International Platform of Registered Systematic Review and Meta-analysis Protocols

INPLASY

Effect of stem cell therapies on tendon–bone healing after anterior cruciate ligament reconstruction in animal models: protocol for a systematic review and meta-analysis

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ADMINISTRATIVE INFORMATION

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Review Stage at time of this submission - The review has not yet started.

Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 30 August 2025 and was last updated on 30 August 2025.

INTRODUCTION

Review question / Objective The objective of this systematic review and meta-analysis is to evaluate the effectiveness of stem cell-based therapies in enhancing tendon-bone healing following anterior cruciate ligament (ACL) reconstruction in animal models. Specifically, we aim to assess the impact of various stem cell sources, delivery methods, and follow-up durations on biomechanical strength, bone integration (assessed by micro-CT), and histological outcomes. This review will synthesize preclinical data to provide a structured evaluation of stem cell therapies and explore factors influencing treatment efficacy.

Rationale Anterior cruciate ligament (ACL) reconstruction is a common procedure for treating ACL injuries, but poor tendon-bone healing remains a major cause of graft failure and long-term joint instability. Stem cell-based therapies have emerged as promising biological adjuvants to enhance tendon-bone integration and improve clinical outcomes. However, the preclinical evidence regarding the efficacy of stem cell therapies for ACL reconstruction is fragmented and inconsistent, with variations in stem cell types, delivery methods, and follow-up durations. A comprehensive synthesis of this evidence is needed to clarify the therapeutic potential of stem cell interventions and guide future clinical applications. This systematic review and meta-analysis will provide a rigorous evaluation of the available animal studies, assess the impact of stem cell therapies on tendon-bone healing, and explore potential factors influencing treatment outcomes.

Condition being studied The condition being studied is anterior cruciate ligament (ACL) injury and the subsequent need for ACL reconstruction

surgery. The focus is on evaluating the effectiveness of stem cell-based therapies in enhancing tendon-bone healing during the recovery phase after ACL reconstruction in animal models.

METHODS

Search strategy A comprehensive search will be conducted in the following databases: PubMed, Embase, Scopus, SPORTDiscus, and the Cochrane Library. The search will cover all articles published from the inception of each database up to August 2025. We will use a combination of MeSH terms and keywords to identify relevant studies. The key search terms will include:

"anterior cruciate ligament" OR "ACL"

"reconstruction" OR "surgery" OR "graft"

"stem cell" OR "mesenchymal stem cell" OR "adipose-derived stem cell" OR "bone marrow stem cell" OR "exosome" OR "extracellular vesicle"

"animal" OR "preclinical" OR "in vivo"
The Boolean operators AND/OR will be used to combine these terms. In addition to the database searches, reference lists of included studies will be manually reviewed to identify any additional relevant articles.

Participant or population The population being studied consists of animal models used to simulate anterior cruciate ligament (ACL) injury and the subsequent ACL reconstruction surgery. Specifically, the study will focus on rodent and rabbit models, as these are commonly used in preclinical research on ACL reconstruction and stem cell-based therapies. These animal models are used to evaluate the effects of stem cell-based interventions (e.g., mesenchymal stem cells, adipose-derived stem cells) on tendon-bone healing after ACL reconstruction.

Intervention The intervention being studied involves stem cell-based therapies used to enhance tendon-bone healing following anterior cruciate ligament (ACL) reconstruction in animal models. The specific interventions include:

Mesenchymal stem cells (MSCs) derived from various sources, including bone marrow, adipose tissue, synovium, and tendon.

Stem cell-derived products, such as extracellular vesicles, exosomes, and scaffold-based delivery systems.

Different delivery methods, including local injection, graft wrapping, and scaffold implantation, are also considered.
The effectiveness of these interventions will be assessed based on their impact on tendon-bone integration, biomechanical strength, and histological outcomes in animal models of ACL reconstruction.

Comparator The comparator in this systematic review and meta-analysis consists of control groups in animal models of anterior cruciate ligament (ACL) reconstruction that do not receive stem cell-based interventions. The control groups may include:

Sham surgery (where the ACL is reconstructed without stem cell treatment).

Placebo or vehicle control (where a non-therapeutic substance is used as a comparison).

ACL reconstruction alone (without any stem cell or other biological intervention).
These control groups will allow for comparison of the effects of stem cell-based therapies on tendon-bone healing and other relevant outcomes.

Study designs to be included This systematic review and meta-analysis will include controlled animal studies that evaluate the effects of stem cell-based therapies on tendon-bone healing after anterior cruciate ligament (ACL) reconstruction. The study designs to be included are:Randomized controlled trials (RCTs) involving animal models.Non-randomized controlled studies, including cohort studies and case-control studies in animal models.Preclinical experimental studies that compare ACL reconstruction with and without stem cell-based interventions.In vivo studies that assess the impact of different stem cell types.

Eligibility criteria
Inclusion Criteria:
We will include controlled animal studies that evaluate the effects of stem cell-based therapies on tendon-bone healing after anterior cruciate ligament (ACL) reconstruction. The inclusion criteria are:

Animal studies (including rats, rabbits, and other species used in ACL reconstruction models).

Studies that evaluate the effects of stem cell-based interventions, including mesenchymal stem cells (MSCs), adipose-derived stem cells (ADSCs), bone marrow-derived stem cells (BMSCs), and stem cell-derived products (e.g., exosomes, extracellular vesicles).

Studies that compare ACL reconstruction with stem cell intervention versus control groups (e.g., no stem cells, sham surgery, placebo).

Studies that report at least one relevant outcome, such as biomechanical strength (e.g., ultimate failure load, stiffness), micro-CT evaluation of bone integration, or histological assessment of tendon-bone healing.

Studies published in peer-reviewed journals or credible sources.

Studies published in English.

Exclusion Criteria:
We will exclude the following studies:

Clinical trials or human studies.

Studies that do not involve ACL reconstruction or stem cell-based therapies.

In vitro studies (studies that do not use animal models).

Studies without a control group (e.g., without comparison to a sham or placebo group).

Studies that do not report relevant outcomes (e.g., biomechanical, radiological, or histological data).

Studies published in languages other than English.

Conference abstracts and reviews.

Information sources
A comprehensive search will be conducted using the following information sources:

PubMed

Embase

Scopus

SPORTDiscus

Cochrane Library

Additionally, we will manually review the reference lists of included studies to identify any additional relevant articles. Grey literature, including dissertations, conference proceedings, and reports from reputable organizations such as UNHCR, UNICEF, WHO, and World Food Program will also be searched using Google Scholar and Google Advanced Search.

All sources will be searched from their inception up to August 2025.

Main outcome(s) The main outcomes of this systematic review and meta-analysis are:

Biomechanical strength: This includes measures of the mechanical properties of tendon-bone healing, such as ultimate failure load and stiffness of the graft-tendon-bone interface.

Tendon-bone integration: Assessed using micro-CT to evaluate bone integration at the graft-tendon-bone interface.

Histological outcomes: Including tissue maturation, collagen fiber alignment, and the extent of fibrocartilage and bone formation at the tendon-bone interface, assessed by histological analysis.

Additional outcome(s) None.

Data management Data management for this systematic review and meta-analysis will follow standard protocols to ensure data integrity and transparency. The process includes:

Data extraction: Data from eligible studies will be independently extracted by two reviewers (SZ and CW) using a pre-specified data extraction form to minimize discrepancies. Any disagreements will be resolved through discussion and, if necessary, consultation with a third reviewer.

Data storage: All extracted data will be stored securely in Microsoft Excel and organized according to study characteristics, outcomes, and risk of bias. The data will be password-protected to ensure confidentiality.

Data analysis: Data will be exported to STATA (V.18) and Review Manager (RevMan V.5.4) for statistical analysis. A random-effects model will be used for meta-analysis, and subgroup analyses will be performed based on stem cell type, delivery method, animal species, and follow-up duration.

Data sharing: The aggregated data and results of the meta-analysis will be made available to other

researchers upon request, in accordance with ethical guidelines.

Quality assurance: To ensure the reliability of the data management process, regular checks and validation of the data will be performed throughout the review process.

Quality assessment / Risk of bias analysis The quality of the included studies will be independently assessed by two reviewers (SZ and CW) using the SYRCLE's risk of bias tool for animal studies. This tool is specifically designed for assessing the methodological quality of animal studies and evaluates ten domains that are crucial for determining risk of bias. These domains include:

Sequence generation: Was the randomization process appropriately conducted?

Baseline characteristics: Were baseline characteristics similar across groups?

Allocation concealment: Was the allocation of animals to different groups adequately concealed?

Blinding: Were the outcome assessors blinded to the intervention?

Random housing: Were animals randomly housed to prevent bias?

Random outcome assessment: Was the outcome assessment process randomized?

Incomplete outcome data: Was the issue of missing data addressed appropriately?

Selective outcome reporting: Were all planned outcomes reported?

Other sources of bias: Were there any other biases not addressed in the study?

Risk of bias in specific outcome measures: Were there any biases in the specific outcome measures, such as the measurement of biomechanical strength or histological analysis?

Each domain will be rated as low risk, high risk, or unclear risk. Any disagreements between the reviewers will be resolved through discussion, and a third reviewer will be consulted if necessary. The overall risk of bias will be taken into account when interpreting the findings of the meta-analysis. Studies will not be excluded based solely on their risk of bias score, but it will be considered in the sensitivity analysis and subgroup analyses.Quality assessment /Risk of bias analysis.

Strategy of data synthesis Data synthesis for this systematic review and meta-analysis will be performed using the following steps:

Descriptive summary: A descriptive summary of the included studies will be provided, detailing the study characteristics, interventions, and outcomes. This will include the animal species used, the type and source of stem cells, the delivery method, and the outcomes assessed (e.g., biomechanical strength, micro-CT evaluation, histology).

Meta-analysis: A random-effects model will be used for the meta-analysis to estimate pooled effect sizes with 95% confidence intervals (CIs) for continuous outcomes (e.g., ultimate failure load, stiffness, bone volume fraction, histological scores) and dichotomous outcomes (e.g., presence of tendon-bone integration).

Heterogeneity assessment: We will assess statistical heterogeneity across studies using the χ^2 test and quantify it using the I^2 statistic. If substantial heterogeneity ($I^2 > 75\%$) is detected, subgroup analyses will be performed to explore potential sources of heterogeneity.

Subgroup analyses: Subgroup analyses will be conducted based on:

Stem cell type (e.g., bone marrow-derived MSCs, adipose-derived stem cells).

Delivery method (e.g., local injection, scaffold, graft wrapping).

Animal species (e.g., rats vs rabbits).

Follow-up duration (≤4 weeks vs >4 weeks).

Sensitivity analysis: A sensitivity analysis will be performed by sequentially omitting one study at a time to assess the robustness of the pooled estimates.

Publication bias: If more than 10 studies are included, publication bias will be assessed visually using funnel plots and further tested using Egger's regression test.

Data presentation: The results will be presented as forest plots for continuous and dichotomous outcomes. The effect sizes will be presented as standardized mean differences (SMDs) for

continuous data and risk ratios (RRs) for dichotomous outcomes.

Subgroup analysis Subgroup analyses will be conducted to explore potential sources of heterogeneity and to assess the impact of various factors on the outcomes. The following subgroups will be analyzed:

Stem cell type:

Bone marrow-derived mesenchymal stem cells (BMSCs)

Adipose-derived stem cells (ADSCs)

Tendon-derived stem cells (TDSCs)

Other types of mesenchymal stem cells

Delivery method:

Local injection

Scaffold-based delivery

Graft wrapping

Animal species:

Rats

Rabbits

Other species used in ACL reconstruction models

Follow-up duration:

≤4 weeks

4 weeks

Outcome measures:

Biomechanical outcomes (e.g., ultimate failure load, stiffness)

Histological outcomes (e.g., collagen fiber alignment, tendon-bone integration)

Imaging outcomes (e.g., micro-CT analysis of bone integration).

Sensitivity analysis Sensitivity analysis will be performed to assess the robustness of the results and evaluate the influence of individual studies on the overall findings. The following steps will be taken:

Excluding individual studies: We will sequentially omit each study to determine if any single study significantly alters the pooled effect size. This will help assess whether the results are sensitive to particular studies or study characteristics.

Assessing the impact of study quality: We will perform sensitivity analysis by excluding studies with high risk of bias (e.g., based on SYRCLE's risk of bias tool). This will help determine whether the overall results are influenced by studies with methodological flaws.

Excluding small studies: Small studies may overestimate the effect size. We will assess the impact of excluding studies with small sample sizes to determine if these studies contribute disproportionately to the overall effect size.

Assessing the impact of statistical methods: Sensitivity analysis will be conducted to assess the influence of different statistical approaches (e.g., random-effects vs fixed-effects model) on the pooled results.

Heterogeneity exploration: We will explore how the exclusion of studies with significant heterogeneity (e.g., $I^2 > 75\%$) impacts the overall results, helping to identify sources of variation across studies.

Language restriction This systematic review and meta-analysis will include studies published in English only. Studies published in other languages will be excluded to ensure consistency and comparability of data extraction and analysis.

Country(ies) involved China.

Keywords Anterior cruciate ligament (ACL) Stem cell therapy Tendon-bone healing Mesenchymal stem cells (MSCs) Animal models.

Dissemination plans Peer-reviewed journal publication: The results will be submitted to a high-impact, peer-reviewed journal in the fields of orthopedics, regenerative medicine, or stem cell therapies.

Academic conferences: The findings will be presented at relevant national and international conferences, such as those focused on orthopedic surgery, regenerative medicine, or stem cell research.

Collaborations with clinicians: We will engage with clinicians in the field of ACL reconstruction and orthopedic surgery to ensure the applicability of the findings to clinical practice.

Research networks: The results will be shared with research networks and collaborators working in the fields of stem cell therapy and orthopedic rehabilitation.

Public outreach: A summary of the findings may be shared with the general public and media outlets to raise awareness about the potential applications of stem cell therapies in orthopedic surgery.

Contributions of each author

Author 1 - Congcong Wang - SZ and CW conceived the study topic and designed the overall review protocol. SZ drafted the manuscript, and CW critically revised it for important intellectual content. Both authors contributed to the development of the search strategy, data extraction framework, and statistical analysis plan.
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Author 2 - Shibo Zhao - SZ and CW conceived the study topic and designed the overall review protocol. SZ drafted the manuscript, and CW critically revised it for important intellectual content. Both authors contributed to the development of the search strategy, data extraction framework, and statistical analysis plan.
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