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Review Article

Graft healing after anterior cruciate ligament reconstruction (ACLR)

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ABSTRACT

Anterior cruciate ligament reconstruction (ACLR) is a commonly performed procedure in Orthopaedic sports medicine. With advances in surgical techniques providing better positioning and fixation of the graft, subsequent graft failure to certain extent should be accounted by poor graft healing. Although different biological modulations for enhancement of graft healing have been tried in different clinical and animal studies, complete graft incorporation into bone tunnels and the “ligamentization” of the intra-articular part have not been fully achieved yet. Based on the understanding of graft healing process and its failure mechanism, the purpose of this review is to combine both the known basic science & clinical evidence, to provide a much clearer picture of the obstacle encountered in graft healing, so as to facilitate researchers on subsequent work on the enhancement of ACL graft healing.

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Introduction

There is an estimated incidence of 200,000 anterior cruciate ligament (ACL) injuries per year in the United States,¹ with approximately 60,000 to 150,000 annually requiring ACL reconstruction (ACLR). However, the rate of graft failure for this commonly performed surgical procedure was still considerably high with reports up to 13.3%,² with evidence suggesting that despite advances in surgical techniques and optimizing rehabilitation protocols, unfavorable healing of graft may probably one of the major contributing factors.

Previous studies have demonstrated different ways of clinical and biological assessment of the healing process of ACL graft at different stages. Clinically, different imaging techniques, arthroscopy and biopsy have been used to detect the changes of the tendon graft after ACLR. Meanwhile, a myriad of histological and biochemical pre-clinical studies have demonstrated the different molecular and cellular response of the tendon graft after ACL reconstruction.

Graft healing in ACL reconstruction has been conventionally categorized as a 3-stage process, namely early healing, proliferation, and maturation phase.^{3,4} The early healing phase is characterized by graft necrosis and hypocellularity without any significant detectable revascularization occurs, followed by the proliferation

phase with the most intensive cell infiltration, and finally a maturation phase with slow matrix remodeling.^{5,6} For ACLR using free tendon grafts, complete tunnel closure and ossification of graft inside bone tunnels have never been truly observed, as only certain graft incorporation into tunnel wall is found as Sharpey's fibers or via fibrocartilage zone only.^{7,8} Also, the biological changes in the intra-articular region of the graft, which is described as “ligamentization”,⁴ could not be fully achieved as well. Because of that, over the years, different biological modulations have been advocated by different researchers, in order to improve the graft healing and thus the final clinical outcome.⁹ A successful ACLR with a tendon graft requires solid healing of the tendon graft in the bone tunnel and fully “ligamentization” in the intra-articular region of the graft as soon as possible after surgery. Enhancing the healing of the tendon graft is crucial to facilitate an early and aggressive rehabilitation and a rapid return to full activity.¹⁰

Based on systematic review on all the previous per-clinical & clinical evidence on the study of ACL graft healing, we are proposing a clearer picture of the whole process of ACL graft healing after reconstruction, which is indeed a multitude of molecular and cellular events, taking place at different region of the graft, at different time points, leading to sequential changes in the original tendon, to become the new ACL. It is thus important to understand the regulation of all these events, and their clinical relevance during the rehabilitation, to facilitate future research directions and established the right targets, on improvement of the graft healing, and thus the final clinical outcome.

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Graft failure as a result of problematic graft healing

The true incidence of ACL graft failure after implantation is unknown at present although as high as 24.4% has been reported.¹¹ Most studies have reported graft failure rates in the range of 0.7%–14%.^{12–14} Several recent systematic reviews by Spindler et al.,¹⁵ Lewis et al.¹⁶ and Wright et al.,¹⁷ have reported failure rates of 3.6%, 4%, and 5.8%, respectively. A study by the University of Pittsburgh¹⁸ showed that after single-bundle ACL reconstruction, the most common rupture pattern seen at the time of revision surgery is proximal rupture, followed by mid-substance rupture. They also¹⁹ classified the mechanisms of ACL graft failure as related to (a) surgical technique; (b) graft incorporation; and (c) trauma. Also, a Multicenter ACL Revision Study (MARS) Group developed a multi-surgeon, multicenter prospective longitudinal study, and the MARS cohort (460 patients) showed the etiology of failure, as deemed by the revising surgeon, including traumatic, technical, biologic, etc.²⁰

Since advances have been made in surgical techniques²¹ and rehabilitation methods,²² when graft failure happens following ACL reconstruction without traumatic events, problematic graft healing should be considered. So, a better understanding of the biological healing process is needed.

Clinical evidence of graft healing

Magnetic resonance imaging (MRI)

Clinically, MRI is the most commonly used imaging technique for monitoring the healing process after ACL reconstruction.²³ Howell et al.²⁴ conducted the first prospective study to serially observe the MR appearance of ACL autografts during the first year of implantation. A four-level grading system (Fig. 2) based on the MR signal of the graft was developed and it was reported that increases in magnetic resonance graft signal were time-dependent, becoming well established by 3 months and remaining unchanged at 1 year. The increased MR signal has been thought to be related to an increase in water concentration representing graft edema.²⁵ Later, the Howell team also designed a study to assess the degree of revascularization after administration of Gd-DPTA contrast agent with T1-weighted MRI.²⁶ The unimpinged ACL graft acquired no discernible blood supply during the 2 years of implantation and the periligamentous soft tissues were richly vascularized and covered the graft by 1 month. However, Biercevicz et al.²⁷ found that the use of signal intensity (SI) as an outcome measure was limited by its dependence on image acquisition parameters and scanner manufacturer. Later, Li et al.²⁸ evaluated the MRI signal/noise quotient (SNQ) of ACL grafts at 3, 6, and 12 months after ACLR (a high graft SI represents high SNQ value, which indicates inferior graft maturity) and demonstrated that the graft SNQ value has a significant negative correlation with postoperative time from 12 to 114 months postoperatively. Recently, with increasing interest in biological treatments to enhance ACL graft healing, there is a clinical need for improved quantitative MRI measures to follow up the healing process. MRI ultra-short echo time T2* (UTE-T2*) is sensitive to collagen matrix integrity and organization.^{29,30} Chu et al.³¹ showed that quantitative MRI ultra-short echo time T2* (UTE-T2*) and T2* mapping suggested substantial changes within the graft during the first 6 months post-surgery and relatively stable graft composition from 6 months to 1 year, consistent with remodeling, followed by decreases from 1 to 2 years, suggestive of continuing maturation. As above-mentioned, MRI results differed greatly across the studies due to the wide heterogeneity of the acquisition and interpretation methods, which will impede the comparison of SI. However, the time frames of the

healing process can still be concluded based on this MRI evidence (Fig. 1), and objective quantitative MRI biomarkers of graft healing would be desirable for further studies.

Computerized tomography (CT)

CT has been recommended to evaluate bone-tunnel changes during the ACL graft healing since plain radiograph is often difficult to reliably identify the tunnel and measure the width of the tunnel.^{32,33} Suzuki et al.³⁴ evaluated the bone plug was almost completely integrated into the rectangular femoral tunnel by 8 weeks after anatomical ACL reconstruction using a bone-patellar tendon-bone (BTB) graft by CT scans. Christian Fink et al.³⁵ used CT sequentially to monitor the time course of changes over 2 years. The percentage of change in tunnel size (Fig. 3) was significantly higher within the first 6 weeks following surgery compared with all other time intervals and the tunnel size was almost stable after 1 year. For autologous hamstring tendons, at a mean follow-up of 10 months, the CT scan showed a 3% femoral tunnel diameter increase, and sclerotic tunnel boundary can be revealed.³⁶ CT imaging has also been used to compare the extent of widening using different tunnel placement methods as well as different fixation methods.^{37,38} Besides, low bone mineral density (BMD) may increase the risk of incident knee osteoarthritis after ACLR, which cannot be detected by the conventional CT scan. Peripheral quantitative computed tomography (pQCT) captures not only the bone mineral content but also volumetric trabecular and cortical bone microstructure which is directly related to bone strength.³⁹ We can see that conventional CT and pQCT detect the properties of bony changes after ACLR, while researchers found that dual-energy computed tomography (DECT)⁴⁰ has the potential to evaluate soft tissue changes by generating gemstone spectral imaging (GSI) images and creating material-specific color mapping and dual-energy bone removal. So, with the development of the CT technique, we may be able to evaluate the bony and soft tissue changes simultaneously with high accuracy.

Second-look knee arthroscopy & biopsy

Since the healing status provided by the non-invasive methods such as MRI and CT scans is still limited, second-look knee arthroscopy after ACL reconstruction is one of the most reliable types of examination to provide valuable information on ACL grafts such as synovialization and vascularization.⁴¹ Nakamae et al.⁴² demonstrated significantly better synovial coverage of the graft 18 months after ACL reconstruction using second-look arthroscopy (Fig. 4). Synovialization plays an important role in graft healing and is considered to positively affect the survival of the graft. Studies reported that hamstring autografts showed considerably better synovial coverage than soft tissue allograft based on second-look arthroscopic evaluation.^{43,44} Furthermore, arthroscopy has been a tool to get biopsy samples for examinations to study the healing process.^{45–47} Histology through biopsy specimens procedure during second-look arthroscopy⁴⁸ has been examined to investigate the fate of ACL allografts on a long-term basis. In the 6-month, the surface blood flow was significantly higher than that for the control ACLs and declined with time from 6 months post-surgery onward, reach a plateau by 12 months, and maintained a level equivalent to that of the normal ACLs. In 24–30, 36–45, 48–89-month grafts, the blood flow values were also statistically insignificant compared with those for the normal control ACLs. Histologically, the specimens at 24–89 months closely resembled those at 18 months, suggesting that the allografts had reached stability by 18 months post-surgery and remained viable thereafter. Besides from synovialization and vascularization, innervation after ACLR also raises

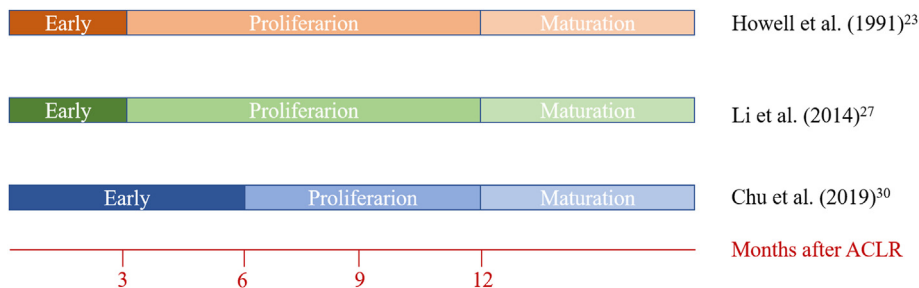


Fig. 1. Graft healing time frames in human grafts demonstrated by MRI.

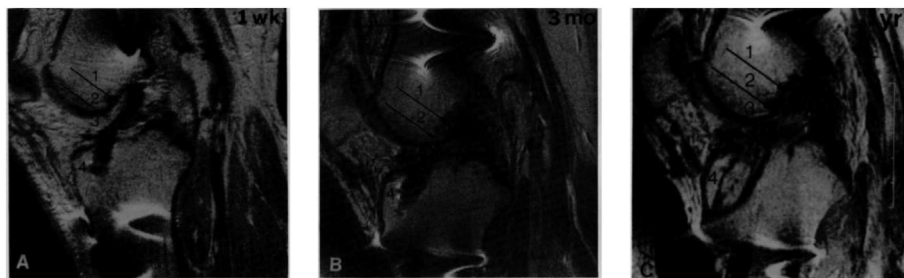


Fig. 2. A, at 1 week post implantation the entire graft had a normal MR signal (Grade I). B, by 3 months, the graft exiting from the femoral tunnel in the proximal intraarticular zone (1) has remained unchanged (Grade I); the middle intraarticular zone (2) had acquired an increased signal involving approximately 50% of the width of the graft (Grade II); the distal intraarticular zone (3) had only a few strands of normal-appearing ligament with more than 50% of the ligament having an increased signal (Grade III). The portion of the graft within the tibial tunnel (4) was normal in appearance (Grade I). C, the increase in the MR signal of the graft persisted at 1 year with no evidence of returning to normal.²⁴

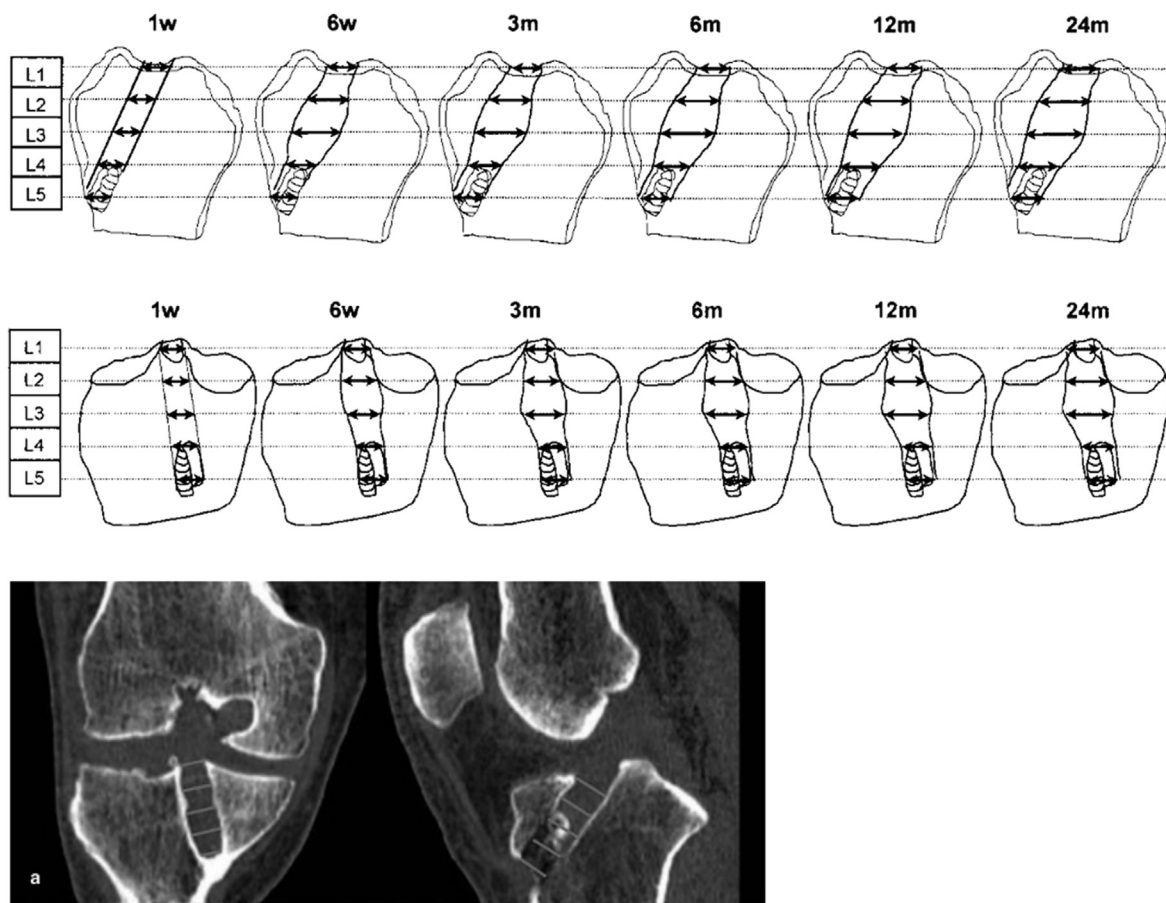


Fig. 3. Tunnel enlargement in the sagittal plane. L1, tibia plateau; L4, proximal end of the bone block; L2, 33% of L1 to L4; L3, 66% of L1 to L4; L5, $\geq 33\%$ of L1 to L4.³⁵

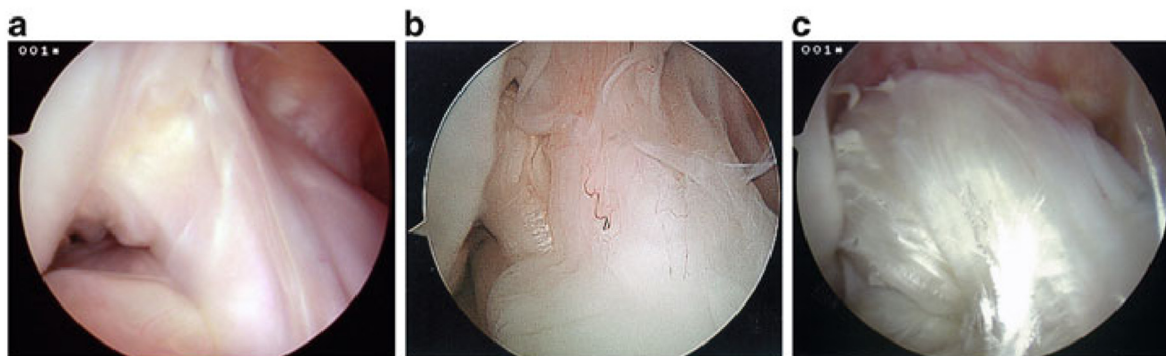


Fig. 4. Second-look arthroscopic view of the reconstructed anterior cruciate ligament. The synovial coverage of the grafts was classified as follows: (a) good (synovial coverage of >80% of the graft), (b) fair (50–80% coverage), and (c) poor (<50% coverage).⁴¹

interests from researchers. However, biopsy after ACLR using Achilles tendon showed that neural tissue analogs could only be found on the H-E stains in the control group and in the Achilles tendon allograft group, mechanoreceptors were not observed.⁴⁹ Although the biopsy can demonstrate the vascular, neural, and other cellular level changes during the healing process, it is an invasive technique and cannot do a follow-up of the healing process frequently.

Despite different imaging techniques such as MRI & CT scan, as well as arthroscopic assessment have been widely employed for assessment of ACL graft healing, the most ultimate solid evidence of proper graft healing still relies on histological & biochemical assessment. Over the years, there have been a lot of scientific work on studying the different cellular & biochemical response of the graft healing, which took place at different parts of the graft, at different time point after the ACL reconstruction.

Histological and biochemical characterization of graft healing

Some papers define the graft healing process as the combination of several biological events, including inflammatory response, graft necrosis, revascularization, cell repopulation, osseous integration, collagen remodeling, and ligamentization.⁵⁰ These biological events can be categorized into three different healing phases, namely early healing, proliferation, and maturation phase.

The early healing phase

The early cellular response following surgical implantation of a tendon graft involves the accumulation of host inflammatory cells. Shortly after graft implantation, neutrophils and ED1+ macrophages⁵¹ are recruited to the periphery of the implanted graft and various cytokines like interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), transforming growth factor-beta (TGF- β) are released.⁵² It has also been shown that the level of matrix-metalloproteinase-1, 13 (MMP-1, 13) released by cells increased after ACL reconstruction,⁵³ which will digest the collagen and help the repopulated cells with infiltration.

At the same time, researchers agree that the tendon graft undergoes avascular necrosis mainly in its central portion.⁵ As part of this necrotic process, several cytokines are released and initiate the cascade of growth factors that guide the different subsequent steps.^{51,54} Different from the inside tunnel part, the intra-articular substance is exposed to synovial fluid, which contains a lot of catalytic enzymes, cytokines, and growth factor inhibitors that interfere with the healing mechanisms.⁵⁵ Such differences may result in extended necrosis, collagen disturbance (disintegration, fragmentation, disorganization), myxoid degeneration in the intra-articular

part in the early healing phase, which may lead to a poor healing outcome if the subsequent healing process is not optimized.

It is important to actually know about the biochemical & cellular response in this early healing phase after ACL reconstruction, as a lot of surgeons are in favor of providing NSAID or COX-2 Inhibitors immediately after ACL reconstruction, to minimize the symptoms (pain & swelling) after the operation. A recent systematic review indeed demonstrated that the selective COX-2 inhibitors could negatively affect the healing process, though some other studies demonstrated no negative effect.⁵⁶ That's why cautions is needed for surgeons when administering NSAIDs/COX-2 inhibitors after ACL reconstruction, probably good to keep the duration and dosage of NSAIDs as short and low as possible to while avoiding unpleasant effect on the graft.⁵⁷

The proliferation phase

Since the ACL graft undergoes necrosis following implantation, adequate revascularization is critical for successful graft healing by allowing cellular repopulation and subsequent matrix remodeling. A deficient revascularization process will result in a lack of available oxygen for cells, thus impeding the cell repopulation.⁵⁰ Researchers have emphasized the importance of the blood supply and revascularization of the autograft in the maintenance of graft viability.^{58,59} Vascular ingrowth forms as early as 3 weeks and infiltrates even the central portion of the autograft. It is suggested that new blood vessels develop from the synovium, the infrapatellar fat pad, and the pseudo-ligamentum mucosum.⁶⁰ And vascular endothelial growth factor (VEGF) expression is accompanied by the level of vascular density. The observed VEGF production in vivo might be induced by the previous Inflammatory reactions in tendon grafts.⁶¹

The replacement cells are from a source other than the autograft. From drilling maneuver, bone marrow stem cells are released in the bone tunnels for osteoblasts and there is no question that some of these cells end up in the intra-articular space and could contribute to graft cellularity. Seeding fibroblasts from the residual stumps of the ACL can survive the synovial fluid and produce the extra-cellular biochemical products of the ACL. Meanwhile, pleuripotential mesenchymal cells from the articular cartilage could potentially express fibroblastic properties which are well suited to survive in synovial fluid. The fibroblast-like cells (Type B cells) originating from the synovial membrane are adapted for survival in synovial fluid and are present within the joint throughout the postoperative period. This suggests that these fibroblasts are the most likely candidates for the source of replacement cells that seed the autograft.⁵⁸ These cells are initially seen at the periphery of the graft, then migrated to more loosely woven areas of the matrix

where they proliferated and finally repopulated the vacant connective tissue matrix.⁵ The cell repopulation correlates with the presence of PDGF-AA, PDGF-BB, and TGF- β 1 in the reconstructed graft.

As shown from the evidence, the blood vessels and cells during this phase are coming from various tissues in the knee joint, such as the synovium, the infrapatellar fat pad, and the pseudoligamentum mucosum, indicating that it is indeed important to preserve these tissues during operation and over-debridement should be prevented. There is also evidence showing that superior postoperative knee stability and clinical outcomes were observed for remnant-preserving ACLR, with the native ACL stump preserved during the operation, when compared with standard ACLR.⁶²

The maturation phase

The changes at the wall of the bone tunnel are similar to the process of endochondral ossification, with the environment of the bone tunnel similar to that of a fracture. Bone morphogenetic proteins (BMP-2, BMP-7) are specifically involved in bone remodeling leading to osseous integration.⁶³ Bone ingrowth plays an important role in graft-to-bone healing because this stage of healing coincides with improved load-to-failures. Several studies have investigated strategies to improve bone ingrowth into a tendon graft. Osteoinductive factors (BMP-2, BMP-7),^{64–66} osteoconductive agents such as calcium-phosphate cement,⁶⁷ and osteoclast inhibition⁶⁸ have been studied as potential strategies to improve bone formation around a tendon graft.

Basic fibroblast growth factor (bFGF) is expressed from the margins of the tendon that signals the migration of spindle-shaped fibroblasts from the bone tunnel into the graft that then produce type III collagen. Then, total collagen content and the non-reducible/reducible crosslink ratio increase during this process. The collagen fibrils in the reconstructed ligament are differently organized than those of the native ACL, having a unimodal, small diameter collagen-fibril diameter profile and the remodeling process never results in exact reproduction of the original ligament organization.⁴⁷ What should be mentioned here is that, Andreas Weiler et al. showed that the histologic data indicated that anatomic interference bio-screw would lead to the development of a direct type of ligament insertion.⁶⁹ Thus, the tendon-to-bone incorporation process may be improved by the use of bio-screw fixation method.

During the above-mentioned whole healing process, Smith et al.⁷⁰ conducted anterior laxity test to see the increase in knee laxity between the day of surgery and each monthly follow-up interval in the first year using tibialis allograft in ACLR. The result showed the maximum increase in anterior laxity was at 6 months, which is correlated with the timeline of the healing process when the graft is between the early healing phase and the beginning of the proliferation phase.⁶⁰ And there is no increase in the knee laxity in the late proliferation phase and the following maturation phase.

ACL graft healing is characterized by matrix remodeling influenced by regional responses at different phases after graft implantation

As above-mentioned, although a pyramid of biological modulations has been tried, the healing outcome has never been perfect. So, a better understanding & differentiation of the graft healing process, in terms of the time and location of the responses, may be helpful for all the researchers in this area. Here, a clearer picture of graft healing, categorized into three different phases (early healing phase, proliferation phase, and maturation phase), followed by

different host and graft responses in the two different sites of the graft (intra-articular part and bone tunnels) has been proposed (Fig. 5):

At the early healing phase, inflammation of the host and cell necrosis of the graft happens immediately as a response to grafting after ACLR. In the next phase of proliferation, depending on the peri-graft environment, different cell types are recruited inside the bone (osteoprogenitors) and in the intra-articular space (fibroblasts). These cells repopulate the necrotic graft tissues with neovascularization to gain nutrients supplies. The subsequent maturation phase represents matrix remodeling processes mediated by these repopulated cells, under mechanical and biochemical influences that exhibit regional variations along with the graft.

Bone tendon junction healing inside bone tunnels and ligamentization in the intra-articular part are reactive matrix remodeling processes influenced by regional responses to grafting. In contrast to the conventional concepts of graft healing in ACLR, we highlight the regional variations in the peri-graft environment that influence the matrix remodeling. Biological modulation should target specifically either advantageous cell repopulation or the favorable regional peri-graft environment to achieve matrix remodeling to regain the original function of ACL.

Biological enhancement of graft healing: are we there yet?

Although tremendous biological modulations have been used to improve the above-mentioned healing process, good ACL graft healing is still far from ideal:

Site of biological enhancement: graft tunnel interface vs. intra-articular mid substance

Biological modulations like mesenchymal stem cells, growth factors, biomaterials, or biophysical intervention have been applied to improve the healing outcome and these biological strategies have long been reviewed.^{71,72} From different reviews, we can conclude that majority targeted graft incorporation inside bone tunnels or tendon-bone interface healing. However, it was reported that the graft ruptured most frequently at the femoral insertion⁷³ and was followed by intra-articular mid-substance.¹⁸ Animal studies have shown the same result.^{74,75} So, more attention may need to be paid to try to modulate the biological events which may improve the healing results of the intra-articular mid-substance of the graft.

Targeted outcomes of biological enhancement

As one of the most important outcomes of the ACL reconstruction, the mechanical property is always measured to compare the results after operation. However, unfortunately, the strength of the graft simulating native ACL has never been achieved. McFarland et al.⁷⁶ developed a dog ACL reconstruction model and by 16 weeks, the grafts remained only 40% as strong as controls. Another study⁷⁷ examined the biomechanics of goats for as long as 3 years after surgery and the strength and stiffness of the grafts were 44 and 49% those of the control ligaments, respectively. Rhesus monkeys⁷⁸ were also studied, the tendon had approximately 80% of the tensile strength that they had before transfer. And for small animals like rats and rabbits, they could achieve around 20% strength when compared with the native ACL.^{74,79} We have to be cautious that indeed the initial ultimate "Strength to failure" of various graft is much higher than that of the native ACL before implantation, which again suggests that the weakest link is at the bone tendon junction, which justify the efforts on researching better ACL graft healing.⁸⁰

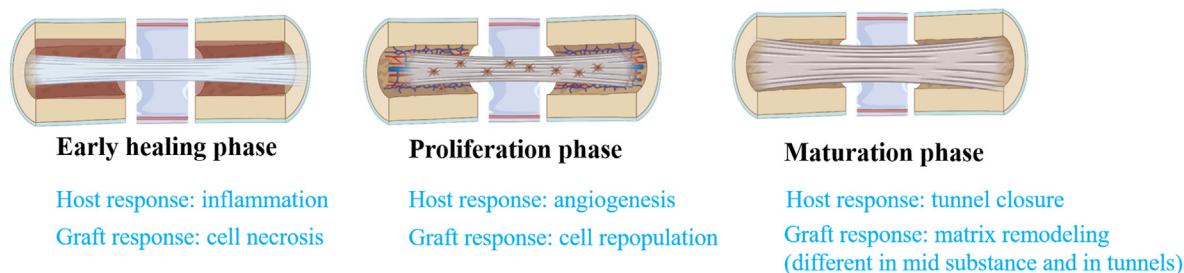


Fig. 5. ACL Graft healing is characterized by matrix remodeling influenced by regional responses at different phases after graft implantation.

Delivery of biological enhancement

Since most of the existing modulations were delivered at the early healing phase but were proposed to act on the proliferation phase or maturation phase, sustained delivery of modulations was required but few studies have demonstrated whether they act on the desired phases. Taking the cell supplementation as examples, the proliferation phase is identified by cell infiltration and repopulation in the graft. To enhance the cell repopulation, mesenchymal stem cells (MSC),⁸¹ adipose-derived stem cells (ADSC),⁸² ACL-derived cells,⁸³ synovial cells, and periosteum progenitor cells⁸⁴ have been delivered and their effects on graft healing have been investigated. The most commonly used methods to transfer stem cells is direct intra-articular and/or bone tunnel injection or embedding within fibrin glue.^{85,86} And Mifune et al. showed that the cell sheet technique is rather a superior strategy to deliver stem cells into the reconstructed ACL compared to direct injection or fibrin glue technique.⁸³ Furthermore, to achieve a continuous and stable concentration of growth factors, gene therapy based on stem cells has been introduced.^{87,88} Although the healing outcomes of these studies have shown improvements, further studies to precisely influence the targeted phases are still needed.

Conclusion

The graft failure rate after ACLR is still relatively high despite advances in surgical techniques and optimizing rehabilitation protocols, and the reason may be because of unfavorable healing process. Based on the evidence provided by clinical and animal studies investigating the healing process, tremendous biological modulations have been applied to enhance the bone-tendon interface healing. However, the mechanical strength achieved is still beyond ideal, and a junctional/mid-substance rupture is still frequently observed. A clearer picture of the healing process with three phases (early healing phase, proliferation phase, and maturation phase), with host and graft responses in two different sites (intra-articular part and bone tunnels) is proposed, aiming to give a new insight for further modulations to be delivered more specifically at targeted time and site to enhance the healing outcome. Biological modulations have promising potential in improving graft healing after ACLR in laboratory and animal studies; however, high-quality clinical studies are needed in the near future, which are closely relevant to surgeons. Surgeons also need to understand these advances background, and how these modulations work, to better facilitate translation and future research even clinical practice.

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Declaration of competing interest

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