Systematic Review

Current Evidence of Adult Stem Cells to Enhance Anterior Cruciate Ligament Treatment: A Systematic Review of Animal Trials

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Purpose: To systematically review the available preclinical evidence of adult stem cells as a biological augmentation in the treatment of animal anterior cruciate ligament (ACL) injury. **Study Design:** Systematic review. **Methods:** PubMed (MEDLINE) and Embase were searched for the eligible studies. The inclusion criteria were controlled animal trials of adult stem cells used in ACL treatment (repair or reconstruction). Studies of natural ACL healing without intervention, in vitro studies, ex vivo studies, and studies without controls were excluded. Evidence level, methodologic quality, and risk of bias of each included study were identified using previously established tools. **Results:** Thirteen animal studies were included. Six of 7 studies using bone marrow–derived mesenchymal stem (stromal) cells (BMSCs) reported a positive enhancement in histology, biomechanics, and biochemistry within 12 weeks postoperatively. Four studies using ACL-derived vascular stem cells showed a promoting effect in histology, biomechanics, and imaging within 8 weeks postoperatively. Two studies focusing on animal tendon-derived stem cells (TDSCs) and human umbilical cord blood–derived mesenchymal stem cells (hUCB-MSCs) reported promotable effects for the early healing in a small animal ACL model. **Conclusions:** BMSCs, ACL-derived vascular stem cells, TDSCs, and hUCB-MSCs were shown to enhance the healing of ACL injury during the early phase in small animal models. **Clinical Relevance:** Results of clinical trials using adult stem cells in ACL treatment are conflicting, and a systematic review of the current best preclinical evidence is crucial to guide further application.

Anterior cruciate ligament (ACL) injury is among the most common orthopaedic trauma, especially in professional and amateur athletics. Surgical treatment is the most widely applied procedure for young and active patients. The incidence of ACL reconstruction in United States increased from 32.9 per 100,000 person-years in 1994 to 43.5 per 100,000 person-years in 2006.[1] Despite the general positive outcomes of ACL reconstruction, graft failure remains a major clinical problem.[2-5] To enhance the graft healing process, biological augmentation using growth factors, stem cells, and scaffolds has been investigated for more than a decade.[6]

With additional application of adult stem cells in ACL treatment, overall positive outcomes in various in vitro and animal studies indicate the potential possibility of clinical translation.[7] In contrast, several uncontrolled clinical case series reported improvements in knee imaging and function after ACL surgery enhanced by autologous bone marrow stem cells compared to preoperative levels,[8-12] whereas other controlled clinical studies presented no superior outcomes of stem cell–augmented ACL healing compared with stem cell–free controls.[13,14]

Systematic review of animal trials contributes heavily to the decision making, safety, and efficacy of the further clinical translation.[15,16] Whether there is any discrepancy between different characteristics of grafts, stem cells, and application techniques remains to be elucidated. The purpose of this review was to systematically summarize the best available evidence in animal studies of adult stem cells as a biological

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augmentation in ACL treatment. We hypothesize that additional application of several types of stem cells effectively promote ligament healing in animal models.

Methods

Eligibility Criteria

The inclusion criteria for studies consisted of the following:

- Study type: Controlled animal trials, concerning the usage of adult stem cells. Studies included in searched reviews were also tracked.
- Study group: Animals with ACL injuries (native ACL dissection and partial or complete ACL transection).
- Intervention type: ACL surgery with application of adult stem cells. Stem cells were not tested with other biological agents or materials (cell factors, synthetic scaffolds, or artificial ligaments). Same interventions without stem cells were taken as positive controls.
- Outcome assessment: The main outcomes were to detect differences in graft-bone integration, graft maturation, and/or knee function between interventions with/without adult stem cells.
- Language: English.

The exclusion criteria were embryonic stem cell, in vitro, ex vivo, clinical studies, and studies without controls.

Literature Search

A comprehensive search was conducted in the electronic databases PubMed (MEDLINE) and Embase, using the Preferred Reporting Items for Systemic Reviews and Meta-Analyses (PRISMA) checklist and flow diagram.[17] The search strategy was composed of three elements: adult stem cells, ACL and animals (see Appendix Table 1, available at www.arthroscopyjournal.org). To broadly capture all studies, the term *stem cell* was used in the literature search phase, then "pluripotent and embryonic stem cell" studies were excluded in the screening phase. Besides, previously established animal filters for both PubMed and Embase were used to identify all animal studies.[18,19] The final search was performed on May 1, 2017. Reference lists of included papers and top hits from Google Scholar were screened for potentially missed papers.

Study Selection

Studies were initially screened on the abstracts and titles. Full texts were then obtained for all studies matching the inclusion criteria and reviewed to reconfirm the eligibility. The study selection was performed independently by 2 authors (R.G. and L.G.), and disagreement was resolved by discussion among all authors.

Methodologic Quality Assessment and Risk of Basis

Scientific level of effectiveness in animal studies is commonly low, and they were further stratified into 5 ranks based on outcome measures according to the previously published review of biological modulation in ACL surgeries.[20]

A: Quantitative outcome measures analogous to clinical outcome measures (e.g., knee laxity, activity level, and gait)

B: Mechanical test of graft complex strength (ultimate load, linear stiffness) as quantitative outcome measures

C: Biochemical measurement as quantitative outcome measures

D: Semiquantitative imaging/histologic assessment

E: Qualitative imaging/histologic assessment

The quality (methodologic score) of animal studies was assessed according to the criteria of the checklist from Fu et al.[20] (see Appendix Table 2, available at www.arthroscopyjournal.org). Studies with $\geq$5 points were recorded as "good methodologic quality" and studies $<$5 points were graded as "poor methodologic quality." The SYstematic Review Centre for Laboratory animal Experimentation's risk of bias tool (SYRCLE's RoB tool), based on the Cochrane risk of bias tool, was used to assess the internal validity of animal studies.[21] Ten signaling questions were used for judging 6 types of bias (selection, performance, detection, attrition, reporting, and other bias). Because of the methodologic issues inherent in animal studies, as well as the significant risks for selection, performance and detection biases, 2 more questions regarding randomization and blinding were added (see Appendix Table 3, available at www.arthroscopyjournal.org). The assessments were performed by 2 authors (R.G. and L.G.) independently. Any discrepancy was discussed with the senior author (B.X.) for the final decision.

Data Synthesis

The following data were extracted from the included studies, including animal species, number in treated and control groups, methods of allocation to treatment group, types of intervention, duration of follow-up, methods to assess efficacy (blinded assessment), and results of treatment. The source of cells, number of applied cells, and application methods were recorded. For each study, we defined whether a positive (beneficial effect) or negative (no difference or deleterious effect) result was reported. Compared with stem cell free controls, significant improvement in histology, biomechanics, imaging, or biochemistry in the stem cell–treated group is defined as "positive effect." In contrast, no significant difference or deterioration is defined as "negative effect."