified silk fibers. J Biomed Mater Res A 2005; 77: 559-570 [PMID: 15467698 DOI: 10.1002/jbm.a.10120]

Altman GH, Horan RL, Lu HH, Moreau J, Martin I, Richmond JC. Kaplan DL. Silk matrix for tissue engineered anterior cruciate ligaments. Biomaterials 2002; 23: 4131-4141 [PMID: 12182315 DOI: 10.1016/S0142-9612(02)00156-4]

Horan RL, Toponarski I, Boepple HE, Weitzel PP, Richmond JC, Altman GH. Design and characterization of a scaffold for anterior cruciate ligament engineering. J Knee Surg 2009; 22: 82-92 [PMID: 19216556 DOI: 10.1055/s-0030-1247730]

Chen X, Qi YY, Wang LL, Yin Z, Yin GL, Zou XH, Ouyang HW. Ligament regeneration using a knitted silk scaffold combined with collagen matrix. Biomaterials 2008; 29: 3683-3692 [PMID: 18542105 DOI: 10.1016/j.biomaterials.2008.05.017]

Fan H, Liu H, Song J, Li Q, Chesler DA, Yuan K, Guerrero-Cazares H, Bamba K, Megges M, Prigione A, Adjaye J, Kassem M, Aldahmash A. Human bone marrow stromal cell and ligament fibroblasts responses on RGD-modified silk fibers. J Biomed Mater Res A 2005; 77: 559-570 [PMID: 15467698 DOI: 10.1002/jbm.a.10120]

MacIntosh AC, Kearns VR, Crawford A, Hatton PV. Skeletal tissue engineering using silk biomaterials. J Tissue Eng Regen Med 2008; 2: 71-80 [PMID: 18385453 DOI: 10.1002/tcr.2008.03.008]

Jin HJ. Kaplan DL. Mechanism of silk processing in insects and spiders. Nature 2003; 424: 1057-1061 [PMID: 12944986 DOI: 10.1038/nature01809]

Kaplan DL. Mechanism of silk processing in insects and spiders. Nature 2003; 424: 1057-1061 [PMID: 12944986 DOI: 10.1038/nature01809]
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2010; 5: 849-863 [PMID: 20431531 DOI: 10.1038/nprot.2010.14]

Cheng MT, Yang HW, Chen TH, Lee OK. Isolation and characterization of multipotent stem cells from human cruciate ligaments. Cell Prolif 2009; 42: 488-460 [PMID: 19489881 DOI: 10.1111/j.1365-246X.2009.03611.x]

Steinert AF, Kunz M, Prager P, Barthel T, Jakob F, Noth U, Murray MM, Evans CH, Porter RM. Mesenchymal stem cell characteristics of human anterior cruciate ligament outgrowth cells. Tissue Eng Part A 2011; 17: 1375-1388 [PMID: 21247268 DOI: 10.1089/ten.TEA.2010.0413]

Nagineni CN, Amiel D, Green MH, Berchuck M, Akeson WH. Characterization of the intrinsic properties of the anterior cruciate and medial collateral ligaments: cells in vitro cell culture study. J Orthop Res 1992; 10: 465-475 [PMID: 1613622 DOI: 10.1002/jor.1100100402]

Huang TF, Chen YT, Yang TH, Chen LL, Chiou SH, Tsai CT, Chen CY, Hwang YY, Yeh CC, Wu CT, Chang YT, Wu I, Tung YL, Chen CY, Chen WJ, Lin HY, Hsu HL, Chao YL, Hwang CH. Augmenting tendon and bone-patellar tendon-bone graft after anterior cruciate ligament reconstruction: a canine model study. Am J Sports Med 2008; 36: 1171-1183 [PMID: 18326832 DOI: 10.1177/0363546508314430]

Marx RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg 2004; 62: 489-496 [PMID: 15085519 DOI: 10.1016/j.joms.2003.12.003]

Chen L, Dong SW, Tao X, Liu JP, Tang KL, Xu JZ. Autologous platelet-rich clot releasate stimulates proliferation and inhibits differentiation of adult rat tendon stem cells towards nonmyocytes lineage. J Int Med Res 2012; 40: 1399-1409 [PMID: 22971491 DOI: 10.1177/03000605124000418]

Mastrangelo AN, Vavken P, Fleming BC, Harrison SL, Murray MM. Reduced platelet concentration does not harm PRP effectiveness for ACL repair in a porcine in vivo model. J Orthop Res 2011; 29: 1002-1007 [PMID: 21376145 DOI: 10.1002/jor.21375]

Fernández-Sarmiento JA, Domínguez JM, Granados MM, Morgaz J, Navarrete R, Carrillo JM, Gómez-Villamandos RJ, Muñoz-Rascón P, Martín de Las Malas J, Millán Y, García-Ballebó M, Cugat R. Histological study of the influence of plasma rich in growth factors (PRGF) on the healing of divided Achilles tendons in sheep. J Bone Joint Surg Am 2013; 95: 246-255 [PMID: 23389788 DOI: 10.2106/JBJS.K.01659]

Figueras F, Figueras B F, Ahumada P X, Calvo R R, Vaisman B A. Use of platelet rich plasma in knee ligament surgery. Rev Med Chil 2013; 141: 1315-1320 [PMID: 24522360 DOI: 10.4067/S0034-98872013000100001]

Yuan T, Zhang CQ, Wang JH. Augmenting tendon and ligament repair with platelet-rich plasma (PRP). Muscles Ligaments Tendons J 2013; 3: 139-149 [PMID: 24367773 DOI: 10.11138/mljt/2013.3.3.139]

Hoffmann A, Pelled G, Turgenman G, Eberle P, Zilberman Y, Shinar H, Blumberg B, Janus N, Yamini A, Winkel A, Shahab S, Navon G, Gross G, Gazit D. Neotendon formation induced by manipulation of the Sma8 signalling pathway in mesenchymal stem cells. J Clin Invest 2006; 116: 940-952 [PMID: 16585960 DOI: 10.1172/JCI22689]

Woos SL, Gomez MA, Sites TJ, Newton PO, Orlando CA, Akeson WH. The biomechanical and morphological changes in the medial collateral ligament of the rabbit after immobilization and remobilization. J Bone Joint Surg Am 1987; 69: 1200-1211 [PMID: 3667649]

Woos SL, Debski RE, Withrow JD, Janaushek MA. Biomechanics of knee ligaments. Am J Sports Med 1999; 27: 533-543 [PMID: 10424228]

Woos SL, Gomez MA, Yao WK, Akeson WH. Mechanical properties of tendons and ligaments. II. The relationships of immobilization and exercise on tissue remodeling. Biochemistry 1989; 28: 197-207 [PMID: 27016891 DOI: 10.1016/0006-2952(89)80176-9]

Tetsunaga T, Furumatsu T, Abe N, Nishida K, Naruse K, Ozaki T. Mechanical stretch stimulates integrin alphaVbeta3-mediated collagen expression in human anterior cruciate ligament cells. J Biomach 2009; 42: 2097-2103 [PMID: 19647831 DOI: 10.1016/j.jbiomech.2009.06.016]

Henshaw DR, Attia E, Bhargava M, Hannafin JA. Canine ACL fibroblast integrin expression and cell alignment in response to cyclic tensile strain in three-dimensional collagen
gels. J Orthop Res 2006; 24: 481-490 [PMID: 16453340 DOI: 10.1002/jor.20050]

94 Berry CC, Shelton JC, Bader DL, Lee DA. Influence of external uniaxial cyclic strain on oriented fibroblast-seeded collagen gels. Tissue Eng 2003; 9: n13-624 [PMID: 13679440 DOI: 10.1089 /107632703768247313]

95 Altman GH, Lu HH, Horan RL, Calabro T, Ryder D, Kaplan DL, Stark P, Martin I, Richmond JC, Vunjak-Novakovic G. Advanced bioreactor with controlled application of multi-dimensional strain for tissue engineering. J Biomech Eng 2002; 124: 742-749 [PMID: 12596643 DOI: 10.1115/1.1519287]

96 Altman GH, Horan RL, Martin I, Farhadi J, Stark PR, Volloch V, Richmond JC, Vunjak-Novakovic G, Kaplan DL. Cell differentiation by mechanical stress. FASEB J 2002; 16: 270-272 [PMID: 11772952 DOI: 10.1096/fj.01-0656fje]

97 Vunjak-Novakovic G, Altman G, Horan R, Kaplan DL. Tissue engineering of ligaments. Annu Rev Biomed Eng 2004; 6: 131-156 [PMID: 15255765 DOI: 10.1146/annurev. bioeng.6.040803.140037]

98 Petrigliano FA, English CS, Barba D, Esmende S, Wu BM, McAllister DR. The effects of local bFGF release and uniaxial strain on cellular adaptation and gene expression in a 3D environment: implications for ligament tissue engineering. Tissue Eng 2007; 13: 2721-2731 [PMID: 17727336 DOI: 10.1089/ten.2006.0434]

99 Berry CC, Cacou C, Lee DA, Bader DL, Shelton JC. Dermal fibroblasts respond to mechanical conditioning in a strain profile dependent manner. Biochemistry 2003; 40: 337-345 [PMID: 12454424]

100 Park SA, Kim IA, Lee YJ, Shin JW, Kim CR, Kim JK, Yang YI, Shin JW. Biological responses of ligament fibroblasts and gene expression profiling on micropatterned silicone substrates subjected to mechanical stimuli. J Biomech Eng 2006; 102: 402-412 [PMID: 17189167 DOI: 10.1263/jb.102.402]

101 Moreau JE, Bramomo DS, Horan RL, Kaplan DL, Altman GH. Sequential biochemical and mechanical stimulation in the development of tissue-engineered ligaments. Tissue Eng Part A 2008; 14: 1161-1172 [PMID: 18380592 DOI: 10.1089 /tea.2007.0147]

102 Rathbone S, Maffulli N, Cartmell SH. Most british surgeons would consider using a tissue-engineered anterior cruciate ligament: a questionnaire study. Stem Cells Int 2012; 2012: 303724 [PMID: 22567023 DOI: 10.1155/2012/303724]

103 Hohrieder M, Teuschl AH, Cicha K, van Griensven M, Redl H, Stampfl J. Bioreactor and scaffold design for the mechanical stimulation of anterior cruciate ligament grafts. Biomed Mater Eng 2013; 23: 225-237 [PMID: 23629535 DOI: 10.3233/BME-130746]

104 Murray MM, Fleming BC. Biology of anterior cruciate ligament injury and repair: Kappa delta ann doner vaughn award paper 2013. J Orthop Res 2013; 31: 1501-1506 [PMID: 23818453 DOI: 10.1002/jor.22420]

105 Vavken P, Fleming BC, Mastrangelo AN, Machan JT, Murray MM. Biomechanical outcomes after bioenhanced anterior cruciate ligament repair and anterior cruciate ligament reconstruction are equal in a porcine model. Arthroscopy 2012; 28: 672-680 [PMID: 22261137 DOI: 10.1016/j.arthro.2011.10.008]

106 Murray MM, Magarian E, Zurakowski D, Fleming BC. Bone-to-bone fixation enhances functional healing of the porcine anterior cruciate ligament using a collagen-platelet composite. Arthroscopy 2010; 26: 549-557 [PMID: 20810092 DOI: 10.1016/j.arthro.2009.12.017]

107 Joshi SM, Mastrangelo AN, Magarian EM, Fleming BC, Murray MM. Collagen-platelet composite enhances biomechanical and histologic healing of the porcine anterior cruciate ligament. Am J Sports Med 2009; 37: 2401-2410 [PMID: 19940313 DOI: 10.1177/ 0363546509339915]

108 Murray MM, Fleming BC. Use of a bioactive scaffold to stimulate anterior cruciate ligament healing also minimizes posttraumatic osteoarthritis after surgery. Am J Sports Med 2013; 41: 1762-1770 [PMID: 23857883 DOI: 10.1177/0363546513483446]
