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Intralesional Secretome Injection in Anterior Cruciate Ligament Reconstruction: A Systematic Review

I Gusti Ngurah Yuda Bagus Aryana[1], I Gusti Ngurah Wien Aryana[2], Febyan[3], Erfan Sanjaya[3]

[1]Faculty of Medicine, Udayana University, Bali, Indonesia
[2]Sport Consultant, Department of Orthopaedic and Traumatology, Faculty of Medicine, Udayana University, Prof Ngoerah General Hospital, Bali, Indonesia
[3]Department of Orthopaedic and Traumatology, Faculty of Medicine, Udayana University, Prof Ngoerah General Hospital, Bali, Indonesia

Corresponding Author: I Gusti Ngurah Yuda Bagus Aryana

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ABSTRACT

Background and Objectives: Traditional ACL reconstruction (ACLR) faces challenges like graft failure, donor site morbidity, and prolonged recovery. Secretome-based therapies, composed of bioactive molecules from stem cells, offer a promising regenerative approach by promoting tissue repair, reducing inflammation, and enhancing graft integration. This systematic review aims to evaluate the efficacy of intralesional secretome injection in ACL graft augmentation compared to traditional ACL reconstruction.

Methods: A systematic review was conducted following PRISMA guidelines. A comprehensive search was performed in PubMed, Scopus, Cochrane Library, Web of Science, and Embase for studies published up to February 10, 2025. Eligible studies included animal studies, randomized controlled trials (RCTs), cohort studies, and case-control studies evaluating the effects of intralesional secretome injection on ACL graft augmentation.

Results: Five preclinical studies were included, all conducted in animal models. The findings indicated that secretome-treated groups exhibited faster bone tunnel closure, higher BV/TV, increased mechanical strength, and enhanced collagen deposition compared to control groups. Histological analysis demonstrated reduced tendon-bone interface width, formation of Sharpey-like fibers, and increased chondrocytes and fibrocartilage, suggesting enhanced tendon-bone integration. Additionally, a study on horses reported a lower reinjury rate (15.38%) in the secretome-treated group compared to untreated controls, with no adverse effects, abnormal tissue growth, or tumor development reported in any of the studies.

Conclusion: Secretome therapy shows promising potential in enhancing ACL graft healing and improving bone regeneration, mechanical properties, and histological integration. The absence of reported adverse effects further supports its safety profile. However, due to the lack of clinical trials, further research is needed to determine the real-world efficacy and long-term outcomes of secretome-based interventions in ACL reconstruction. Future studies should focus on well-designed human clinical trials, standardized secretome application protocols, and functional outcome

assessments to validate their role in clinical practice.

Keywords: Bisphosphonates; ONJ; Pamidronate; Zoledronic acid

INTRODUCTION

Anterior cruciate ligament (ACL) injuries are among the most common and debilitating knee injuries, particularly in athletes and active individuals.1 If not correctly managed, these injuries often result in joint instability, reduced functional capacity, and an increased risk of osteoarthritis. Traditional treatment for ACL tears has primarily relied on surgical reconstruction using autografts or allografts. While ACL reconstruction (ACLR) has demonstrated success in restoring knee stability, it is not without limitations, including graft failure, donor site morbidity, and prolonged rehabilitation periods.[2]

In recent years, regenerative medicine has emerged as a promising alternative or adjunct to conventional surgical approaches. Among these advancements, secretomes have gained attention for their potential to promote tissue repair, modulate inflammation, and enhance regeneration. Secretome refers to the collection of all bioactive molecules secreted by stem cells, including proteins, cytokines, growth factors, extracellular vesicles (such as exosomes), lipids, and enzymes. Intralesional injection of secretomes into the ACL graft site represents a novel approach to augment healing and improve outcomes. This technique aims to harness the paracrine effects of stem cells without the complexities associated with cell-based therapies.[3,4]

Despite the growing interest in secretome-based therapies, there is a lack of comprehensive evidence comparing their efficacy and safety to traditional ACL reconstruction. Current literature on intralesional secretome injection for ACL graft augmentation is fragmented, with studies varying in methodology, outcomes, and populations. To date, no systematic review has comprehensively evaluated the efficacy and outcomes of intralesional secretome injection in ACL graft augmentation. A systematic evaluation of the available evidence is essential to determine whether secretome-based approaches can offer a viable alternative or complement to ACLR, potentially reducing complications and improving functional recovery. This systematic review aims to compare the outcomes of intralesional secretome injection in ACL graft augmentation versus traditional ACL reconstruction.

MATERIALS AND METHODS

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The study protocol was registered with PROSPERO (CRD420250653842).

Search Strategy

A comprehensive literature search was performed in major electronic databases, including PubMed, Scopus, Cochrane Library, Web of Science, and Embase, to identify relevant studies published up to 10th February 2025. A structured search strategy was designed using relevant keywords and Medical Subject Headings (MeSH) terms, including "anterior cruciate ligament," "ACL reconstruction," "secretome," "stem-cell conditioned medium," and "graft augmentation."

Inclusion and Exclusion Criteria

Studies were included if they met the following criteria:

1. Population: Studies involving animal models or human subjects undergoing ACL graft augmentation or ACL reconstruction.
2. Intervention: Use of secretome injection as a treatment strategy.
3. Comparison: Studies comparing secretome-treated groups with control groups (traditional ACL reconstruction techniques).
4. Study Design: Randomized controlled trials (RCTs), cohort studies, case-control studies, and experimental animal studies.
5. Language: Published in English.

Studies were excluded if the design were case reports, review articles, conference abstracts, editorials, and letters to the editor. Studies were also excluded if they provided insufficient data or lacked relevant outcome measures necessary for analysis. Additionally, in vitro or cell-based studies that only examined cell cultures without in vivo animal or human models were excluded. Research focusing on non-ACL applications of secretome therapy was also excluded.

Data Extraction

Two independent reviewers screened titles and abstracts for eligibility, followed by a full-text review of potentially relevant studies. A research team extracted the data using a standardized data collection form, with each selected article reviewed independently by two reviewers. Any disagreements regarding the inclusion or exclusion of a study were resolved through consensus, with a third reviewer consulted if necessary.

Risk of Bias and Quality Assessment

Two reviewers independently assessed the risk of bias using the Cochrane Risk of Bias tool for RCTs and the Newcastle-Ottawa Scale (NOS) for observational studies. Disagreements were resolved through consensus and discussion with a third reviewer.

Data Synthesis

A qualitative synthesis was performed to summarize findings across studies. If sufficient homogeneous data were available, a meta-analysis was conducted using appropriate statistical methods to determine pooled effect sizes.

RESULTS

Study selection

Initially, 65 records were identified through database searching, while no additional records were obtained from other sources. After removing duplicates, 35 records remained and were subjected to screening. During this screening phase, 28 records were excluded based on predefined criteria. Subsequently, seven full-text articles were assessed for eligibility, out of which two were excluded due to irrelevance to the study objectives. Finally, five studies met the inclusion criteria and were incorporated into the qualitative synthesis. This structured selection ensures that only relevant and high-quality studies contribute to the systematic review's findings, following the PRISMA guidelines (Figure 1).

The included studies are listed in Table 1. All of the studies were experimental studies performed in laboratory animals. The outcomes varied, including biomechanical, radiological, histological, and clinical parameters. The study's characteristics are summarized in Table 2, detailing the population, sample size, source of secretome, delivery method, time of delivery, and follow-up period. All studies were conducted in animal models, with four using rodent models (three studies using Sprague–Dawley rats and one study using Wistar rats) and one study conducted in horses. The sample sizes varied significantly, ranging from 13 to 120 subjects.

Figure 1. Flow diagram describing the strategy for conducting this study based on PRISMA guideline.

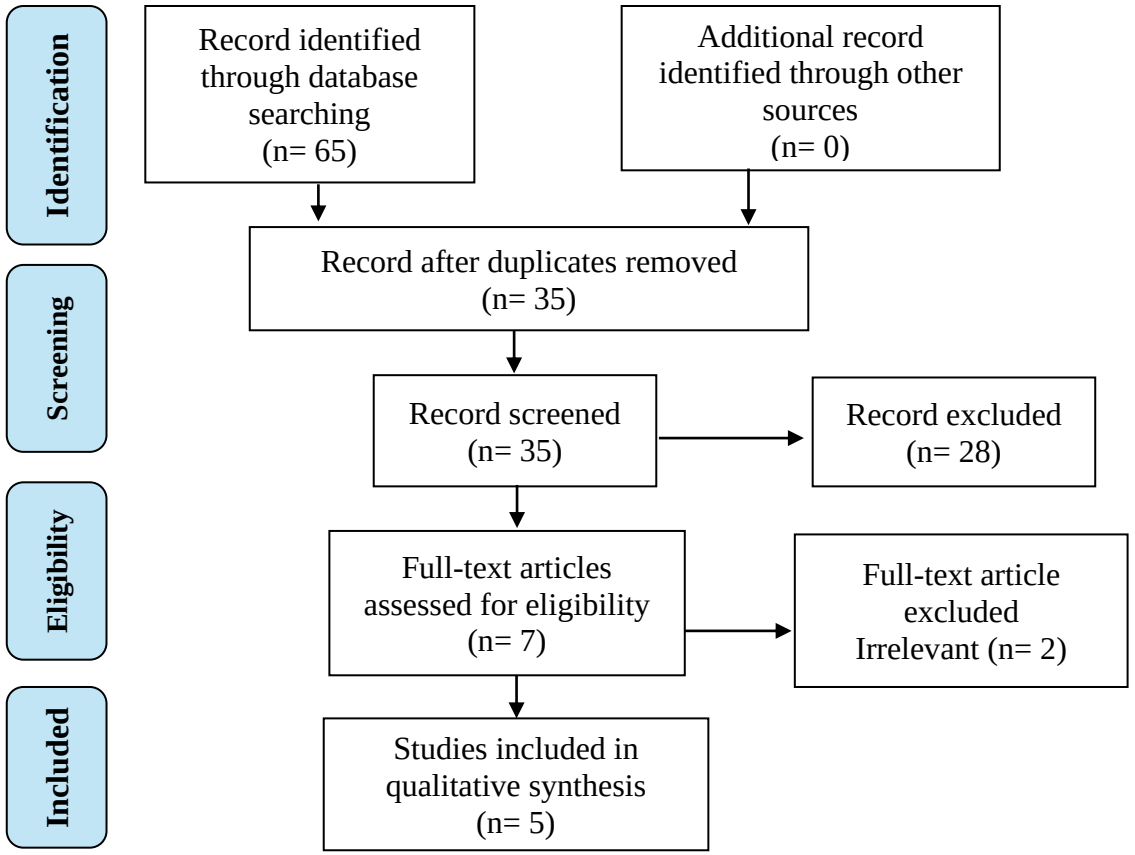

Table 1 Characteristics of study

No.	Reference	Journal	Study Design	Population	Sample Size	Outcome	Follow Up Duration
1	Sun et al., 2019	The American Journal of Sports Medicine	True Experimental (In Vivo)	Sprague–Dawley rats	120	Maximal failure load, stiffness, histology, bone tunnel area	8 weeks
2	Ghebes et al., 2018	Tissue Engineering	True Experimental (In Vivo)	Sprague–Dawley rats	15	Bone tunnel closure	6 weeks

3	Li et al., 2022	Stem Cell Research and Therapy	True Experimental (In Vivo)	Wistar Rat	90	Bone tunnel closure, interface between graft and bone, BV/TV, collagen level, mechanical strength	8 weeks
4	Zhang et al., 2022	Journal of Orthopedic Translation	True Experimental (In Vivo)	Sprague–Dawley rats	87	BV/TV, failure load, stiffness, histology, bone tunnel areas	8 weeks
5	Lange-Consiglio et al., 2013	Stem Cells and Development	True Experimental (In Vivo)	Horse	13	Clinical outcome, USG, adverse effect	2 years

Table 2 Characteristics of ACL Rupture Preparation and Source of Graft

No.	Reference	ACL Rupture Preparation	Source of Graft
1	Sun et al., 2019	ACL resection	Ipsilateral flexor digitorum longus tendon
2	Ghebes et al., 2018	ACL resection	Ipsilateral flexor digitorum longus tendon
3	Li et al., 2022	ACL resection	Peroneus longus tendon
4	Zhang et al., 2022	ACL resection	Ipsilateral flexor digitorum longus tendon
5	Lange-Consiglio et al., 2013	Sport injuries	N/A

Table 3 Characteristics of Secretome Injection

No.	Reference	Source of secretome	Delivery	Dose	Time of Delivery
1	Sun et al., 2019	Human Bone Marrow	Intra-articular injection	50 µL	Every week, starting at 7 days after surgery until 8 weeks
2	Ghebes et al., 2018	Human Muscle	Intra-articular injection	50 µL	Day 7, 14, 21, 28, and 35 after surgery
3	Li et al., 2022	Human Bone marrow	Intra-articular injection	10^{11} particles/ml	Day 0, 3, and 7 after surgery
4	Zhang et al., 2022	Human Bone Marrow	Adhesive hydrogel injection	300 µL, 10 mg/ml	During surgery (before wound closing)
5	Lange-Consiglio et al., 2013	Horse Amniotic Membrane	Intralesional injection	N/A	8-30 days after injury

Table 3 The Outcome of The Included Studies

No.	Ref	Bone Tunnel Closure	Bone Tunnel Area	BV/TV	Mechanical Strength	Collagen	Histology	Clinical Outcome	USG	Adverse effect
1	Sun et al., 2019	N/A	The tunnel area is smaller in the secretome group compared to the control group (femoral side 50.3% vs. 76.4%;	BV/TV is higher in the secretome group compared to the control	Higher maximal failure load in the secretome group compared to the control group (14.91 vs. 10.60) Higher stiffness in the secretome	More collagen type 1 in the secretome group compared to the control group	Less width of the interface between the host bone and graft in the secretome group compared to the control group (26.63% vs.	N/A	N/A	N/A

			tibial side 27.8% vs. 42.5%)	group (0.37 vs 0.27)	group compared to the control group (12.83 vs. 8.86)	(598.75% vs 229.90%)	52.25%) Sharpey-like fibers formed in secretome group			
2	Ghebes et al., 2018	faster femoral bone tunnel closure compared to control No significant differences in tibial bone tunnel closure	N/A	N/A	N/A	There is higher collagen in the secretome group compared to the control group.	Sharpey-like fibers formed in all group	N/A	N/A	N/A
3	Li et al., 2022	Faster tibial and femoral bone closure compared to control	N/A	Increased BV/TV compared to control	Increased mechanical strength compared to control	Increased collagen type II alpha 1 compared to control	Less width of the interface between the host bone and graft in the secretome group Increase chondrocytes and fibrocartilage at the tendon-bone interface of the secretome group.	N/A	N/A	N/A
4	Zhang et al., 2022	N/A	Smaller femur and tibia bone tunnel area in secretome group compared to control	Increased BV/TV in the secretome group compared to the control	The higher failure load and stiffness in the secretome group compared to the control	N/A	Higher histological scores for the grafted tendon-bone tunnel integration in the secretome group	N/A	N/A	N/A
5	Lange-Consiglio et al., 2013	N/A	N/A	N/A	N/A	N/A	N/A	Fewer reinjuries (15.38%) compared to untreated horses	No abnormal tissue growth or tumor development in both groups	None in both groups

The source of secretome differed across studies, with human bone marrow-derived secretome being the most commonly used. Other studies utilized human muscle-derived secretome and horse amniotic membrane-derived secretome. Regarding the delivery method, most studies administered the secretome through intra-articular injection, while one study used an adhesive hydrogel injection applied during surgery. Another study employed intralesional injection for direct application at the injury site. The delivery time varied, with some studies administering injections immediately post-surgery, while others implemented delayed injections at specific time intervals after surgery. The follow-up periods also differed, ranging from 6 to 8 weeks in rodent studies, while the horse study had the most extended follow-up period of 2 years.

The outcomes of the studies are summarized in Table 3. Bone tunnel closure was evaluated in several studies, with results showing faster closure in the secretome-treated group compared to the control group, particularly at the femoral and tibial sites. However, one study reported no significant difference in tibial bone tunnel closure between groups.[5] Bone tunnel areas were smaller in the secretome group compared to the control group in two studies.[6,7] For BV/TV, studies consistently demonstrated higher BV/TV in the secretome group, suggesting improved bone regeneration.[6–8] Similarly, mechanical strength was generally more significant in the secretome group, as indicated by higher maximal failure load and stiffness than controls. Collagen deposition was also increased in the secretome group, with elevated levels of collagen type I and type II alpha 1, contributing to better tissue integration.[5,6,8]

Histological findings revealed that the secretome-treated group exhibited less interface width between the host bone and graft, along with the presence of Sharpey-like fibers, increased chondrocytes, and fibrocartilage formation, indicating enhanced tendon-bone healing.[5–8] Clinical outcomes assessed in one study involving horses showed a lower reinjury rate (15.38%) in the secretome group compared to untreated horses. No abnormal tissue growth or tumor development was reported, and no adverse effects were observed in any of the included studies.[9]

DISCUSSION

This systematic review provides insights into the potential benefits of intralesional secretome injection for ACL graft augmentation, highlighting its effects on bone regeneration, mechanical strength, collagen deposition, and tendon-bone integration. The included studies demonstrated that secretome-treated groups exhibited faster bone tunnel closure, higher BV/TV, improved mechanical properties, and enhanced histological outcomes compared to controls. These findings suggest that secretome therapy could enhance ACL graft healing and integration, potentially addressing some limitations of traditional ACL reconstruction (ACLR), such as delayed graft incorporation and biomechanical instability.

One of the key findings was the improved bone tunnel closure in secretome-treated groups, particularly at the femoral and tibial sites.[8] Faster tunnel closure is associated with better graft stability and reduced risk of tunnel widening, a common issue in ACLR that can compromise long-term outcomes. Similarly, BV/TV ratios were consistently higher in the secretome-treated groups, suggesting enhanced bone remodeling and integration of the graft into the host bone.[6–8] These findings align with previous research indicating that stem cell-derived secretome plays a crucial role in bone regeneration by secretion of growth factors and extracellular vesicles that modulate osteogenesis.[7]

Biomechanically, secretome therapy led to higher maximal failure load and stiffness, indicating stronger graft integration and improved mechanical properties. This improvement is likely attributed to the enhanced collagen deposition, mainly collagen type I and type II alpha 1, critical in tendon and ligament regeneration.[5,6,8]

Additionally, histological findings revealed narrower tendon-bone interface width, the presence of Sharpey-like fibers, increased chondrocyte formation, and fibrocartilage development, all of which contribute to better graft healing and integration.[5–8]

Secretome therapy offers several advantages over stem cell-based therapy, making it a promising alternative for ACL graft augmentation. Unlike stem cells, which require viability, expansion, and differentiation, secretome consists of bioactive molecules such as cytokines, growth factors, extracellular vesicles (including exosomes), and proteins that mediate tissue repair through paracrine signaling. One major advantage of secretome therapy is its cell-free nature, which eliminates the risks associated with immune rejection, tumorigenesis, and ethical concerns related to stem cell transplantation. Additionally, secretome therapy is more stable, easier to store, and scalable, as it does not require the complex culture and expansion processes that stem cells do. Secretome-based treatments also bypass the risk of uncontrolled cell differentiation, which can sometimes lead to fibrosis or inappropriate tissue formation in stem cell therapies. Furthermore, standardization of secretome products is more feasible compared to live stem cells, allowing for consistent therapeutic effects and regulatory approval for clinical use. These benefits position secretome therapy as a safer, more practical, and potentially more cost-effective option for enhancing graft healing in ACL reconstruction and other musculoskeletal applications.[9,10]

Research specifically investigating secretome injection as graft augmentation in ACL reconstruction remains limited, with most available studies focusing on general ligament and tendon healing rather than ACL-specific applications. A systematic review by Rhatomy et al. highlighted the promising effects of secretome therapy in ligament and tendon healing, demonstrating its potential to enhance tissue regeneration, reduce inflammation, and improve biomechanical properties. However, that review did not specifically focus on ACL reconstruction.[3] Therefore, this is the first systematic review investigating the efficacy of intralesional secretome injection in ACL graft augmentation compared to traditional ACL reconstruction.

Despite promising findings, this study has several limitations that should be acknowledged. First, all included studies were preclinical (animal-based) experiments, which may limit the direct translation of findings to human clinical applications. Differences in biological responses, healing mechanisms, and biomechanics between animal models and humans could influence the outcomes, necessitating further validation in clinical trials. Second, variability in study designs, including differences in animal species, sample sizes, secretome sources, delivery methods, and follow-up periods, may introduce heterogeneity, making direct comparisons across studies challenging. Additionally, the lack of standardized protocols for secretome preparation and administration could impact treatment efficacy and reproducibility. Another limitation is the absence of long-term follow-up data in most studies, making it difficult to assess the sustained effects of secretome therapy on ACL graft integration and functional recovery. Furthermore, none of the included studies evaluated patient-reported outcomes or biomechanical function in a clinical setting, which is crucial for determining the real-world effectiveness of this approach. Lastly, potential publication bias and the limited number of available studies may affect the comprehensiveness of this systematic review. Future research should focus on well-designed human clinical trials, standardized secretome application protocols, and long-term evaluations to confirm the efficacy and safety of secretome therapy in ACL reconstruction and graft augmentation.

CONCLUSION

This systematic review highlights the potential of secretome therapy in enhancing

bone regeneration, mechanical strength, collagen deposition, and tendon-bone integration in ACL graft augmentation and reconstruction. Findings from preclinical studies suggest that secretome-treated groups exhibited faster bone tunnel closure, higher BV/TV, improved mechanical properties, and enhanced histological outcomes compared to control groups. Additionally, no adverse effects, abnormal tissue growth, or tumor development were reported, indicating the safety of this approach. However, the lack of clinical studies, variability in experimental protocols, and limited long-term follow-up data underscore the need for further research. Future studies should focus on well-designed human clinical trials, standardized secretome application protocols, and functional outcome assessments to determine the real-world efficacy of secretome therapy in ACL reconstruction. While promising, translating these findings into clinical practice requires further validation to ensure optimal graft healing, biomechanical stability, and long-term patient outcomes.

Declaration by Authors

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REFERENCES

1. Montalvo AM, Schneider DK, Webster KE, et al. Anterior Cruciate Ligament Injury Risk in Sport: A Systematic Review and Meta-Analysis of Injury Incidence by Sex and Sport Classification. J Athl Train 2019; 54: 472–482.
2. Siegel L, Vandenakker-Albanese C, Siegel D. Anterior cruciate ligament injuries: anatomy, physiology, biomechanics, and management. Clin J Sport Med Off J Can Acad Sport Med 2012; 22: 349–355.
3. Rhatomy S, Prasetyo TE, Setyawan R, et al. Prospect of stem cells conditioned medium (secretome) in ligament and tendon healing: A systematic review. Stem Cells Transl Med 2020; 9: 895–902.
4. Yanuar A, Agustina H, Budhiparama NC, et al. Prospect of Exosome in Ligament Healing: A Systematical Review. Stem Cells Cloning 2023; 16: 91–101.
5. Ghebes CA, Groen N, Cheuk YC, et al. Muscle-Secreted Factors Improve Anterior Cruciate Ligament Graft Healing: An In Vitro and In Vivo Analysis. Tissue Eng - Part A 2018; 24: 322–334.
6. Sun Y, Chen W, Hao Y, et al. Stem Cell–Conditioned Medium Promotes Graft Remodeling of Midsubstance and Intratunnel Incorporation After Anterior Cruciate Ligament Reconstruction in a Rat Model. Am J Sports Med 2019; 47: 2327–2337.
7. Zhang T, Yan S, Song Y, et al. Exosomes secreted by hypoxia-stimulated bone-marrow mesenchymal stem cells promote grafted tendon-bone tunnel healing in rat anterior cruciate ligament reconstruction model. J Orthop Transl 2022; 36: 152–163.
8. Li Z, Li Q, Tong K, et al. BMSC-derived exosomes promote tendon-bone healing after anterior cruciate ligament reconstruction by regulating M1/M2 macrophage polarization in rats. Stem Cell Res Ther 2022; 13: 295.
9. Lange-Consiglio A, Rossi D, Tassan S, et al. Conditioned medium from horse amniotic membrane-derived multipotent progenitor cells: Immunomodulatory activity in vitro and first clinical application in tendon and ligament injuries in vivo. Stem Cells Dev 2013; 22: 3015–3024.
10. Wang H-D, Li Z, Hu X, et al. Efficacy of Stem Cell Therapy for Tendon Graft Ligamentization After Anterior Cruciate Ligament Reconstruction: A Systematic Review. Orthop J Sport Med 2022; 10: 23259671221098364.

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