ARTHROSCOPY AND SPORTS MEDICINE

ACL injuries and stem cell therapy

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Abstract Tears of the anterior cruciate ligament (ACL)
are very frequent injuries, particularly in young and active
people. Arthroscopic reconstruction using tendon auto- or
allograft represents the gold-standard for the management
of ACL tears. Interestingly, the ACL has the potential to
heal upon intensive non-surgical rehabilitation procedures.
Several biological factors influence this healing process as
local intraligamentous cytokines and mainly cell repair
mechanisms controlled by stem cells or progenitor cells.
Understanding the mechanisms of this regeneration process
and the cells involved may pave the way for novel, less
invasive and biology-based strategies for ACL repair. This
review aims to focus on the current knowledge on the
mechanisms of ACL healing, the nature and potential of
ligament derived stem/progenitor cells as well as on the
potential and the limitations of using mesenchymal stem
cells (MSCs) for treating injured ACL.

Keywords ACL regeneration · Stem/progenitor cells ·
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Introduction

As tendon and ligament injuries are among the most fre-
quent problems seen in everyday orthopaedic surgical
practice, regeneration strategies for these tissues are of
broad interest. Particularly in athletes, this kind of injury
commonly occurs following rapid changes of direction,
deceleration or landings. Clayton et al. prospectively col-
lected data on musculoskeletal injuries presenting to the
Edinburgh Orthopaedic Trauma Unit over 5 years and
found that nearly 40 % of injuries are tendon and ligament
related lesions. In their cohort, ACL ruptures have an
incidence of 8.1/100.000 per year [1].

Several factors influence the incidence of ACL ruptures
e.g. knee intensive sports such as basketball, soccer or
skiing show a high injury rate. Other factors increasing the
risk of tendon and ligament ruptures include systemic
cortisone intake or metabolic diseases. Female athletes
have a three times higher risk sustaining an ACL rupture
compared to males in soccer and other sports intensively
loading the knee [2]. This sex difference might be due to a
greater strain maximum in AM-ACL strain in female knees
compared to male knees, as it was shown in a biome-
chanical study [3].

Surgery is particularly recommended to any active
patients facing high load on the knee in their daily living;
and especially to athletes involved in stop-and-go sports
with multiple changes in direction like soccer, ice hockey,
rugby or tennis. Conservative treatment includes orthotics,
stabilising and proprioceptive training and muscle
strengthening to increase knee stability.

Frobell et al. recently published a 5 year follow-up
study of a prospective randomised controlled trial, showing
that the long term outcome of non-surgical ACL-rupture
treatment with intense rehabilitation as primary treatment

is equal to surgical ACL reconstruction, measured by evaluation of the knee injury and osteoarthritis outcome score [4]. Spontaneous ACL healing can occur as well, as shown by Costa-Paz et al. They retrospectively analysed 14 patients, where ACL surgery was recommended but postponed. All of these patients had a follow-up MRI and showed end-to-end healing and a negative pivot test, indicating that a spontaneous ACL healing had occurred [5].

In a 12-year follow-up, Lohmander et al. showed in a cohort of female soccer athletes, that 82 % had radiographic signs of osteoarthritis in their injured knee [6]. In contrast, successful reconstruction of isolated ACL-injuries in high-level active soccer and ski sportsmen who returned to their pre-injury sports level has been reported to prevent development of posttraumatic osteoarthritis compared to the uninjured contralateral knee [7].

In the past, suturing of the ACL was performed resulting in an unsatisfactory outcome, as over 90 % of the patients reported persistent instability [8]. The current gold standard in ACL reconstruction is the use of autograft like hamstrings (semitendinosus and/or gracilis) or patellar tendon, with no significant clinical outcome difference reported between these graft alternatives regarding stability [9].

In 2006, Steadman et al. published a novel method to promote healing of proximal ACL tears in skeletally immature athletes [10]. The so-called "healing response technique" (HRT) is based on microfractures next to the ACL femoral insertion, causing a blood clot which may support reattachment of the ACL at its origin. They also report this technique to be effective in a patient group older than 40 years with complete proximal ACL tears [11].

In contrast, in 2013 Wasmaier et al. report a cohort study including 372 patients, either treated conservatively ($n = 127$), with primary ACL reconstruction ($n = 127$) or HRT ($n = 30$). They report a non- significantly reduced revision rate in the HRT group, but no better results of HRT compared to conservative treatment in the patient that did not receive a revision reconstruction [12].

Despite relatively good success rates achieved by the currently applied methods for treating ACL rupture, novel tools augmenting these methods in order to accelerate healing or graft remodelling, decrease laxity and improve graft vascularization are desirable.

Factors influencing the healing process of the anterior cruciate ligament

The healing process of tendons and ligaments occurs slowly and takes place in three consecutive overlapping phases: (1) tissue inflammation, (2) cell proliferation, (3) remodelling. Over a period of about 8 weeks, a fibrous, scar like tissue is formed [13, 14].

A particular limitation of ACL regeneration might be that an injured ACL does not form a blood clot, which is crucial for the first healing phase, and for the induction of the proceeding phases. This clot is a product of hemostasis formed by aggregation of platelets and activation of the humoral coagulation system [15].

The intra-articular environment also plays an important role in ACL healing. Therefore the composition of the synovial fluid after ACL tear was intensively studied in order to identify factors influencing ACL healing. An increase of Matrix Metalloproteinase 3 (MMP-3), Interleukin 6 (IL-6) and Tissue Inhibitor of Metalloproteinase 1(TIMP-1) was observed in synovial fluid of patients with an isolated ACL tear [16, 17]. MMPs and TIMPs are important enzymes in tissue remodelling. A higher MMP/TIMP ratio was shown to render the knee more susceptible to cartilage destruction [16]. This imbalance might also have an effect on ACL healing. Tang et al. concluded that intra-articular accumulation of MMPs is a reason for impaired ACL repair [18]. Higuchi et al. showed that after ACL injury the concentration of MMP-3 remained high, independent of the length of time passed since the injury, whereas the TIMP-1 levels decreased. The authors suggest that the timing of the treatment of an ACL-injured knee might be of importance [16].

Stem cells

The term "stem cells" involves both embryonic and adult stem cells. The origin of embryonic stem cells is the inner cell mass of blastocysts and their characteristics are pluripotency and the ability to replicate indefinitely [19, 20]. In orthopaedic research, foetal derived embryonic-like stem cells have been used for example to successfully regenerate flexor tendonitis in a horse model [21]. In humans however, research on the use of embryonic stem cells in medical therapy is shadowed by ethical concerns as well as by the potential risk of malignant changes [22].

In order to avoid these risks and substantial ethical limitations, new concepts involving mesenchyme-derived stem cells have been developed, also in the field of ACL research. Therefore, the following chapters will only focus on mesenchymal stem cells and ligament regeneration.

Mesenchymal stem cells (MSCs) are adult stem cells from various sources, being multipotent and having the capacity of self renewal. MSCs can differentiate into mesoderm-associated cell types such as chondrocytes, adipocytes or osteoblasts. In vivo, they are often located in the perivascular area [23].

The International Society for Cellular Therapy defined specific criteria to identify MSCs: (1) plastic-adherence, (2) expression of CD73, CD90 and CD105, (3) no expression of CD11b, CD14, CD19 or CD 79alpha, CD34, CD45, HLA DR as these markers hallmark hematopoietic cells and (4) to possess the potential to differentiate into adipocytes, osteoblasts and chondrocytes. Surface marker as CD13, −29, −44, −90 and −105 are associated with MSCs [24].

Tendon and ligament derived stem and progenitor cells

Besides the option of treating injured ACL using MSCs in the future, there is another aspect of stem cells playing a role in tendon and ligament injury and regeneration: In 2003, Salingcarnboriboon et al. were the first to isolate multipotent stem cells from murine tendon; these cells have similarities with MSCs, such as their capacity to differentiate into adipocytes, osteoblasts and chondrocytes [25]. Bi et al. further characterised these cells and identified the extracellular matrix to play a key role in maintaining the tendon stem cell niche [26]. A similar cell type was identified in human ACL by Cheng et al. [27]. The authors show that these cells have a higher proliferation rate and a higher capacity to undergo osteogenic differentiation in vitro than bone marrow MSCs [27].

Matsumoto et al. isolated cells from the ACL with multi-lineage differentiation potential. These cells expressed several markers of MSCs, i.e.CD44, CD73, CD90 and CD146. Furthermore, they showed a septum between the two bundles (anteromedial and posterolateral bundle) of the foetal ACL, which contains CD34, CD146 and α-SMA positive cells. In adult ACL this septum remains as a thin capillary which is considered to be a possible source for ligament-specific stem cells [28].

The precise phenotype of tendon derived stem cells (TDSCs) remains unclear. In an own study the authors show that perivascular cells of the human supraspinatus tendon express both stem cell markers such as CD29, CD44, CD133, Mushashi-1, Nestin as well as tendon associated markers like Scleraxis and Collagens type 1 and 3 [29]. Interestingly, Musashi 1 and Nestin are proteins commonly associated with stem cells from the neural lineage. Whether these cells represent the same cell type as the ACL derived stem cells still needs further investigations.

Until now, the role of TDSCs in ACL regeneration following trauma is poorly understood. Steinert et al. characterised cells from human ACL in vitro and showed that the cells migrating out of a torn ACL exhibit characteristics of MSCs. The authors demonstrate some differences between cells migrating out of a piece of ACL and

bone marrow derived MSCs in terms of marker expression and further concludes that these cells actually could have the potential regenerate ligament tissue [30].

Using a TDSC sheet treated with connective tissue growth factor and ascorbic acid wrapped around an ACL graft in a rat model, Lui et al. showed beneficial effects in early graft healing. They observe increased mineralized tissue formation inside the bone tunnel as well as superior ultimate failure load and stiffness in the TDSC group [31].

According to their different anatomical nature, tendons and ligaments differ in their morphological and biochemical phenotype. Ligaments for example, have more cellular nuclei, collagen fibre cross-links and express a higher amount of type III collagen [32]. Moreover, the fibrils are not uniformly orientated and there is even a difference between intra- and extra-articular ligaments. Compared to the cells from the MCL, ACL cells contain more glycosaminoglycan (GAG), which might be due to the GAG rich synovial fluid [32, 33].

Compared to the commonly used autograft tendons like the semitendinosus tendon, the ACL fibroblast density is almost twice as high; also the degree of vascularisation is higher in the ACL [34].

MSCs and ACL repair studies

The use of autologous MSCs in order to accelerate ACL healing is tempting. However, it has not yet progressed beyond the state of preclinical research and small trials involving only a limited number of patients.

Animal models

In several animal studies, mesenchymal stem cells have been injected intra-articularly to investigate their regeneration potential.

A major challenge prior to clinical application of stem cells in cell therapy is determining the fate of the cells after injection. It is still a matter of debate whether systemic administration of stem cells is a feasible way of administration, whether cells home to the site of injury or whether they get trapped in other tissues.

Becerra et al. administered MSCs labelled with TC^{99m} to investigate the cell survival and cellular distribution after different application routes in a tendon overstrain injury horse model. No "homing" to the injury site was observed after intravenous administration, however, cell trapping in the lungs was observed. Intra-lesional injection was shown to be the most effective way of administration in this model, with 24 % of the cells being present in the tendon after 24 h. The authors reported no adverse reactions to the cells [35].

Both in a rat [34] and a caprine [35] model of knee injury/osteoarthritis engraftment of the implanted cells was observed. Agung et al. merely showed that intra-articular injection of MSC leads to cell engraftment in cartilage, meniscus and ACL in a dose-dependent manner [36]. In contrast, in their caprine osteoarthritis model, Murphy et al. observed regeneration of the meniscal tissue and retarded cartilage destruction, but no effect on the ACL [37].

Kanaya et al. report a positive effect of intrarticular MSC injection on the histological score of a partly transected rat ACL. Also the biomechanical properties were shown to slightly be improved. These authors did not follow-up on the cell fate after injection [38].

Besides the question how to direct stem cells to the site of injury, it still is a matter of debate whether stem cells in successful cell therapy mediate regeneration by immunomodulation and trophic factors or whether they integrate into the tissue and form functional tissues.

There is evidence that MSCs produce MMPs (-2, -3, -10, -11, -13) and TIMP-2 and respond to their MMP environment in terms of migration, quiescence and response to mechanical load [39].

A previously published paper of Xie et al. assumes that the high amount of MMPs and lower expression of lysyl oxidases (LOXs) (induced by TGFβ-1 in an injured ACL) intra-articular, compared to the MCL, might be a reason why the healing abilities of the MCL are that superior in contrast to the ACL [40]. TGFβ-1 acts as a chemical mediator in wound healing, but is also assumed to induce the expression of LOXs and MMPs. LOX plays an important role in cross-linking both in elastin and collagen [41].

Besides intra-articular MSC injection, the use of suture supporting scaffolds seeded with MSCs possibly represents a superior strategy for ACL repair. In a rabbit model of total ACL transection, collagen scaffolds seeded with MSCs supporting the suture led to total ACL regeneration in a third of the samples, whereas regeneration failed in the suture and scaffold alone groups. In this rather descriptive work, the authors do not show biomechanical data, nor do they follow-up cell fate [42].

Stem cells in human ACL repair

Silva et al. carried out a study focussing on the graft-to-bone healing in ACL reconstruction. 20 patients undergoing ACL reconstruction received an intra-operative infiltration of their graft with non-cultivated adult bone marrow-derived stem cells (BMSCs). MRI evaluation showed no difference between the treatment and the control group. Two patients required further arthroscopy for meniscectomy, one of the control and the other of the intervention group. Biopsies of the femoral tunnel have been taken; no difference in cellular and collagen content or vascularity was found after histologic examination. The authors speculate that the number of stem cells injected might have been too low to accelerate healing. However, the number of implanted cells was not determined [43].

Intra-articular MSC injections for the treatment of osteoarthritis have already been performed in a few patients. These clinical trials partly support the effects described in animal studies. The treatment resulted mainly in a decrease of pain. Further, the quantification of cartilage quality was assessed with MRI measurements and showed an improvement of the cartilage quality and a decrease of poor cartilage areas [44, 45].

Regarding osteoarthritis in ACL injuries, a phase 2 clinical trial is planned to evaluate the safety and tolerability of MSB-CAR001, a mixture of mesenchymal precursor cells and hyaluronan, compared to hyaluronan injection alone on patients who had undergone an ACL reconstruction. So far patients have not been recruited (NCT01088191).

Safety is a key issue for the clinical use of any stem cell product, so tumorgenicity and the potential to form unwanted, ectopic tissue needs to be carefully examined prior to clinical application. Centeno et al. followed-up a cohort of patients who were treated with cultured, autologous bone marrow derived mesenchymal stem cells for various orthopaedic defects. Mean follow-up was 3 years; both groups had prospective surveillance for complications. Group 1 additionally underwent MRI. No formation of neoplastic tissue was observed at the site of stem cell injection [46].

Conclusions

Despite the high hopes pinned on cell therapy approaches not only in ACL repair but in musculoskeletal regeneration in general, the use of stem cells is still far from reaching everyday clinical practice. Besides regulatory obstacles, it still needs to be defined which cell type is most beneficial for regenerating injured ligament tissue. So far it remains unclear, whether trophic factors deriving from stem cells improves regeneration or if the cells themselves form new tissue.

The best suited source for stem cells for ACL regeneration remains to be determined as well as the optimum time point for their implantation.

Despite reports on successful application of MSCs to treat damaged ACL in various animal models, further high quality studies are required to address these issues.

Any cell therapy approach is elaborate and expensive, requiring an outcome of trials showing a much higher

efficacy and benefit compared to treatments currently available justifying their introduction in clinical practise.

So far, no clinical study has been performed to either support or reject the hypothesis, that MSCs or ligament-derived stem cells support the healing of ACL ruptures with an increased need for future investigation in this interesting field of basic science.

Conflict of interest None.

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