

Platelets and Adipose Stroma Combined for the Treatment of the Arthritic Knee

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Abstract: Knee osteoarthritis (OA) leads to significant pain and disability, prompting new cell-based injections to lessen the symptoms. Biological therapies such as autologous microfragmented adipose tissue (AMAT) and a stromal vascular fraction (SVF) are a common source for harvesting mesenchymal and progenitor cells. Platelet-rich plasma (PRP) is associated with cytokines and growth factors. Recent studies have reported good clinical outcomes with AMAT, SVF, and PRP in knee osteoarthritis treatment. However, the preparation, processing, and application technique are vital to achieving satisfactory results. Many studies have examined outcomes after AMAT, SVF, or PRP injection, with encouraging results. Still, there is a lack of studies describing a technique that combines both methods, the timing, and the amount of SVF or PRP injected. This technical note's objective was to describe a standardized new technique composed of platelet and adipose stroma to treat knee osteoarthritis (OA) and the processing method.

Introduction

Osteoarthritis of the knee can diminish the quality of life and cause joint organ degeneration.¹ Current surgical treatments include osteotomies and partial or total knee replacement. Nowadays, many patients prefer nonsurgical options. Autologous microfragmented adipose tissue (AMAT), stromal vascular fraction (SVF), and platelet-rich plasma (PRP) are common sources to obtain mesenchymal stem cells (MSCs), progenitor cells, and associated cytokine/growth factors, respectively. Because the use of AMAT/SVF and PRP are minimally manipulated therapies with homologous use and only a minor change during the preparation and currently approved by the U.S. Food and Drug Administration, they represent a viable treatment option using progenitor cells and growth factors as an injection for OA.^{1,2}

Adipose-derived cell therapies have gained recent popularity as a treatment. Compared to peripheral blood, adipose tissue has 25,000 times more reparative

cells.³ In the bone marrow, MSCs represent a small fraction (.001–.01%) of nonhematopoietic, multipotent cells.⁴ Adipose tissue has been reported to have larger quantities of mesenchymal and progenitor cells.⁵

PRP contains a high concentration of growth factors, including platelet-derived growth factor (PDGF), transforming growth factor- β (TGF- β), and vascular endothelial growth factor (VEGF), having anabolic and anti-inflammatory effects responsible for the beneficial symptomatic pain relief with this biologic approach. One of OA treatment research's primary foci in the last decade has been regenerative cellular therapy, primarily medicinal signaling cells (MSCs) and growth factors.^{6,7} Some clinical in vivo human studies showed encouraging results and a safe profile in mild knee OA treatment.^{1,8-12}

Nevertheless, harvesting and processing techniques are vital to achieving optimal MSCs and growth factors for satisfactory results. This technical note's objective was to describe the technique named P.A.S.T.A. for platelets and adipose stroma combined for the treatment of arthritis and the processing method.

Technique and Processing Method

[Video 1](#) shows the procedure.

Autologous Adipose Tissue Aspiration

With the patient in the prone position, under sedation, aseptic conditions, and local anesthesia ([Fig 1, A and B](#)), adipose tissue was harvested using a bilateral gluteus lipoaspiration approach ([Fig 1C](#)). The procedure

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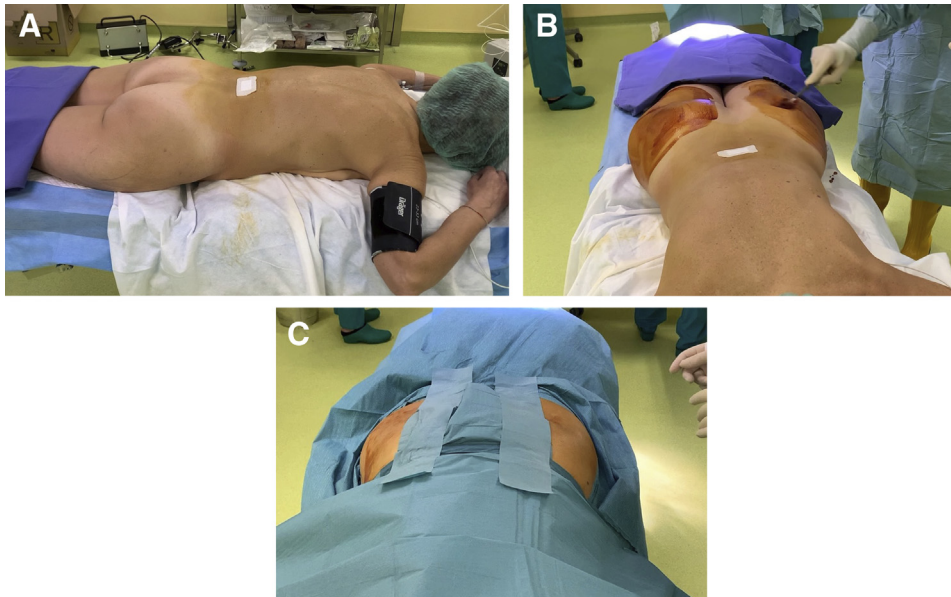


Fig 1. Platelets and adipose stroma combined for the treatment of the arthritic knee. Surgical technique first Step: Patient positioning and site preparation for adipose tissue harvesting. A) Patient prepared in the prone position in the surgical room, under sedation and epidural anesthesia. B) Bilateral gluteus antiseptics for a double-side approach. C) Sterile dressing proposal for bilateral approach.

started with a small stab incision using an 11 blade, and a subcutaneous fat infiltration throughout the whole harvesting area is performed using an infiltration cannula 2.1 mm \times 15 cm (Tulip, Medical Products, San Diego, CA)

A tumescent solution composed of 500 mL of saline with 30 mL of lidocaine 2% plus epinephrine 1:200,000 plus 3 mL of bicarbonate (6.8%) was used (Fig 2, A and B). We allowed the tumescent fluid to take effect for 15 minutes. Following this, 30 mL of adipose tissue and tumescent fluid were aspirated through a Carraway Harvester cannula, 2.1 mm \times 15 cm (Tulip) connected to the syringe (Fig 3A), pulling back the syringe plunger and using the Johnnie Snap locking system (Tulip). We maintained a constant vacuum throughout the harvesting process (Fig 3B). Finally, 15 mL of lipoaspirate was transferred to each ACP double syringe, using the

2.4-mm transparent transfer adapter for micro fat (Fig 3C) (Arthrex, Munich, Germany).

Autologous Adipose Tissue Processing

The ACP double syringes filled with 15 mL of decanted lipoaspirate were centrifuged at 2,500 rpm with a centrifuge Hettich Rotofix 32A with a swing-out rotor, 220 V (Andreas Hettich, KG Tuttlingen, Germany), for 4 minutes at room temperature (Fig 4A). The syringes were removed from the centrifuge, taking care to keep in an upright position to avoid mixing (Fig 4B). Subsequently, the oil is transferred into the smaller inner syringe to be discarded (Fig 4C), and the aqueous fraction is drained (Fig 4D). Twenty milliliters of condensed lipoaspirate was obtained (Fig 4E) and transferred into two 10-mL syringes using a GEMS transparent transfer, 2,4 (Tulip) (Fig 5A).

Fig 2. Platelets and adipose stroma combined for the treatment of the arthritic knee. Surgical technique second step: tumescent solution infiltration for adipose tissue harvesting. A) Shows the tumescent solution infiltration with a 2.1 mm \times 15 cm cannula. B) Tumescent solution (500 mL of saline, 30 mL of lidocaine 2%, 1 mL epinephrine 1:300,000, and 3 mL of bicarbonate 6.8%) gluteus application.

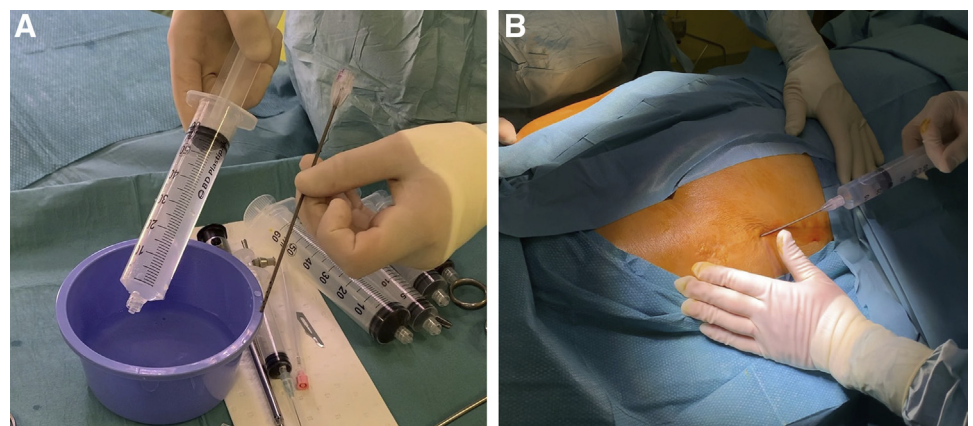


Fig 3. Platelets and adipose stroma combined for the treatment of the arthritic knee: Surgical technique third step: Adipose tissue harvesting. (A) Shows gluteus lipoaspiration with a 2.1 mm × 15 cm harvester cannula connected to syringe with Johnnie snap locking system. (B) The arrow illustrates the Johnnie snap locking system for a constant vacuum. (C) Transference of 15 mL of lipoaspirate to ACP double syringe with 2.4 mm adapter for micro fat.

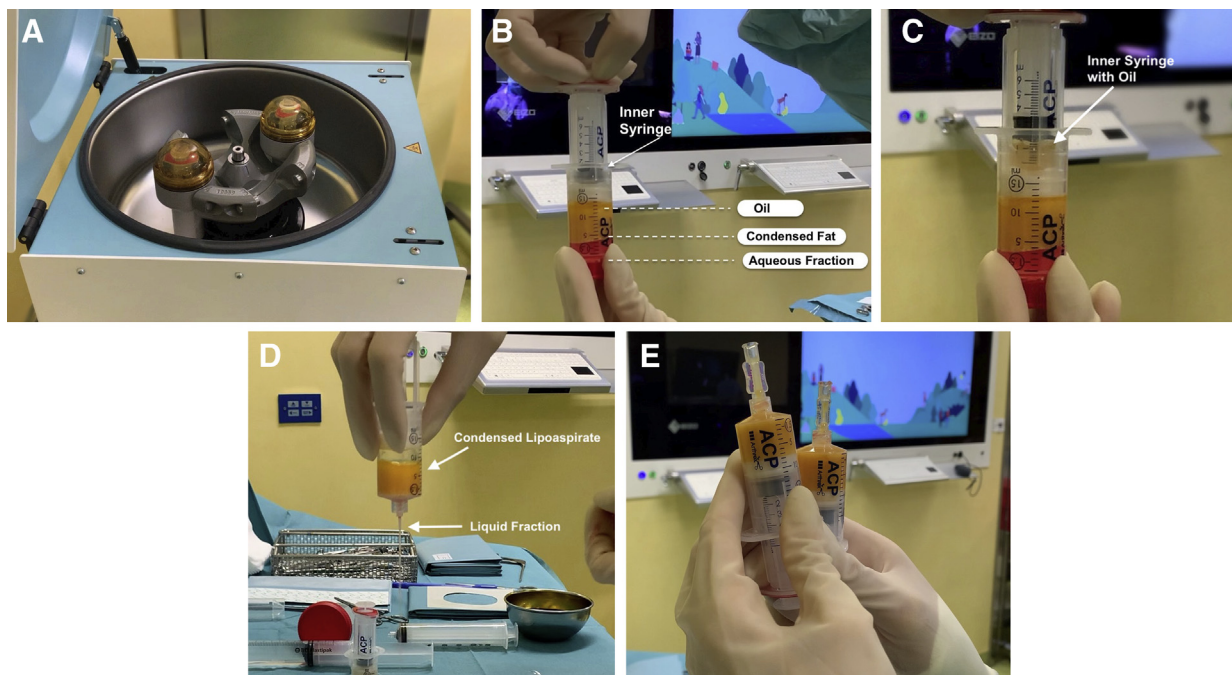
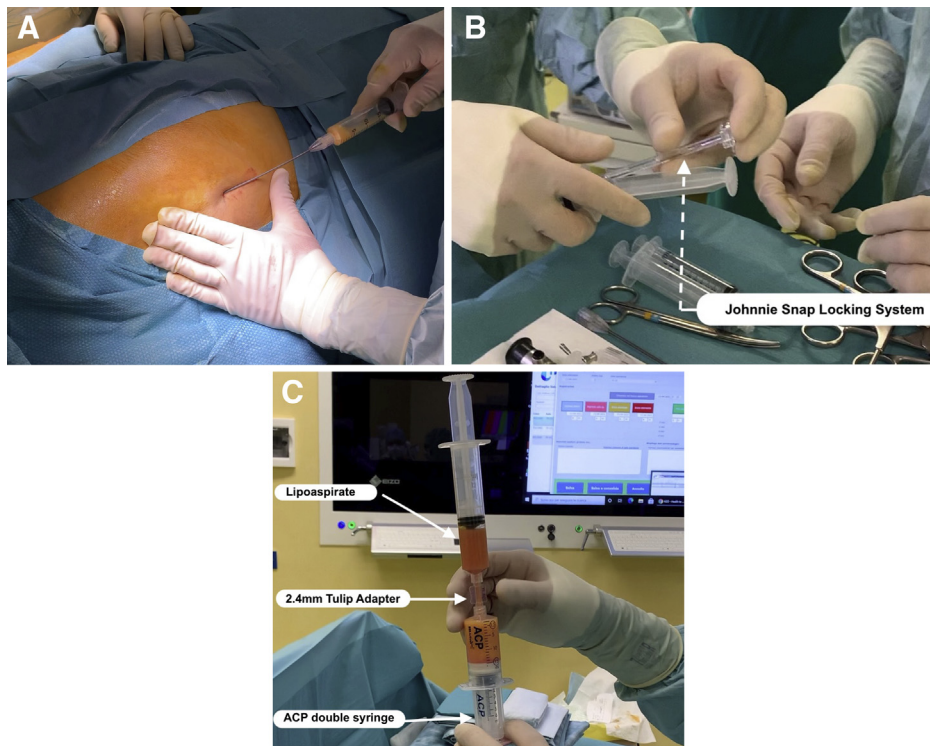


Fig 4. Platelets and adipose stroma combined for the treatment of the arthritic knee: Surgical technique fourth step: First-round centrifugation for adipose tissue processing. (A) Shows two ACP double syringes, with 15 mL of lipoaspirate, centrifuged at 2,500 rpm, 4 minutes at room temperature (Hettich Rotofix 32A, swing-out rotor, 220 V). (B) Shows the FRC product: upper oil fraction, middle condensed lipoaspirate, and lower aqueous fraction. (C) Upper oil fraction inner syringe extraction. (D) Aqueous fraction drainage. (E) Final condensed lipoaspirate.

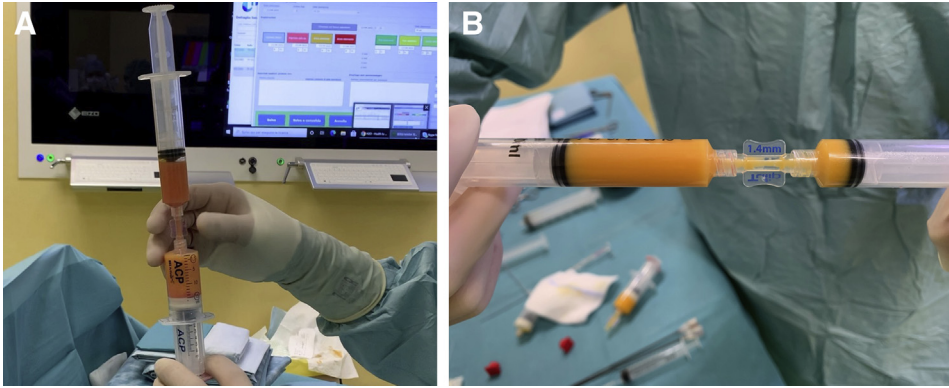


Fig 5. Platelets and adipose stroma combined for the treatment of the arthritic knee: Surgical technique fifth step. Mechanical adipose tissue fragmentation. (A) Lipoaspirate transfer from ACP system to a 10-mL syringe with a GEMS 2.4-mm tulip (B). Swooshing of condensed lipoaspirate in a 10-mL syringe connected by a GEMS 1.4-mm tulip.

Microfragmentation was performed by swooshing the condensed lipoaspirate 40 times forward and back over the GEMS transparent transfer, 1.4 mm (Fig 5B). The second round of centrifugation using the same parameters yielded three fractions: disrupted adipocytes turned into oil (65 vol%), 3 mL of tissue SVF (30 vol%), and a liquid fraction of the infiltration fluid with a small pellet (5 vol%) (Fig 6, A-D).

LP-PRP Preparation

The PRP was obtained after 15 mL of whole blood was drawn from the patient using the ACP double syringe and processed according to the manufacturer's

instructions in the same centrifuge at 1,500 rpm 340 g for 5 minutes at room temperature (Fig 7A). No citrate was added to the blood sample as the prepared PRP was injected within 10 minutes after preparation. Five cubic centimeters of PRP was obtained into the ACP double syringe system resulted in an LP-PRP with 2.7-fold, similar to other studies (Fig 7B).^{13,14} The PRP was not activated before injection.

Platelets and Adipose Stroma Combined Application

The final product was produced by combining 5 mL of LP-PRP and 1 mL of SVF into a 10-mL syringe (Fig 8A),

Fig 6. Platelets and adipose stroma combined for the treatment of the arthritic knee: Surgical technique sixth step: Second round centrifugation (SRC) for adipose tissue processing. (A) Lipoaspirate transfer back to ACP double syringe for the 2nd centrifugation at 2,500 rpm, for 4 minutes at room temperature. (B) SRC product: 65% oil, 30% of tissue stromal vascular fraction (SVF), and 5% liquid fraction of the infiltration fluid. (C) Oil transfer to the inner syringe for discard. (D) Final SVF fraction.

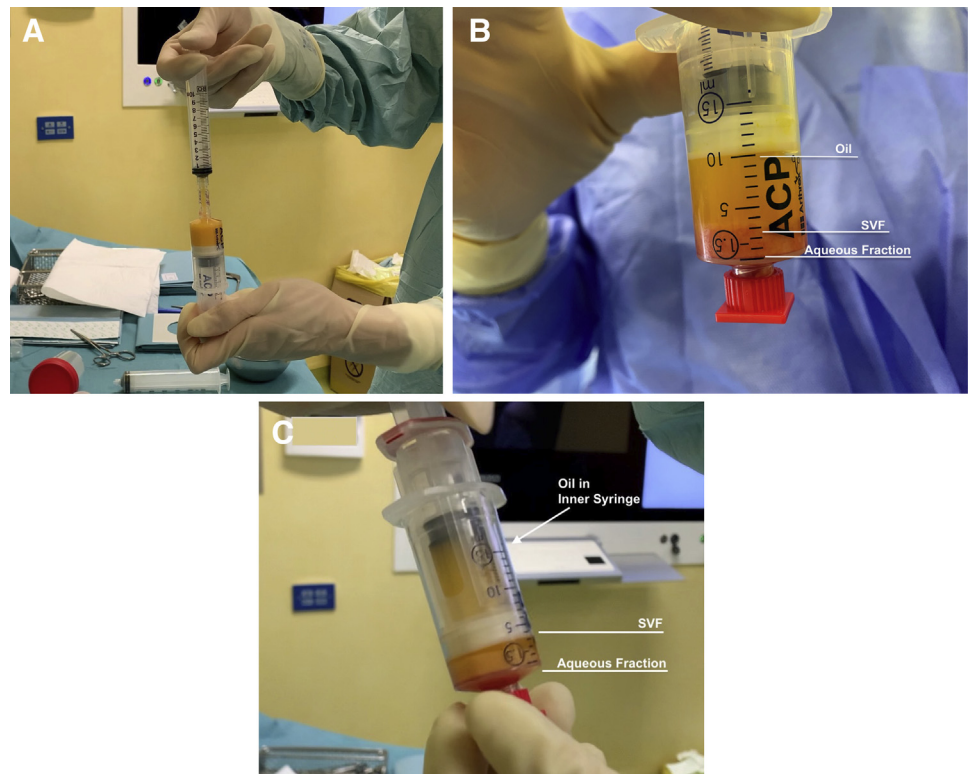
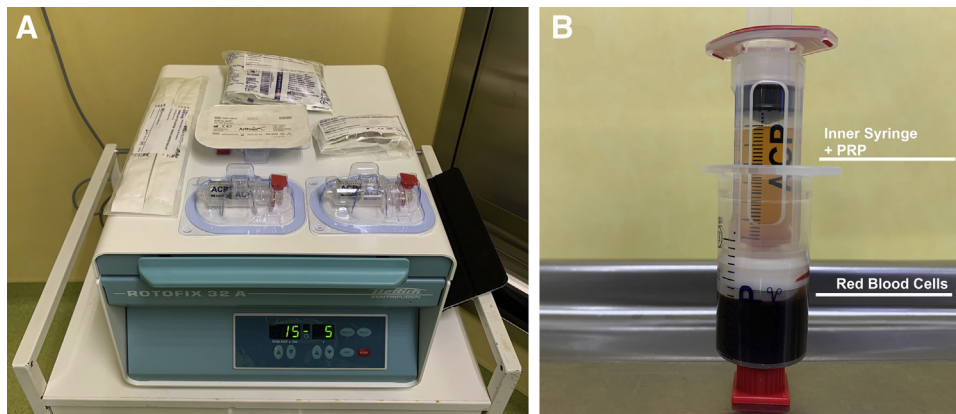


Fig 7. Platelets and adipose stroma combined for the treatment of the arthritic knee: Surgical technique seventh step: leucocyte-poor platelet-rich plasma (LP-PRP) processing. (A) Shows the 15 mL of whole blood autologous sample, centrifuged at 1,500 rpm for 5 minutes at room temperature (Hettich Roto-fix 32A, swing-out rotor, 220V). (B) ACP double system syringe product after centrifugation, with the inner syringe containing PRP.



gently emulsified and turned into 6 mL. Then it was injected into the knee joint through the AM portal under dry arthroscopy visualization after injecting 50 mL of air to create joint distension (Fig 8, B-D).

The gluteus harvest sites incision and knee portals were closed with one stitch using a synthetic absorbable suture, Ethicon Vicryl Rapide 3-0 (Johnson & Johnson International), followed by a sterile dressing.

Postprocedure

After the procedure, patients were allowed weight-bearing, and local ice application was recommended for 20 minutes every 2 to 3 hours for 24 hours.

Vigorous activities of the knee were discouraged for at least 48 hours.

Discussion

A standardized technique for SVF and PRP preparation and injection is reported in this technical note. Recent clinical studies have reported on the processing protocols and clinical applications of PRP and adipose-derived cell therapies in orthopaedic conditions. Most recent studies have reported lipoaspirate 60 mL of AMAT and a different concentration of

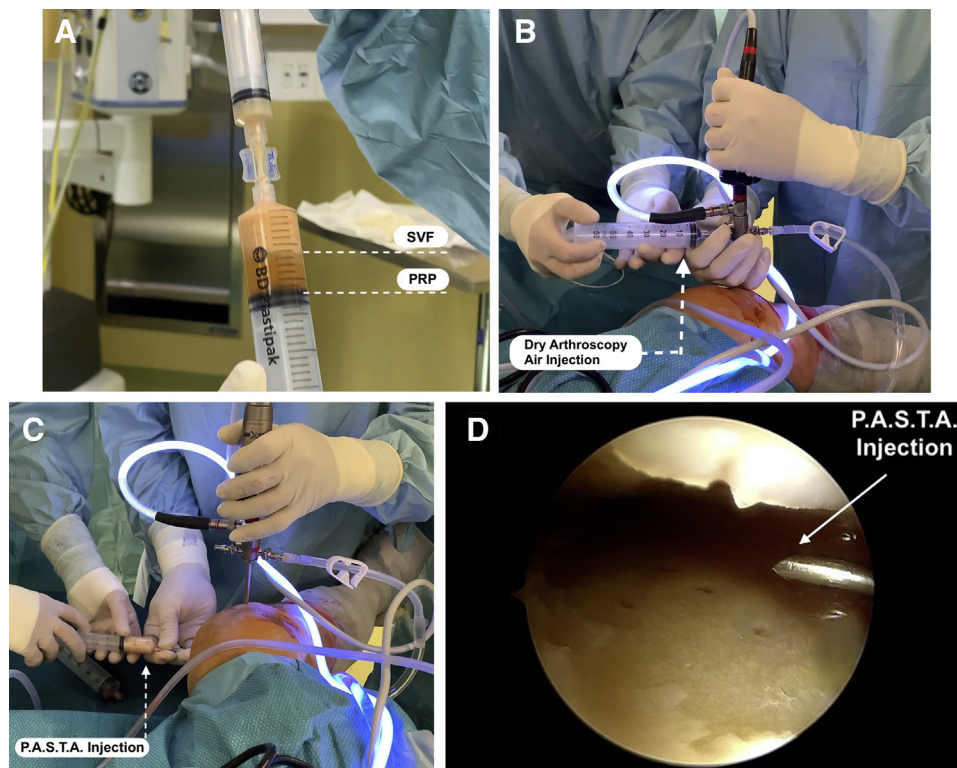


Fig 8. Platelets and adipose stroma combined for the treatment of the arthritic knee: surgical technique eighth step: product application. (A) Combination of 5mL of leucocyte-poor platelet-rich plasma (LP-PRP) and 3mL of SVF into a 10-mL syringe to create the final product. (B) 50-mL air injection for joint distension. (C) Combined solution injection under dry arthroscopy. (D) Arthroscopic anterolateral view of product injection through anteromedial portal.

platelet and leucocytes in the PRP for their clinical applications.^{1,2,9,13}

In the present technical note, 30 mL of autologous adipose tissue was lipoaspirate and later processed to obtain 1 mL of SVF. Regarding the PRP part, 15 mL of whole blood was drawn to get 5 mL of LP-PRP 2.7-fold and then mixed to obtain 6 mL of the final product.

Some studies report the use of activation of the PRP.¹³ The technique presented herein does not use activation before application.

Despite encouraging initial clinical studies, the optimal use for SVF plus PRP in knee OA has not been identified. Moreover, studies have been limited in identifying the component of SVF responsible for its desired clinical effects. The results may come from the new vision of medicinal signaling cells (MSCs) proposed by Caplan.⁶ It was hypothesized that MSCs, rather than participating in tissue formation, work as site-regulated "drugstores" in vivo by releasing trophic and immunomodulatory factors activated by local injury.^{6,7} Nonetheless, the mechanism by which MSCs produce their effect is still unclear.

The use of SVF in patients with OA in the knee has been scarcely delineated. Garza et al. published a double-blinded prospective randomized controlled clinical trial. Thirty-nine patients with symptomatic knee OA were eligible. They reported significantly decreased knee OA symptoms and pain at 6 months and one year.¹⁵ Recently, Gobbi et al., in a multicentric international study, shows that a single dose of microfragmented adipose tissue injection leads to clinical, functional, and quality of life improvement at two years in seventy-five elderly patients, in KL grades 2 to 4 of knee osteoarthritis.¹

This study highlights a potential treatment option more effective for patients with Kellgren-Lawrence grade 2 than for patients with advanced OA (Kellgren-Lawrence grade 4), indicating a relationship between AMAT administration effect size. The majority of studies reporting on AMAT and PRP application in patients with knee OA have reported good outcomes. These studies used different outcome scores and treatment protocols, making interstudy analysis difficult. Some have used adipose-derived cells harvested from the fat pad.⁸ Other studies harvest from the periumbilical fat.^{1,12,16} These studies used different outcome scores and treatment protocols, making interstudy analysis difficult. There is no consensus regarding the use of SVF, AMAT, or PRP injections. However, given the positive results of the studies mentioned earlier, further investigation is warranted. In patients with OA, improved outcomes after adipose-derived cell therapies or PRP injections have been reported; however, these studies used a variable number of treatments and had limited follow-up intervals.^{1,2,8}

There is also a lack of literature addressing the optimal augmentation method for SVF or AMAT applications. Platelet-rich plasma has emerged as a promising augmentation method given its positive healing effects in degenerative knee pathologies.¹⁷ The combination of SVF and PRP may provide an additive stimulatory effect to support angiogenesis and accelerate the wound-healing process.¹⁸ However, more studies are needed to elucidate the effects of platelet-rich plasma augmentation on SVF effectiveness.

Recent clinical evidence based on 78 randomized control trials (RCTs) comparing PRP to control found that PRP led to a significant reduction of knee OA pain.¹⁹

A new prospective comparative study by Dallo et al. examined the clinical efficacy of repeated doses of leucocyte-poor platelet-rich plasma (LP-PRP) plus hyaluronic acid (HA) to a single dose of autologous microfragmented adipose tissue (AMAT) injections in patients with early osteoarthritis symptoms. The study highlights the value of a combined biological approach in treating patients with osteoarthritis.¹²

Microfragmented adipose tissue, also known as adipose stromal vascular fraction (SVF) therapy, has gained recent popularity as a treatment. Adipose tissue has been reported to have larger quantities of different types of progenitor cells.⁵ Koh et al. analyzed the group of adipose-derived cells at two years. They noted that the whole-organ MRI score had significantly improved from 60.0 points to 48.3 points ($P < .001$), particularly in cartilage which improved from 28.3 points to 21.7 points.⁹ In another study of 30 patients with knee OA, Adriani et al. demonstrated significant improvements in pain, quality of life, and function at 12 months after ultrasound-guided injection of AMAT. Twelve males and 18 females; mean age of 63.3 years; mean body mass index of 25.1, and without prior treatment over the last 12 months. The patients were evaluated at baseline and 1, 3, 6, and 12 months after treatment using the visual analog scale (VAS) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). The average VAS was 7.7 at baseline and improved to 4.3 at a 3-month follow-up. However, a slight deterioration (VAS 5.0) was noted at 1 year. Total WOMAC score was 89.9 at baseline, 68.6 at 3 months, and 73.2 at 12-month follow-up.¹⁰ Recently, Russo et al. showed that clinical improvement using AMAT to treat diffuse degenerative knee OA was maintained at 3 years of follow-up.¹¹

The safety of MSC's injected with SVF and AMAT is of the utmost importance and remains an issue that requires further study. Nonetheless, the procedure is safe with a low rate of complications, such as hematoma and discomfort in the area of liposuction.²⁰ Self-limited pain and swelling were the most commonly reported

Table 1. Advantages and Disadvantages of the Technique

Advantages	Disadvantages
Source of growth factors	Limited proliferation and differentiation potential in comparison with pluripotent or expanded MSCs.
Microvascularized tissue with abundant pericytes and progenitor cells	Lack of consensus regarding preparation, doses, the timing of SVF and PRP
Synergistic effect	Very few clinical studies reporting the combination of SVF and PRP for OA
Minimal morbidity	Short-term clinical outcomes
Minimal manipulation minimizing the risk of infection.	Randomized control trials are needed to understand patient outcomes better
Immunomodulatory, antiapoptotic, and anti-inflammatory effects.	
No enzymes—mechanical processing whereby the SVF can be collected as a pellet	

MSCs, mesenchymal stem cells; OA, osteoarthritis; PRP, platelet-rich plasma; SVF, stromal vascular fraction.

adverse events at the injection site.^{1,9} Other studies are needed to develop a comprehensive SVF and AMAT safety profile. Advantages and disadvantages are discussed in Table 1.

In conclusion, a step-by-step biological approach, preparation, and subsequent injection are presented. (Table 2) Further study is necessary to clarify the number, volume, and timing of injections of this treatment for specific knee OA. Moreover, standardization of the techniques of obtaining and processing SVF and PRP is needed. The clinical role for the presented approach is noted, and we encourage the further

Table 2. Step-by-Step Process for Harvest and Processing

1. Skin preparation – Asepsis and anesthesia
2. Infusion of tumescent solution
3. Blood extraction: 15 mL of peripheral
4. Centrifugation to obtain 5 mL of LP-PRP
5. Fat tissue harvesting from the gluteus donor site
6. Transfer the fat from 2 syringes to 2 ACP syringes
7. Isolation of fat graft after 1st centrifugation
8. Processing: eliminating oil and liquid fraction
9. Microfragmentation with 2 ACP syringes
10. Isolation of SVF after 2nd centrifugation and the elimination of the oil
11. Homogenizing LP-PRP and SVF
12. Platelets and adipose stroma combined application—knee infiltration
13. The skin incisions are closed, followed by a sterile dressing

ACP, autologous conditioned plasma; LP-PRP, leukocyte-poor platelet-rich plasma; SVF, Stromal vascular fraction.

study of adipose-derived and LP-PRP mixed injections in patients with OA.

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