

Effect of Mesenchymal Stem Cell-Derived Secretome Injection on Functional Outcomes After Anterior Cruciate Ligament Reconstruction Using Peroneus Longus Tendon Autograft: A Double-Blind Randomized Controlled Trial

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Abstract

Introduction: Graft healing after anterior cruciate ligament (ACL) reconstruction remains challenging, as inadequate ligamentization and tendon–bone integration can lead to graft failure. Mesenchymal stem cell (MSC)-derived secretome, a cell-free conditioned medium containing bioactive factors, has demonstrated promising regenerative potential in preclinical studies. This study compared six-month functional outcomes of ACL reconstruction using a peroneus longus tendon (PLT) autograft with and without adjuvant secretome injection.

Methods: A double-blind randomized controlled trial enrolled 24 participants undergoing arthroscopic single-bundle ACL reconstruction with a PLT autograft, randomly assigned to receive intralesional secretome injection into the graft (secretome group, $n = 12$) or saline placebo (control group, $n = 12$). Functional outcomes were assessed preoperatively and at six months using the International Knee Documentation Committee (IKDC) Subjective Knee Evaluation Form and Tegner–Lysholm Knee Scoring Scale. Between-group comparisons used the independent-samples t-test or Mann–Whitney U test, with significance at $p < 0.05$.

Results: Baseline IKDC scores were comparable (secretome 48.57 ± 4.91 vs. control 47.51 ± 4.45 ; $p > 0.05$). At six months, the secretome group showed significantly higher IKDC scores (86.89 ± 5.11 vs. 78.27 ± 3.91 ; $p < 0.01$) and Tegner–Lysholm scores (87.08 ± 5.16 vs. 79.33 ± 2.67 ; $p < 0.01$). Post hoc power analysis confirmed adequate statistical power (0.85 for IKDC; 0.89 for Tegner–Lysholm). No major adverse events occurred; transient swelling and mild pain resolved spontaneously.

Conclusion: Adjuvant MSC-derived secretome injection during PLT autograft ACL reconstruction yielded significantly superior six-month functional outcomes. Secretome may serve as a promising cell-free biologic adjuvant to enhance graft healing and early recovery following ACL reconstruction.

Level of evidence Level 1

Registry: ClinicalTrials.gov, NCT07723625, Registration date: 19 July 2026.

INTRODUCTION

Anterior cruciate ligament (ACL) injuries represent one of the most prevalent ligamentous injuries of the knee, accounting for approximately 50% of all knee ligament injuries [1]. An estimated 200,000 ACL reconstruction procedures are performed annually worldwide, yet graft failure rates remain as high as 10%, primarily due to inadequate graft ligamentization, resulting in re-rupture, persistent instability, or ongoing postoperative pain [2, 3]. Successful ACL reconstruction depends on two fundamental biological processes: tendon-to-bone integration within the osseous tunnel and intra-articular graft ligamentization [4]. The peroneus longus tendon (PLT) has emerged as a viable autograft option, offering favorable biomechanical properties, adequate graft diameter, and lower donor-site morbidity compared

with conventional graft choices [5, 6]. However, regardless of the graft source, the quality of biological healing remains a critical determinant of long-term clinical success.

Orthobiologic augmentation strategies have attracted considerable interest as adjunctive approaches to enhance graft maturation. Among these, mesenchymal stem cell (MSC)-derived secretome—also referred to as conditioned medium (CM)—has demonstrated substantial regenerative potential [7]. The secretome comprises a complex mixture of soluble proteins, growth factors including transforming growth factor beta 1 (TGF- β 1), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), and platelet-derived growth factor (PDGF), cytokines, and extracellular vesicles secreted by MSCs through paracrine signaling [8]. Preclinical studies have demonstrated that secretome administration promotes tendon–bone integration, accelerates graft remodeling, enhances type I collagen deposition, and improves the biomechanical properties of the reconstructed graft without the tumorigenic risks associated with direct cell transplantation [9–11].

Validated patient-reported outcome measures (PROMs), including the International Knee Documentation Committee (IKDC) Subjective Knee Evaluation Form and the Tegner–Lysholm Knee Scoring Scale, are widely used to assess knee function and activity levels following ACL reconstruction [12, 13]. Despite encouraging preclinical evidence, no clinical trial has yet evaluated the effect of secretome injection on functional outcomes following ACL reconstruction using a PLT autograft in human.

The aim of this study was to compare six-month functional outcome between patients who underwent ACL reconstruction with PLT autograft augmented with intralesional secretome injection and those who received reconstruction without secretome augmentation. We hypothesized that the secretome group would demonstrate significantly higher IKDC and Tegner–Lysholm scores at six months postoperatively.

METHODS

Study Design

This prospective, double-blind, randomized, placebo-controlled trial was conducted between June 2025 and June 2026 at the Department of Orthopaedic and Traumatology, at Ngoerah Hospital, a tertiary academic medical center affiliated with Udayana University, Denpasar, Indonesia. Randomization and treatment allocation were performed by medical personnel who were not involved in any other aspects of the study. Registry: ClinicalTrials.gov, NCT07723625, Registration date: 19 July 2026. This study was approved by the Institutional Review Board of Faculty of Medicine, Udayana University (2761/UN.2.2.VII.14/LT/2025). Written informed consent was obtained from all participants before enrollment. This study was conducted and reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

Participants

Twenty-four patients with confirmed complete ACL rupture who were scheduled to undergo arthroscopic single-bundle ACL reconstruction using a peroneus longus tendon (PLT) autograft were consecutively enrolled. The diagnosis was established through clinical examination (positive Lachman, anterior drawer, and pivot shift tests) and confirmed by magnetic resonance imaging (MRI). Patient criteria were based on previous clinical trial by Rhatomy et al. 2019 [14]:

Inclusion criteria: (1) age 18–40 years; (2) unilateral, complete ACL rupture confirmed by MRI; (3) no prior ipsilateral knee ligament surgery; and (4) willingness to comply with the postoperative rehabilitation protocol and follow-up schedule.

Exclusion criteria: (1) concomitant multi-ligament knee injury; (2) ipsilateral or contralateral lower-extremity fracture; (3) systemic inflammatory or autoimmune disease; (4) active infection; (5) previous corticosteroid injection in the affected knee within three months; and (6) known allergy to biologic products.

Randomization and Blinding

Participants were randomly allocated in a 1:1 ratio to either the secretome group (Group A, n = 12) or the control group (Group B, n = 12) using a computer-generated randomization and sealed opaque envelopes. The secretome and placebo (normal saline) injections were prepared in visually identical syringes by a dedicated nurse who was not involved in the surgical procedures, outcome assessment, or data analysis. The operating surgeon, participants, and outcome assessors remained blinded to treatment allocation throughout the study period.

Sample Size Calculation

The minimum required sample size was calculated using a two-sample, one-sided t-test formula with a significance level (α) 0.05 and a statistical power of 80%, based on previously reported mean IKDC scores and standard deviations from Wang et al. [15] The calculation indicated that a minimum of 11 participants was required per group. Accounting for a 10% anticipated dropout rate, 12 patients were enrolled per group (total N = 24).

Preparation of UM-MSCs (secretome)

The secretome used in this study was derived from human umbilical cord mesenchymal stem cells (UC-MSCs) and already processed by PT. Prodia StemCell Indonesia (ProStem) (USEPro®). The conditioned medium was collected from MSC cultures (10×10^6 cells) maintained for 48–72 hours, subsequently filtered through a 0.22- μ m membrane filter, and stored at -80°C until use. Each syringe contained 1.5 mL of concentrated secretome (equivalent to the secretory products of 10×10^6 UC-MSCs). The placebo consisted of 1.5 mL sterile normal saline prepared in an identical syringe (Fig. 1.).

Surgical Technique

All procedures were performed by a single sports orthopaedic surgeon (IGNWA) under regional anesthesia with tourniquet application. Standard anterolateral and anteromedial arthroscopic portals were established. Diagnostic arthroscopy confirmed the ACL rupture. The PLT autograft was harvested through a 2–3 cm longitudinal incision posterior to the lateral malleolus. The peroneus longus tendon was identified, and a distal tenodesis to the peroneus brevis was performed using non-absorbable sutures. The tendon was harvested proximally using a closed tendon stripper, prepared as a double- or triple-strand graft to achieve a minimum diameter of 8 mm, and both ends were whipstitched with Ethibond sutures. The patient was positioned in a supine position with the knee flexed to 90 degrees, using a leg support that allows the knee to be flexed up to 120 degrees. Anteromedial and anterolateral portals are created to provide arthroscopic access. Femoral and tibial tunnels were created using standard anatomic single-bundle technique. The graft was fixed on the femoral side using an Endobutton device and on the tibial side with a biodegradable interference screw. (Fig. 2.) In the secretome group, 0.5 mL of the study solution was injected into each end of the graft (proximal and distal) prior to tunnel insertion. Following graft fixation, residual arthroscopic fluid was evacuated, and the remaining 0.5 mL was injected intra-articularly around the graft under arthroscopic visualization and participants in the control group underwent an identical injection protocol with normal saline (Fig. 3).

Postoperative Rehabilitation

All participants followed a standardized rehabilitation protocol at our hospital. Perioperative nonsteroidal anti-inflammatory drugs (NSAIDs) were avoided to minimize potential interference with the early inflammatory phase of graft healing. The protocol included progressive range-of-motion exercises, quadriceps strengthening, proprioceptive training, and activity-specific functional exercises, return to sports was permitted from six months postoperatively based on individual progress.

Outcome Measures

The primary outcome measures were the IKDC Subjective Knee Evaluation Form score and the Tegner–Lysholm Knee Scoring Scale score, assessed preoperatively and at six months postoperatively. The IKDC score ranges from 0 (worst) to 100 (no limitation), while the Tegner–Lysholm score ranges from 0 to 100, categorized as poor (< 65), fair (65–83), good (84–90), or excellent (> 90) [12, 13]. All scores were calculated using the validated online scoring platform (www.orthopaedicscore.com). Adverse events were systematically recorded at each follow-up visit.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 29.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean $\pm$ standard deviation (SD), and

categorical variables as frequencies and percentages. Normality was assessed using the Shapiro–Wilk test. Baseline demographic and clinical characteristics were compared using the chi-square test for categorical variables and the independent-samples t-test or Mann–Whitney U test for continuous variables, as appropriate. Between-group comparisons of functional outcome scores were performed using the independent-samples t-test or Mann–Whitney U test. A post hoc power analysis was conducted. Statistical significance was set at $p < 0.05$.

RESULTS

Baseline Characteristics

All 24 enrolled participants completed the study with no dropouts or protocol violations. The two groups were comparable at baseline with respect to age, sex distribution, body mass index (BMI), mechanism of injury, and time from injury to surgery (Table 1). The mean age was similar between the secretome group and the control group. No significant differences in baseline demographic or clinical variables were observed between groups (all $p > 0.05$).

Table 1
Baseline demographic and clinical characteristics of participants

Characteristics	Category	Frequency	Percentage
Sex	Male	10	41.7%
	Female	14	58.3%
Year Group	20–24 years old	6	25%
	25–30 years old	18	75%
Occupation	Student	4	16.6%
	Doctor	1	4.2%
	Housewife	3	12.5%
	Employee	9	37.5%
	Self-employed	7	29.2%

Functional Outcome (IKDC) Scores

Preoperative IKDC scores were comparable between the secretome group (48.57 ± 4.91) and the control group (47.51 ± 4.45 ; $p > 0.05$). At the six-month follow-up, the secretome group demonstrated a significantly higher mean IKDC score (86.89 ± 5.11) than the control group (78.27 ± 3.91 ; $p < 0.01$). Both groups showed substantial improvement from baseline, with a mean improvement of 38.32 points in the secretome group and 30.76 points in the control group (Table 2).

Table 2
Comparison of IKDC scores between groups

Outcome Measure	Secretome Group (n = 12)	Control Group (n = 12)	p-value
IKDC Preoperative (mean $\pm$ SD)	48.57 $\pm$ 4.91	47.51 $\pm$ 4.45	> 0.05
IKDC 6-month Postoperative (mean $\pm$ SD)	86.89 $\pm$ 5.11	78.27 $\pm$ 3.91	< 0.01

Functional Outcome (Tegner- Lysholm) Scores

Preoperative Tegner–Lysholm scores were also comparable between groups (secretome 53.42 $\pm$ 7.50 vs. control 52.02 $\pm$ 5.91; $p > 0.05$). At six months, the secretome group demonstrated a significantly higher Tegner–Lysholm score (87.08 $\pm$ 5.16) than the control group (79.33 $\pm$ 2.67; $p < 0.01$). Based to the Tegner–Lysholm classification, the mean postoperative score in the secretome group was categorized as good (84–90), whereas the control group remained in the "fair" category (65–83) (Table 3).

Table 3
Comparison of Tegner–Lysholm scores between groups

Outcome Measure	Secretome Group (n = 12)	Control Group (n = 12)	p-value
Tegner–Lysholm Preoperative (mean $\pm$ SD)	53.42 $\pm$ 7.50	52.02 $\pm$ 5.91	> 0.05
Tegner–Lysholm 6-month Postoperative (mean $\pm$ SD)	87.08 $\pm$ 5.16	79.33 $\pm$ 2.67	< 0.01

Post Hoc Power Analysis

Post hoc power analysis demonstrated statistical power values of 0.85 for IKDC scores and 0.89 for Tegner–Lysholm scores, indicating that the study had adequate power to detect the observed between-group differences.

Adverse Events

No major adverse events, infections, systemic reactions, or graft failures were observed in either group during the six-month follow-up period. Minor transient adverse events in the secretome group included localized swelling ($n = 3$) and mild pain at the injection site ($n = 2$), all symptoms resolved resolved spontaneously within 3–5 days without additional intervention.

DISCUSSION

The principal finding of this study is that adjuvant secretome injection during ACL reconstruction with PLT autograft resulted in significantly superior six-month functional outcomes, as measured by both IKDC and Tegner–Lysholm scores, compared with reconstruction without secretome augmentation. To

our knowledge, this is the first randomized controlled trial to evaluate the clinical efficacy of MSC-derived secretome as an adjunct to ACL reconstruction using a PLT autograft in human.

The orthobiologic field has undergone a paradigm shift from cell-based therapies toward cell-free approaches. The secretome offers several advantages over direct MSC transplantation, including the absence of tumorigenic risk, ease of standardization, improved storage stability, and avoidance of immunological complications [7, 8]. The bioactive molecules within the secretome—including TGF- β 1, VEGF, IGF-1, and PDGF—have been shown to modulate the local microenvironment by promoting anti-inflammatory macrophage polarization (M1-to-M2 transition), enhancing angiogenesis, stimulating fibroblast proliferation and collagen synthesis, and facilitating tendon-to-bone integration [9, 11, 15].

The IKDC score improvement observed in the secretome group (mean increase of 38.32 points) exceeded that of the control group (mean increase of 30.76 points), representing a clinically meaningful difference. The IKDC Subjective Knee Evaluation Form is a validated and responsive instrument for assessing knee function following ligament reconstruction, and a change exceeding 11.5 points is generally considered the minimal clinically important difference (MCID) [12, 16]. Both groups surpassed this threshold; however, the significantly greater improvement in the secretome group suggests an provides additional biological benefit of the secretome on graft healing and functional recovery.

Similarly, the Tegner–Lysholm scores at six months demonstrated a significant between-group difference. The secretome group achieved a mean score classified as "good" (87.08), while the control group scored in the "fair" range (79.33). This difference has practical clinical significance, as it may reflect enhanced joint stability, reduced pain, and a greater capacity to resume higher-level functional activities, which are paramount goals of ACL reconstruction [13, 17].

Our findings are consistent with preclinical evidence. Sun et al. [9] demonstrated that intra-articular injection of hBMSC-derived conditioned medium in a rat ACL reconstruction model significantly enhanced ultimate failure load, stiffness, bone tunnel closure, and Sharpey-like fiber formation at the tendon–bone interface at four and eight weeks. Wang et al. [10] in a systematic review confirmed that stem cell-derived therapies improved both biomechanical and histological outcomes of ACL grafts in animal models. Aryana et al. [11] further reported that secretome-treated grafts exhibited narrower tendon–bone interface width, increased chondrocyte density, and improved fibrocartilage formation in preclinical settings. The current study provides the first clinical evidence supporting these preclinical findings in the context of human ACL reconstruction [18–20].

The favorable safety profile observed in this trial is reassuring. No major adverse events, infections, or allergic reactions were documented. The mild, self-limiting injection-site reactions are consistent with previous reports of intra-articular biologic injections [14, 18, 21]. These findings align with preclinical toxicity studies demonstrating no local or systemic toxicity and no tumor formation with secretome administration for up to 47 weeks [10, 11].

LIMITATIONS

This study has several limitations that should be acknowledged. First, the relatively small sample size, although adequately powered for the primary outcome measures, limits the generalizability of the findings and the ability to detect differences in less common secondary outcomes. Second, the follow-up period of six months, while capturing the critical early phase of graft healing and functional recovery, does not allow assessment of long-term graft survival, return-to-sport rates, or re-injury risk. Third, this was a single-center study performed by a single surgeon, which enhances internal consistency but may limit external validity. Fourth, objective assessments of graft maturation such as MRI evaluation of graft signal intensity or second-look arthroscopy were not performed, precluding direct correlation between functional outcomes and biological graft healing. Fifth, the secretome composition may vary between batches, and standardization of the biologic product remains a challenge for broader clinical application. Future multicenter trials with larger sample sizes, longer follow-up durations, objective imaging endpoints, and dose-response analyses are warranted to validate these preliminary findings.

CONCLUSION

Adjuvant intralesional injection of MSC-derived secretome during ACL reconstruction with a peroneus longus tendon autograft was associated with significantly superior functional outcomes at six months postoperatively, as measured by both the IKDC and Tegner–Lysholm scores, compared with standard reconstruction without biologic augmentation.

These results suggest that secretome may serve as a safe and effective cell-free adjuvant to enhance early graft healing and functional recovery following ACL reconstruction. Larger, multicenter studies with longer follow-up are needed to confirm these findings and establish the optimal treatment protocol, including dosage and timing of administration.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This research was conducted in accordance with the Good Clinical Practice. This study was approved by the Institutional Review Board of Faculty of Medicine, Udayana University (2761/UN.2.2.VII.14/LT/2025). Written informed consent was obtained from all participants before enrollment. The completed CONSORT checklist has been provided as a supplementary document to ensure transparency, completeness, and adherence to the recommended reporting standards for randomized controlled trials.

CONFLICT OF INTEREST

The author clarifies that there is no conflict of interest

FUNDING

Author Contribution

IGNWA, FBN, IWSD, MBK IKS contributed to the conceptualization and study design. Supervision was provided by CRS, IWS, CGOD. Data collection was performed by FBN, while statistical analysis was conducted by IWSD, MBK. Manuscript drafting was undertaken by IGNWA, FBN.

DATA AVAILABILITY

All data relevant to the study are included in the article.

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Figures



Figure 1

Each syringe contained 1.5 mL of concentrated secretome (equivalent to 10×10^6 cells) and the placebo consisted of 1.5 mL sterile normal saline prepared in an identical syringe

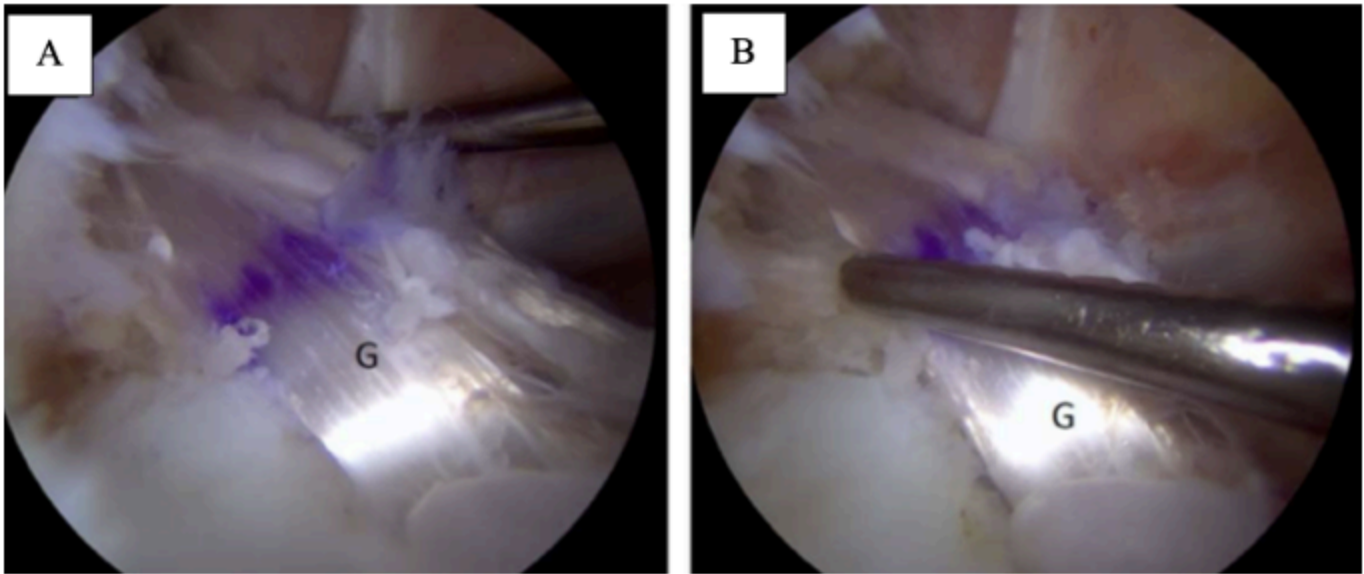


Figure 2

The graft is pulled through the tunnels and fixed on both the femoral and tibial sides.

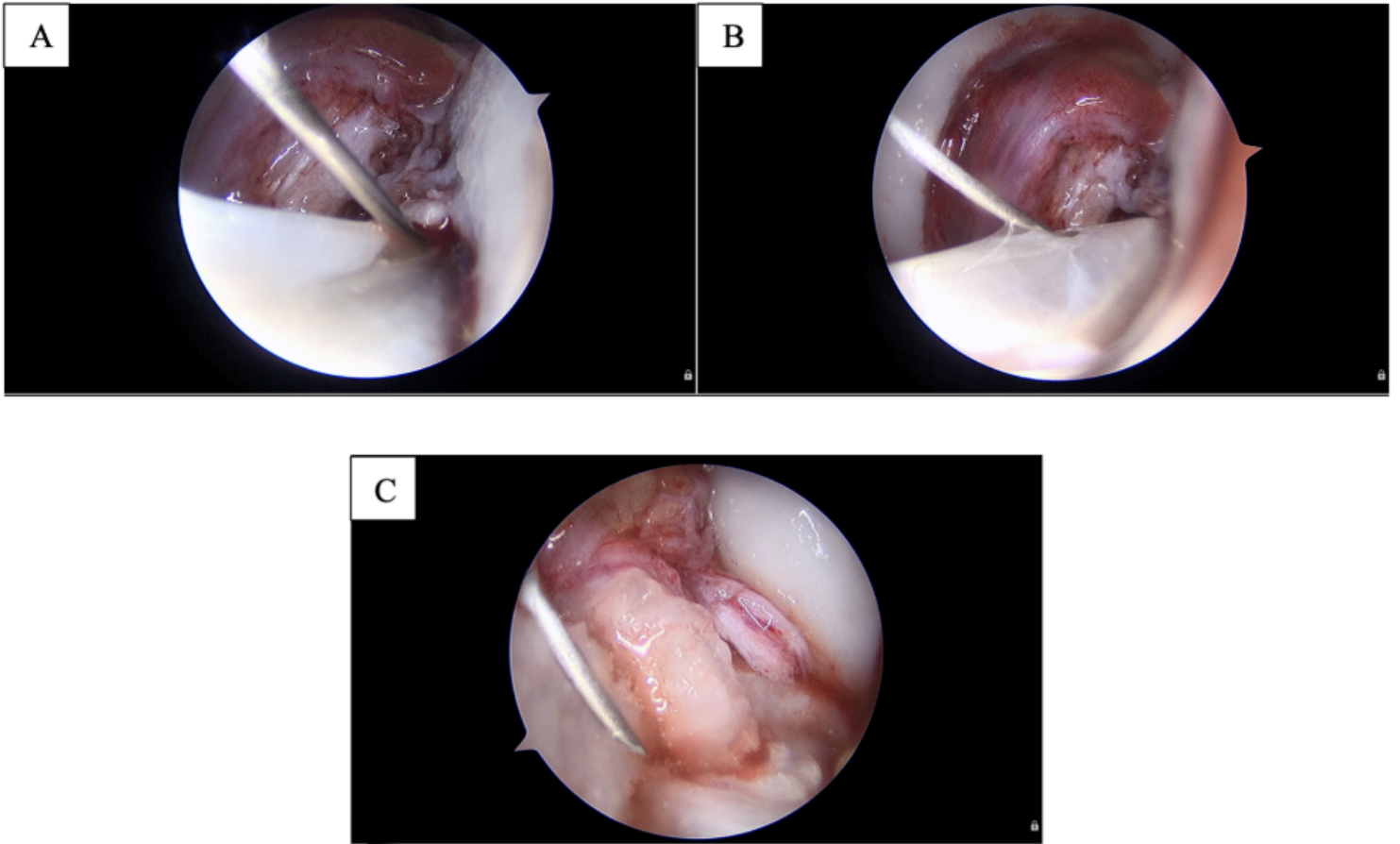


Figure 3

(A) Femoral Intratunnel Injection for Graft (Proximal). (B) Midgraft injection. (C) Tibial Graft Injection (Distal)