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A Review of Manual and Automated Stromal Vascular Fraction (SVF) Isolation Techniques

Muhamad Fahbel Ilham Jamra
Universitas Indonesia, Indonesia
jamrafahbel@gmail.com

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ABSTRACT

The stromal vascular fraction (SVF) is a heterogenous cell population isolated from adipose tissue that offers significant therapeutic potential in regenerative medicine. Efficient, safe, and standardized isolation methods are critical to ensuring consistent cell quality and optimal clinical outcomes at the point of care. This narrative review was conducted through a structured literature search of peer-reviewed studies published in major scientific databases, including PubMed, Scopus, and Web of Science. Articles were selected based on their relevance to SVF isolation techniques, reporting of cell yield, viability, safety, standardization, and suitability for clinical point-of-care applications. This review evaluates and compares conventional open-system isolation techniques against modern closed-system technologies. Conventional methods include enzymatic digestion (the "gold standard" for high yield and viability, such as the patented H-Remedy process) and mechanical processing (a faster, cost-effective alternative utilizing physical shear forces). These manual, lab-dependent methods are compared with closed systems, which range from automated enzymatic platforms (e.g., CellUnit, TGI 1000) to mechanical disruption devices (e.g., Lipogems, Rotating Blades system). Conventional enzymatic methods yield superior cell dissociation but face regulatory hurdles and contamination risks in open environments. Open mechanical methods offer regulatory compliance via "minimal manipulation" but suffer from lower yields and poor standardization. Conversely, closed-system technologies successfully minimize contamination and human error. Automated enzymatic devices yield high, reproducible cell counts, while closed mechanical systems preserve the extracellular matrix and vital intercellular connections, displaying robust cell viability and strong multipotent differentiation potential. The reviewed evidence indicates that closed-system technologies provide a more consistent and clinically applicable approach by combining sterility, reproducibility, and regulatory compliance while maintaining satisfactory cell recovery and functionality Transitioning to closed-system technologies addresses critical safety and regulatory challenges, providing a sterile, standardized, and GMP-ready pathway for effective point-of-care SVF clinical applications.

INTRODUCTION

The stromal vascular fraction (SVF) is defined as a heterogenous population of cells isolated from adipose tissue (Karina, Pawitan, et al., 2020; Karina, Rosliana, et al., 2020). Unlike purified and expanded mesenchymal stem cells (MSCs), SVF consists of a diverse population including adipose-derived stem cells (ADSCs), endothelial cells, pericytes, fibroblasts, macrophages, and other immune cells such as T cells and leukocytes (Rosadi et al., 2019). This distinctive composition allows complementary interactions between different cell types, which may enhance therapeutic outcomes through multiple mechanisms, including angiogenesis, immunomodulation, and the secretion of essential extracellular matrix (ECM) proteins (Min et al., 2022; Wu et al., 2025).

The clinical applications of fresh, point-of-care SVF have significantly expanded due to its ability to be isolated and administered in a single-day procedure, typically within two to three hours (Karina, Pawitan, et al., 2020; Williams et al., 2017). It had been reported that SVF is widely utilized

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in several clinical fields such as orthopedics, wound healing, and soft tissue reconstruction (Karina et al., 2023). Additionally, its therapeutic application has broadened to encompass metabolic conditions like type 2 diabetes (Karina et al., 2023), autoimmune disorders (Karina, Rosliana, et al., 2020), and neurological diseases, including autism (Karina et al., 2026).

There is a critical need for efficient, safe, and standardized isolation of SVF to ensure consistent clinical outcomes. Successful therapy often depends on delivering an optimal dose of viable cells to the target tissue to compensate for reduced biological functions in patients (Tsubosaka et al., 2021). However, achieving this requires standardized protocols, which are essential for maintaining cell quality across diverse patient demographics and varying pathological conditions (Cremona et al., 2023).

Conventional methods for SVF isolation are often lab-dependent, requiring specialized infrastructure, expensive consumables, and significant expertise. Furthermore, traditional open-system techniques, while effective in controlled environments, are open to potential contamination from external reagents or handling. These limitations have driven the development of closed system automated biomedical devices. These systems aim to simplify the procedure for non-experts, reduce costs, and provide a sterile, GMP-ready environment for point-of-care applications.

Although numerous studies have reported the effectiveness of individual SVF isolation techniques, the available literature remains fragmented, with most reports focusing on either enzymatic or mechanical approaches, or on specific commercial devices. Furthermore, previous reviews have primarily emphasized cell yield and viability outcomes without comprehensively evaluating how different isolation platforms influence standardization, sterility, regulatory compliance, workflow efficiency, and clinical applicability. As closed-system technologies continue to evolve and gain acceptance in regenerative medicine, a critical knowledge gap remains regarding the comparative advantages and limitations of conventional open-system methods versus modern closed-system platforms. A comprehensive synthesis of these approaches is therefore needed to support evidence-based selection of SVF isolation strategies for both research and clinical settings.

This review compares conventional isolation methods, including both open-system enzymatic digestion and mechanical processing, with modern closed systems, which range from semi-automated devices to fully closed, GMP-ready platforms. While enzymatic methods like the patented H-Remedy process have been optimized for high yield and viability in a laboratory setting (Moegni et al., 2019), closed systems provide standardized, sterile alternatives. Examples include mechanical closed systems like Lipogems, which process lipoaspirates using mild mechanical forces without chemical additives (Karina, Pawitan, et al., 2020), and automated enzymatic closed systems like the CellUnit SVF isolation system, which integrates digestion, washing, and centrifugation within a single sterile chamber (Koo et al., 2022; Min et al., 2022).

CONVENTIONAL (OPEN) SVF ISOLATION TECHNIQUES

Enzymatic Methods

Classic enzymatic isolation of SVF is considered as the “gold standard” for processing adipose tissue due to its high efficiency in disrupting the ECM. This biochemical approach allows for a more complete release of cells from their perivascular niches compared to physical disruption (Senesi et al., 2019). In an “open system” or manual laboratory setting, this process is meticulously performed using sterile containers, and syringes rather than fully automated, closed-circuit devices (Choudhery et al., 2025).

The primary agents utilized in this process are collagenases, predominantly type I or type IV. These enzymes are typically prepared in phosphate-buffered saline (PBS) solution containing calcium and magnesium ions (Ca^{2+}/Mg^{2+}), which are essential for activating the enzymatic activity required to hydrolyze the ECM (Choudhery et al., 2025). A significant innovation in this field is the patented H-Remedy recombinant enzyme, which is used in advanced protocols to standardize the isolation process

and ensure high cell recovery. Additionally, some proprietary reagents, such as the Matrase reagent used in specific kits, are employed to facilitate tissue digestion.

The manual isolation protocol involves several specific stages to ensure high cell yield and purity (Choudhery et al., 2025; Senesi et al., 2019). The freshly harvested lipoaspirate is washed multiple times (usually 3 – 5 times) with PBS to eliminate contaminants like blood, oil, and anesthetic debris. The cleaned tissue is then mixed with an equal volume of enzymatic solution and incubated at a physiological temperature of 37°C for 30 – 60 minutes (or up to 1 hour specifically for H-Remedy) (Karina et al., 2025). Periodic agitation is required during this phase to facilitate the release of cells from the stromal framework. Following digestion, the enzyme is neutralized using a serum-containing medium or a human albumin solution to prevent further proteolytic activity that could damage the cells. The suspension is centrifuged (typically at 300 – 600g for 5 – 15 minutes) to separate the SVF-enriched cellular pellet from the supernatant containing oil and floating adipocytes (Agostini et al., 2018). The resulting pellet is washed again to remove residual enzymes and passed through a sterile strainer (40 – 100 μm) to eliminate any remaining tissue fragments. The final SVF is resuspended in a physiologically compatible solution, such as saline, autologous plasma, or culture medium.

The most notable advantage of enzymatic processing is its superior yield and viability, consistently providing higher cell recovery and better dissociation compared to mechanical methods, with cell viability often exceeding 90%. It produces a predictable, standardized single-cell suspension that demonstrates strong colony-forming activity and robust trilineage differentiation potential. Furthermore, biochemical action effectively liberates cells from perivascular niches that mechanical forces often fail to reach.

However, this method faces significant regulatory hurdles, as it is often classified as “more-than-minimal manipulation” by organizations like the US Food and Drug Administration (FDA) and European Union, which can restrict its clinical application. There is also safety concern regarding residual enzymes the final product requires exhaustive washing to ensure no proteolytic agents remain to damage the patient’s host tissue. Finally, the process is time-consuming and cost-intensive, requiring expensive GMP-grade enzymes, specialized laboratory equipment, and increased labor compared to rapid mechanical techniques. Manual processing in an open environment also increases the risk of contamination compared to closed-system devices.

Mechanical Methods

The inclusion criteria consisted of original research articles, comparative studies, technical reports, and relevant review articles that provided methodological or clinical information on SVF isolation systems. Studies were excluded if they focused exclusively on unrelated stem cell sources, lacked sufficient methodological detail, did not report outcomes relevant to SVF quality or isolation performance, or were not available in English. Data were synthesized narratively by grouping the evidence into two major themes: classic mechanical isolation methods and closed-system SVF isolation technologies. Within each theme, findings were compared based on isolation principle, processing time, regulatory implications, contamination risk, cell yield, viability, immunophenotypic profile, and functional characteristics. Because of heterogeneity in protocols, devices, sample sources, and outcome reporting, meta-analysis was not performed.Mechanical isolation of the SVF is an alternative to enzymatic digestion that relies on physical disruption rather than biochemical agents to release regenerative cells from adipose tissue. This approach is increasingly favored in clinical settings because it typically falls under the “minimal manipulation” regulatory category, avoiding hurdles associated with using proteolytic enzymes like collagenase. While enzymatic methods remain the “gold standards” for high cell yield and viability, mechanical methods provide a faster, bedside-compatible strategy for obtaining therapeutic cell products (Uguten et al., 2024).

This method utilizes shear forces, filtration, or micro-fragmentation to break down the adipose tissue’s ECM. While procedures vary significantly among practitioners, a typical “open” mechanical isolation follows as structured sequence. It starts with washing the harvested lipoaspirate with PBS or normal saline to remove debris and infiltration fluid, this step often assisted by gravity decantation or sedimentation. The washed tissue is then subjected to physical force, such as inter-syringe shuffling or vibration, to break down the adipose clusters. The emulsified mixture frequently passed through a mesh

filter to remove large fragments and then centrifuged (typically between 1200g and 1600g for 6 – 10 minutes) to separate and concentrate the cells. The resulting pellet is resuspended, and in some protocols, it undergoes lysis or a second round of centrifugation to achieve a cleaner cellular fraction (Sforza et al., 2025).

Classic mechanical isolation methods offer several distinct advantages in clinical settings, primarily centered on regulatory compliance and procedural efficiency. As mentioned before, these methods are typically classified as "minimal manipulation" by the FDA, because they rely on physical force rather than chemical dissociation, which simplifies the path to clinical implementation. These systems are highly time-efficient, with many protocols requiring only 8 to 20 minutes for completion, compared to the 50 to 210 minutes necessary for enzymatic digestion (Uguten et al., 2024). This speed, combined with the removal of high-cost enzymatic reagents, makes mechanical isolation a much more cost-effective option. Furthermore, these techniques enhance safety by avoiding the potential risks and legal complexities associated with introducing foreign enzymes into the patient. A notable biological advantage is the preservation of the ECM and vital intercellular connections in "tissue-like SVF" (tSVF), which can enhance the regenerative secretome and prevent cells from and prevent cells from rapidly vacating the treatment site.

Despite these benefits, classic mechanical systems face significant drawbacks, most notably a lower total cell yield compared to enzymatic methods. Because physical force is less efficient at fully dissociating the dense adipose tissue matrix, the resulting cell counts are consistently lower. The "open" nature of these systems, involving manual transfers between syringes and exposure to the clinical environment, also introduce a higher risk of microbial contamination compared to automated closed devices. Additionally, there is a profound lack of standardization in mechanical protocols, where heterogeneity in the duration and intensity of applied forces, such as shuffling passes or vibration speed, may lead to significant variability in the quality and composition of the isolated fraction between practitioners Finally the repetitive physical stress used to disrupt the tissue can compromise cell integrity and the long-term proliferative potential of ADSCs (Mundluru et al., 2024).

CLOSED SYSTEM TECHNOLOGIES FOR SVF ISOLATION

The urgency of adopting closed-system technologies for SVF isolation is driven by the need to minimize contamination risks and bypass the severe regulatory and temporal burdens associated with ex vivo cell expansion. Standard laboratory isolation methods are often labor-intensive, prone to human error and require specialized clean-room environments that are not always available in a clinical setting. Closed-system technologies for the isolation of stromal vascular fraction (SVF) include several distinct devices designed to minimize contamination and regulatory burdens, ranging from fully automated enzymatic systems to mechanical disruption devices. The Cellunit and TGI 1000 are described as computer-controlled, fully automated systems that automate the entire process of washing, enzymatic digestion, and centrifugation (Min et al., 2022; Williams et al., 2017). In contrast, the Lipogems system is a simple, disposable device that utilizes mild mechanical forces in a manual but completely closed immersion system. The rotating blades (RBs) system technology integrates a surgeon-independent actuator (engine) to ensure consistent and reproducible mechanical fat disruption (Bianchi et al., 2013; Solodeev et al., 2023).

The operational principles of these devices fall into two primary categories: enzymatic digestion and mechanical isolation. Automated enzymatic systems like the Cellunit and TGI 1000 work by mixing harvested fat with proprietary or standard enzymes, such as collagenase, to disrupt the extracellular matrix and release SVF cells from adipocytes(Huh et al., 2025; Koo et al., 2022). Mechanical systems, such as Lipogems, work through mild tissue cluster size reduction (from 1–3.5 mm down to 0.2–0.8 mm) using stainless steel marbles and gravity-driven saline counterflow to wash away oil and blood residues while preserving the vascular stroma (Bianchi et al., 2013). The RBs system uses engine-driven rotating blades to inflict controlled shear forces, separating cell aggregates from the adipose tissue in under 15 minutes, which is significantly faster than the 45 – 90 minutes typically required for enzymatic systems (Solodeev et al., 2023).

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The results regarding cell yield, viability, and characterization vary across these technologies, though all generally produce high-quality, functional cells. Cell yields for the TGI 1000 average approximately 1 x 10^5 cells per cc of fat, while the RBs system achieves yields of roughly 2.01 x 10^5 nucleated cells per mL, which is comparable to clinical-grade enzymatic protocols. The Cellunit system has reported yields ranging from 2.7 x 10^5 to 9.7 x 10^5 cells/mL (Kim et al., 2020). Cell viability remains consistently high; the Cellunit reports approximately 83.4% viability, while Lipogems and TGI 1000 maintain viability levels near 100% or within FDA-recognized standards (Williams et al., 2017).

Characterization and functional testing demonstrate that these systems effectively isolate multipotent stem cells. Lipogems-derived SVF is notably enriched in pericytes (CD146+/CD90+/CD34-) and mesenchymal stem cells (CD90+/CD29+/CD34-), with a significantly lower percentage of hematopoietic elements compared to standard lipoaspirates. Isolated cells via the RBs system contain approximately 22.7% CD45-CD31-CD34+ stem cell progenitors, which is similar to enzymatic controls. Functionally, SVF from all these systems demonstrates the classic ability to differentiate into adipogenic, osteogenic, and chondrogenic lineages. Furthermore, Lipogems-derived cells have shown a significantly higher degree of vasculogenic gene expression (e.g., VEGF, HGF) compared to enzymatically isolated cells, possibly due to the preservation of the cell glycocalyx by avoiding digestive enzymes (Bianchi et al., 2013; Solodeev et al., 2023).

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