World Journal of Stem Cells

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World J Stem Cells 2024 August 26; 16(8): 784-798

DOI: 10.4252/wjsc.v16.i8.784

ISSN 1948-0210 (online)

MINIREVIEWS

Understanding and controlling the variables for stromal vascular fraction therapy

Naveen Jeyaraman, Sandeep Shrivastava, VR Ravi, Arulkumar Nallakumarasamy, Aditya Pundkar, Madhan Jeyaraman

Specialty type: Cell and tissue engineering

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade B, Grade C, Grade C, Grade D

Novelty: Grade B, Grade B, Grade B, Grade C

Creativity or Innovation: Grade B, Grade C, Grade C, Grade C

Scientific Significance: Grade B, Grade B, Grade B, Grade C

P-Reviewer: Li SC; Liu Y; Lu H; Mubarak M; Shen Y

Received: January 21, 2024

Revised: June 13, 2024

Accepted: July 25, 2024

Published online: August 26, 2024

Processing time: 217 Days and 15.6 Hours

Naveen Jeyaraman, Arulkumar Nallakumarasamy, Department of Orthopaedics, Datta Meghe Institute of Higher Education and Research, Wardha 442004, Maharashtra, India

Naveen Jeyaraman, VR Ravi, Arulkumar Nallakumarasamy, Madhan Jeyaraman, Department of Regenerative Medicine, Mother Cell Regenerative Centre, Tiruchirappalli 620017, Tamil Nadu, India

Sandeep Shrivastava, Aditya Pundkar, Department of Orthopaedics, Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education and Research, Wardha 442004, Maharashtra, India

Madhan Jeyaraman, Department of Orthopaedics, ACS Medical College and Hospital, Dr MGR Educational and Research Institute, Chennai 600077, Tamil Nadu, India

Corresponding author: Madhan Jeyaraman, MS, PhD, Assistant Professor, Research Associate, Department of Orthopaedics, ACS Medical College and Hospital, Dr MGR Educational and Research Institute, Velappanchavadi, Chennai 600077, Tamil Nadu, India.
madhanjeyaraman@gmail.com

Abstract

In regenerative medicine, the isolation of mesenchymal stromal cells (MSCs) from the adipose tissue's stromal vascular fraction (SVF) is a critical area of study. Our review meticulously examines the isolation process of MSCs, starting with the extraction of adipose tissue. The choice of liposuction technique, anatomical site, and immediate processing are essential to maintain cell functionality. We delve into the intricacies of enzymatic digestion, emphasizing the fine-tuning of enzyme concentrations to maximize cell yield while preventing harm. The review then outlines the filtration and centrifugation techniques necessary for isolating a purified SVF, alongside cell viability assessments like flow cytometry, which are vital for confirming the efficacy of the isolated MSCs. We discuss the advantages and drawbacks of using autologous *vs* allogeneic SVF sources, touching upon immunocompatibility and logistical considerations, as well as the variability inherent in donor-derived cells. Anesthesia choices, the selection between hypodermic needles *vs* liposuction cannulas, and the role of adipose tissue lysers in achieving cellular dissociation are evaluated for their impact on SVF isolation. Centrifugation protocols are also analyzed for their part in ensuring the integrity of the SVF. The necessity for standardized MSC isolation protocols is highlighted,

promoting reproducibility and successful clinical application. We encourage ongoing research to deepen the understanding of MSC biology and therapeutic action, aiming to further the field of regenerative medicine. The review concludes with a call for rigorous research, interdisciplinary collaboration, and strict adherence to ethical and regulatory standards to safeguard patient safety and optimize treatment outcomes with MSCs.

Key Words: Mesenchymal stromal cells; Stromal vascular fraction; Adipose tissue; Autologous stromal vascular fraction; Stromal vascular fraction isolation

Core Tip: Stromal vascular fraction isolation is essential for extracting mesenchymal stromal cells (MSCs) in regenerative medicine. Optimizing this process requires improved liposuction techniques, immediate processing, precise enzymatic digestion, and efficient filtration and centrifugation. Quality control is verified through flow cytometry to ensure cell viability and purity. The necessity for standardized MSC isolation protocols is emphasized to ensure reproducibility and clinical success.

Citation: Jeyaraman N, Shrivastava S, Ravi V, Nallakumarasamy A, Pundkar A, Jeyaraman M. Understanding and controlling the variables for stromal vascular fraction therapy. *World J Stem Cells* 2024; 16(8): 784-798
URL: https://www.wjgnet.com/1948-0210/full/v16/i8/784.htm
DOI: https://dx.doi.org/10.4252/wjsc.v16.i8.784

INTRODUCTION

The extraction of mesenchymal stromal cells (MSCs) from stromal vascular fraction (SVF) is a cornerstone in the burgeoning field of regenerative medicine. The therapeutic efficacy of interventions utilizing MSCs is critically dependent on the successful isolation of these cells in terms of both quality and quantity[1]. SVF, sourced from adipose tissue, is a heterogeneous cellular conglomerate that provides a repository for MSCs, celebrated for their intrinsic regenerative capabilities. The complex process of MSC isolation from SVF demands a comprehensive understanding of various influential factors to optimize cell yield and ensure the development of effective regenerative therapies.

The technique of adipose tissue extraction through liposuction is pivotal in preserving the integrity and viability of the SVF[2-5]. The selection of a liposuction method that reduces adipocyte trauma is essential for maintaining a cell composition conducive to a substantial yield of MSCs. The choice of anatomical site for tissue harvest is also significant, as different regions may vary in MSC concentrations, affecting their regenerative potential. The critical time interval between adipose tissue collection and the initiation of SVF isolation inversely affects cell viability, necessitating optimized processing times to maintain MSC functionality[6-8]. Control of temperature during processing and transport is equally crucial to avert cell damage and maximize MSC yield.

Enzymatic digestion is a pivotal step in SVF isolation, and the choice and balance of enzymes, such as collagenase and dispase, are vital[8]. This stage must be meticulously controlled to prevent cellular damage that would reduce MSC viability and yield. The subsequent separation of SVF from the digested tissue matrix, utilizing precise filtration and centrifugation parameters, is critical for MSC recovery[9]. The washing and resuspension of isolated cells are further essential steps, requiring the use of isotonic buffers and appropriate culture media to support cell survival and maximize MSC yield[9]. Assessing cell viability and purity is fundamental, with techniques like the trypan blue exclusion test and flow cytometry providing crucial metrics of MSC quality[10]. The culture conditions post-isolation, including growth media composition, supplemental growth factors, and incubator environmental conditions, significantly impact MSC proliferation and yield[11]. The inherent biological variability among donors adds another dimension of complexity to MSC yield and quality, indicating the potential need for personalized isolation protocols[12].

This narrative review aims to systematically dissect the multifaceted process of MSC isolation from SVF, exploring the impact of each stage on the yield and viability of MSCs. It will delve into current methodologies and technologies, assess the influence of procedural variables, and consider donor variability on the quality of MSCs isolated. Furthermore, this review seeks to underscore the necessity of standardizing protocols to harness the full therapeutic potential of MSCs. Through this lens, the objectives of this review are to consolidate existing knowledge, identify gaps in the current understanding, and suggest directions for future research to refine MSC isolation techniques, thus facilitating the transition of MSCs from laboratory research to clinical application.

ADIPOSE TISSUE BIOLOGY

Adipose tissue, an intricate and specialized form of connective tissue, plays a quintessential role in the physiological landscape of mammals, offering a multifaceted suite of functions that transcend mere fat storage. Within this tissue, a

complex network comprising adipocytes, immune cells, vasculature, and an extracellular matrix (ECM) orchestrates a series of critical bodily functions. These include the regulation of energy balance, the secretion of hormones, the provision of thermal insulation, and the mechanical protection of organs. The primary cellular constituents of adipose tissue are the adipocytes, which are specialized in the storage and liberation of energy as triglycerides. These cells are structurally defined by a singular lipid droplet that occupies the majority of the cytoplasm, dynamically expanding and contracting in response to the organism's metabolic demands[11]. This capability to modulate lipid storage and mobilization positions adipocytes as pivotal actors in the maintenance of energy homeostasis, particularly evident during fluctuations in caloric intake and expenditure.

The dichotomy within adipose tissue is characterized by the distinction between white adipose tissue (WAT) and brown adipose tissue (BAT), which are anatomically and functionally distinct entities. WAT, the predominant form, is primarily tasked with energy storage. Its distribution is extensive, encompassing subcutaneous layers that insulate the body and visceral depots that enshroud internal organs, thereby serving as an energy reservoir. Beyond its storage capacity, WAT exerts profound endocrine effects through the secretion of adipokines, hormones that actively participate in the regulation of metabolic pathways, immune responses, and appetite[12]. In contrast, BAT serves a specialized function in thermoregulation, primarily through the process of non-shivering thermogenesis. This heat generation is a critical physiological response, especially in neonates and hibernating species, where maintenance of body temperature is vital for survival. The brown coloration of this tissue type is attributed to its high mitochondrial content, with mitochondria being rich in iron-containing cytochromes[12].

The dynamic nature of adipose tissue is further exemplified by its ability to remodel and adapt. Factors such as diet, physical activity levels, and hormonal milieu can precipitate structural changes within the tissue, manifesting either as hypertrophy of existing adipocytes or as hyperplasia, the formation of new fat cells[13]. In certain instances, this remodeling process involves the "browning" of white adipocytes, where they begin to mimic the thermogenic properties of brown adipocytes, a phenomenon that has garnered interest for its potential implications in energy expenditure and obesity management.

Adipose tissue's endocrine function is underscored by its role in the secretion of various adipokines, which have systemic effects on multiple organ systems[14]. These hormones play an instrumental role in maintaining metabolic equilibrium, influencing processes such as glucose uptake, fat oxidation, and appetite control. Disruption in the homeostatic secretion of adipokines has been implicated in the pathogenesis of metabolic disorders, highlighting the critical role of adipose tissue in the broader context of metabolic health. Within this complex tissue resides a reservoir of adipose-derived stem cells (ADSCs), which exhibit the potential to differentiate into a variety of cell types, including but not limited to, adipocytes, chondrocytes, and osteoblasts[15]. This multipotent characteristic of ADSCs, combined with their relative abundance and accessibility, renders them an attractive candidate for therapeutic applications in the burgeoning field of regenerative medicine. The regulation of adipose tissue is pivotal for metabolic health, with imbalances in this system contributing to the pathophysiology of obesity. Excessive accumulation of adipose tissue, particularly WAT, is associated with a spectrum of adverse health outcomes, including cardiovascular diseases, type 2 diabetes, and certain cancers. Conversely, therapeutic strategies that promote the activation of BAT or the browning of WAT have shown promise in combatting metabolic syndrome by increasing basal energy expenditure.

The exploration of adipose tissue biology is thus a field of immense importance, bearing significant implications for public health. The intricate mechanisms governing adipose tissue function are a subject of intensive study, promising to unveil a new understanding of obesity and metabolic diseases. This, in turn, is anticipated to catalyze the development of novel therapeutic interventions, tailored to exploit the regenerative and metabolic potential of adipose tissue in disease amelioration and prevention. Having explored the fundamental biology of adipose tissue, we now turn our attention to the specific regenerative products that can be derived from this versatile tissue source. These products form the basis for various therapeutic applications in regenerative medicine.

REGENERATIVE PRODUCTS OF ADIPOSE TISSUE

Adipose tissue, traditionally viewed as a passive storage site for triglycerides, has been reconceptualized as a dynamic and versatile source of regenerative materials. Its diverse cellular composition and rich milieu of bioactive compounds have positioned it at the forefront of regenerative medicine, offering a spectrum of applications from tissue repair to modulating inflammatory responses[13]. The therapeutic regenerative products derived from adipose tissue are tabulated in Table 1[16-22]. The regenerative potential of adipose tissue is primarily determined by ADSCs, which possess multipotent characteristics and can differentiate into various cell types including adipocytes, chondrocytes, osteoblasts, and endothelial cells[23,24]. These cells are not only remarkable for their differentiation capacity but also for their ease of harvest and subsequent expansion, which has spurred extensive research into their application in tissue engineering and cell-based therapies. The versatility of ADSCs lends itself to a myriad of regenerative medicine applications, from reconstructive surgeries to the treatment of chronic wounds, underscoring their significance as a therapeutic tool.

SVF represents another regenerative product obtained from adipose tissue[25]. This heterogeneous cell population, isolated through enzymatic digestion and centrifugation, encompasses a variety of cell types including ADSCs, endothelial progenitor cells, pericytes, fibroblasts, and immune cells. The SVF is particularly valued for its high content of growth factors and its ability to promote repair and regeneration across various tissue types. SVF acts *via* paracrine signaling by secreting growth factors, cytokines, and extracellular vesicles (EVs)[26]. It plays a crucial role in immunomodulation by secreting regenerative factors, modulating inflammation, and promoting tissue repair, including cartilage regeneration[27,28]. The chondrogenic differentiation of SVF cells enhances cartilage regeneration and supports

Table 1 Therapeutic regenerative products derived from adipose tissue

Regenerative product from adipose tissue	Description	Potential applications
Adipose-derived stem cells[16,17]	Multipotent stem cells capable of differentiating into multiple cell types	Tissue engineering, cell-based therapies
Stromal vascular fraction[18]	A heterogeneous cell population, including adipose-derived stem cells and other cell types	Wound healing, tissue repair, regenerative treatments
Extracellular vesicles[19]	Vesicles contain bioactive molecules like proteins and nucleic acids	Angiogenesis, immunomodulation, tissue healing
Adipokines[20]	Hormones and signaling molecules secreted by adipose tissue	Regulation of appetite, insulin sensitivity, inflammation, and lipid metabolism
ECM scaffolds[21]	Decellularized scaffolds maintain adipose tissue's structural components	Tissue engineering, support for tissue regeneration
Microfat and nanofat[22]	Adipose tissue forms rich in regenerative cells and growth factors	Soft tissue augmentation, wound healing, tissue repair

ECM: Extracellular matrix.

subchondral bone regeneration through the production of collagen type I[29]. Additionally, SVF mechanisms involve angiogenesis, where ADSCs produce vascular endothelial growth factor for migration and platelet-derived growth factor for proliferation, which supports endothelial cells in forming vasculature-like structures[25]. Furthermore, SVF contains multiple cell populations, including pericytes and ADSCs, which release growth factors and ECM particles, contributing to its regenerative potential[30,31]. These multifaceted actions of SVF underline its capability to mediate tissue repair, regulate inflammatory and immune responses, and promote chondrocyte proliferation and chondrogenesis[28,32]. Its application in wound healing and tissue repair is particularly promising, offering a minimally invasive option to stimulate the body's intrinsic repair mechanisms.

EVs derived from adipose tissue are yet another avenue through which adipose tissue contributes to regenerative processes[33]. Comprising exosomes and microvesicles, these EVs are laden with proteins, lipids, and nucleic acids that facilitate intercellular communication. Their role in regeneration is attributed to their ability to carry and transfer these bioactive molecules to target cells, thereby initiating processes such as angiogenesis, immunomodulation, and tissue repair. The therapeutic potential of adipose-derived EVs is vast, with research delving into their ability to enhance the body's repair mechanisms in a targeted and controlled manner.

The role of adipose tissue as an endocrine organ is characterized by its secretion of adipokines[34]. These signaling molecules, including adiponectin, leptin, and resistin, have been implicated in a host of physiological processes that extend to the regulation of appetite, insulin sensitivity, and lipid metabolism. Their contribution to regenerative processes, particularly tissue repair and angiogenesis, opens new therapeutic avenues, particularly in conditions where these processes are compromised, such as in diabetic wound healing. The development of adipose tissue-derived ECM scaffolds has introduced a novel approach to tissue engineering[35]. These scaffolds, created through the decellularization of adipose tissue, maintain the complex composition of growth factors and structural proteins inherent to the native tissue. They provide a conducive environment for cell attachment, proliferation, and differentiation, essential for the regeneration of damaged tissues.

The refinement of adipose tissue into microfat and nanofat grafts has expanded the utility of adipose tissue in regenerative medicine[36]. These preparations are rich in regenerative cells and growth factors and have shown effectiveness in soft tissue augmentation, wound healing, and tissue repair. The process of creating microfat and nanofat involves emulsification and filtration, which not only concentrates the regenerative components but also renders the fat more malleable for precise applications. The regenerative products derived from adipose tissue encapsulate a promising and rapidly evolving area of research, offering therapeutic possibilities that were previously unattainable. These products, each with their unique properties and mechanisms, present a diverse toolkit for clinicians and researchers aimed at promoting tissue repair, regenerating damaged tissues, and ultimately improving patient outcomes. As the field advances, ongoing research will be paramount to fully elucidate the mechanisms of action of these regenerative products and to optimize their clinical applications, thereby harnessing the full therapeutic potential of adipose tissue in regenerative medicine. To better understand the complexities of SVF isolation. Figure 1 illustrates the key variables that influence this process. Various methods of lipolysis for SVF-MSC isolation are depicted in Figure 2.

AUTOLOGOUS SOURCE OF SVF

The sourcing of SVF from autologous or allogeneic donors is a critical consideration in the field of regenerative medicine, each with distinct advantages and challenges. Autologous SVF, harvested from the patient's adipose tissue, is a cornerstone in tissue engineering due to its inherent compatibility with the recipient's immune system. In the context of autologous SVF, the process begins with the extraction of adipose tissue from the patient, typically through liposuction

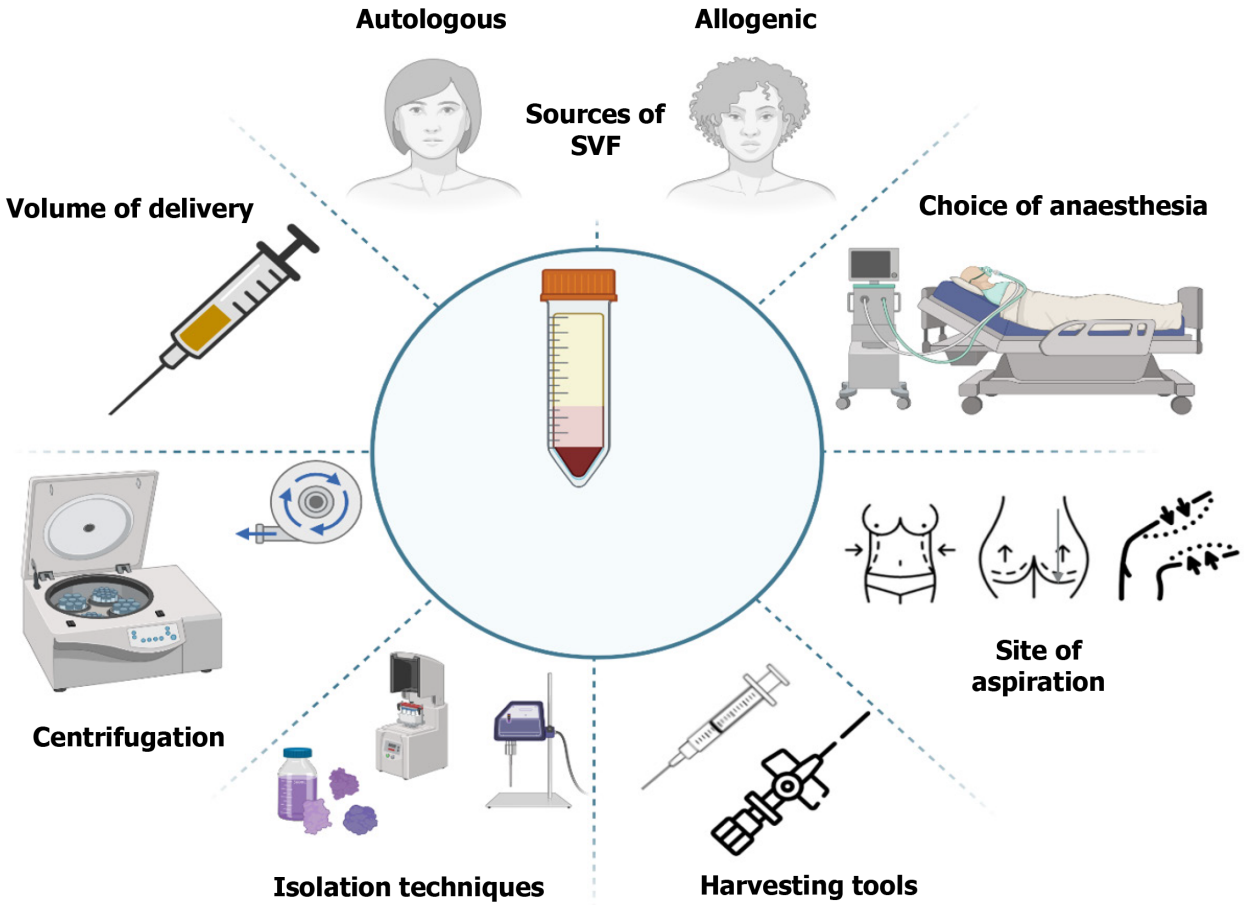

Figure 1 Variables of mesenchymal stem cell isolation in stromal vascular fraction derived from adipose tissue. SVF: Stromal vascular fraction.

[37]. This procedure, while minimally invasive, is a surgical intervention that requires careful consideration of the patient's overall health and comfort. Once the adipose tissue is collected, it undergoes processing to isolate the SVF. While the immunological advantages of autologous SVF are clear, there are practical considerations that must be weighed when choosing this approach.

The advantages of autologous SVF are rooted in its immunological profile, being derived from the patient's cells, thereby virtually eliminating the risk of immune rejection. This biocompatibility is of paramount importance in cell transplantation, as it ensures the integration of the transplanted cells without eliciting an adverse immune response. Furthermore, the autologous route circumvents the risks associated with disease transmission from donor to recipient, a concern particularly relevant in the context of infectious pathogens. Autologous SVF also allows for the possibility of personalized therapies. The cells can be manipulated or expanded *in vitro* to enhance their regenerative properties or to tailor them to address specific patient needs. This bespoke approach holds the potential for more effective and targeted regenerative outcomes.

However, the approach is not without its limitations. The requirement of a liposuction procedure for tissue harvesting introduces additional complexity to the treatment regimen. This step not only poses surgical risks but also may not be well-received by all patients, particularly those who are averse to invasive procedures or those with conditions that elevate surgical risk. Moreover, the variability in SVF yield and cellular potency is a significant factor; these attributes are influenced by the patient's age, comorbidities, lifestyle, and the intrinsic quality of the adipose tissue. Such variability necessitates a careful assessment of the harvested tissue's viability and may require subsequent interventions to secure a sufficient quantity of therapeutically effective cells.

In weighing the benefits and drawbacks of autologous SVF, it is essential to consider the individualized nature of the treatment. Personalized medicine, while offering tailored solutions, must also reconcile the practicality of such approaches, balancing the procedural demands with the expected therapeutic benefits. The development of standardized protocols for the harvesting, processing, and application of autologous SVF is therefore a critical step forward, ensuring the reproducibility and efficacy of regenerative therapies. As the field evolves, the continuous refinement of these techniques and a deeper understanding of the biological behavior of SVF will be instrumental in maximizing the potential of autologous cell therapies in regenerative medicine.

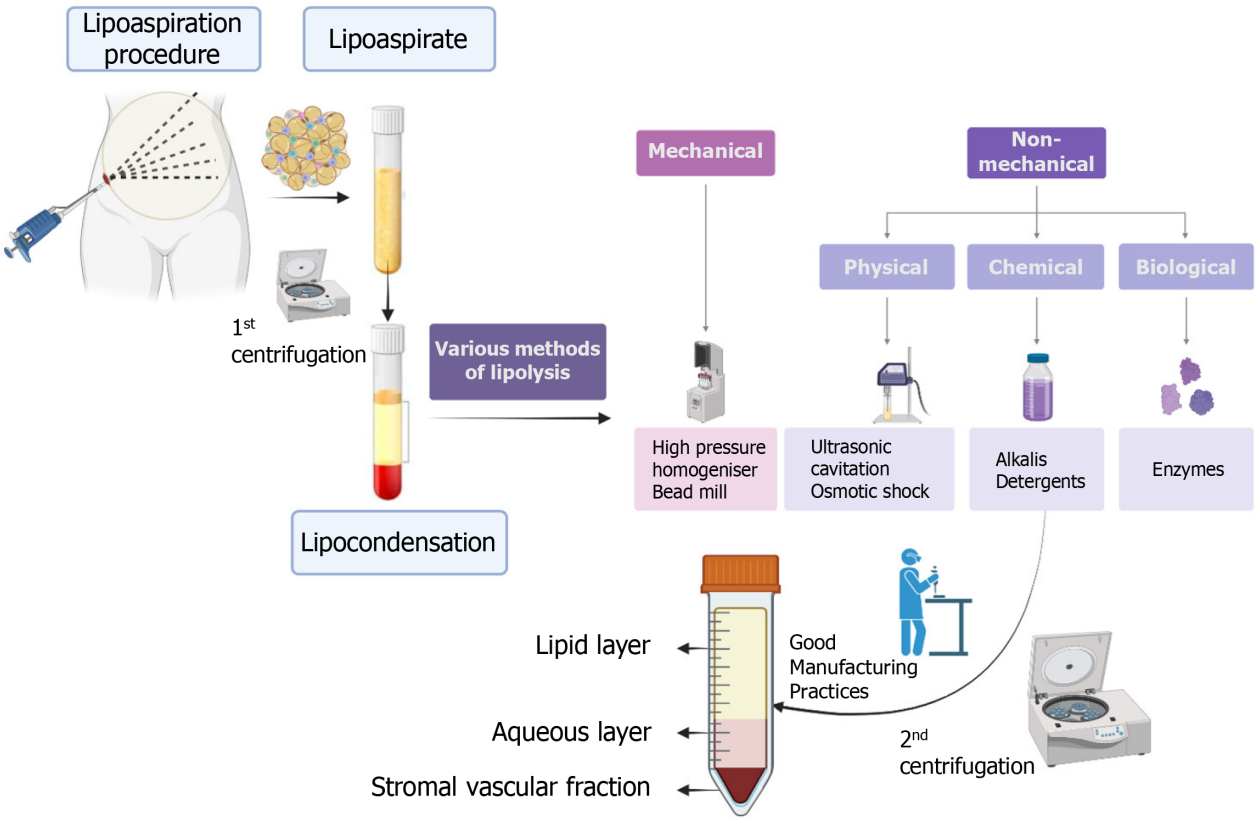

Figure 2 Various methods of lipolysis for mesenchymal stem cell isolation in stromal vascular fraction derived from adipose tissue.

ALLOGENIC SOURCE OF SVF

Allogeneic SVF represents a paradigm shift in the availability and application of regenerative cellular therapies, where SVF is sourced from donors rather than the recipients themselves. This approach leverages the adipose tissue of healthy individuals, which upon extraction and processing, yields SVF that can be administered therapeutically to patients. The allogeneic SVF, once isolated, can be cryopreserved, thus offering a stockpile of regenerative material that can be tapped into as needed[38]. This method stands out for its logistical efficiency, providing a uniform and on-demand supply of SVF, obviating the need for individualized tissue harvesting through surgical procedures.

The benefits of using allogeneic SVF are multifaceted. Primarily, it eliminates the necessity for each patient to undergo a surgical procedure for adipose tissue extraction, thereby reducing the overall invasiveness of the treatment and the associated risks of surgery. Furthermore, it assures a steady and consistent source of SVF, which is particularly advantageous when treating multiple patients or in scenarios where immediate treatment is required. Allogeneic SVF is also a vital resource for patients who may not have an adequate amount of adipose tissue for autologous SVF extraction, such as those with low body fat or certain medical conditions. Despite these advantages, allogeneic SVF is not without potential drawbacks. The risk of immune rejection is a concern, given the possible disparity in human leukocyte antigens between the donor and the recipient, which can provoke an immunological response. Moreover, the transmission of diseases remains a risk factor that necessitates rigorous donor screening, testing protocols, and adherence to stringent regulatory standards to mitigate potential complications.

When considering the use of autologous *vs* allogeneic SVF, the decision-making process must integrate an assessment of the risks and benefits tailored to the individual patient's condition and the intended therapeutic application. While autologous SVF is typically favored for its reduced risk of immune complications, allogeneic SVF offers practical benefits in contexts where autologous tissue is not viable or when there is a need for large-scale or immediate treatment regimens. The employment of allogeneic SVF, therefore, requires a comprehensive framework for donor selection, tissue processing, and quality control to maximize safety and therapeutic efficacy. This holistic approach ensures that the potential of allogeneic SVF can be harnessed effectively, expanding the scope of regenerative medicine. The comparative analysis of autologous *vs* allogenic SVF sources is tabulated in Table 2.

CHOICE OF ANESTHESIA

The management of anesthesia during the procurement of SVF for regenerative medical applications is a nuanced area that currently lacks uniform guidelines. The options of anesthesia for SVF isolation procedures are written in Table 3[39-43]. This absence of consensus leaves the choice of anesthesia to be tailored to the specifics of the SVF isolation procedure,

Table 2 Comparative analysis of autologous *vs* allogenic stromal vascular fraction sources

SVF source	Advantages	Disadvantages
Autologous SVF	Eliminates immune rejection. No donor disease transmission. Enables personalized treatments	Requires surgical adipose extraction. Variable SVF quality and quantity
Allogenic SVF	Avoids surgery for each patient. Steady, ready supply. Beneficial for patients with scant adipose tissue	Possible immune response. Risk of disease if donor screening is lax

SVF: Stromal vascular fraction.

Table 3 Anesthesia options for stromal vascular fraction isolation procedures

Anesthesia type	Description	Application in SVF isolation
Local anesthesia [39]	Involves injecting anesthetic agents like lidocaine at the harvest site. Patients remain conscious and have minimal systemic side effects	Preferred for small-scale SVF isolation; ensures patient comfort and cooperation
Tumescent anesthesia[40]	Diluted lidocaine with epinephrine and saline is injected to anesthetize, minimize bleeding, and ease tissue harvest	Common in liposuction to obtain adipose tissue; provides extended anesthesia and vasoconstriction
General anesthesia [41-43]	Induces complete sedation and unconsciousness, used in comprehensive surgical settings	Rarely used solely for SVF isolation; considered if combined with other surgical interventions

SVF: Stromal vascular fraction.

the patient's health profile, and the combined judgment of the patient and the medical team. During SVF isolation, adipose tissue is harvested primarily from subcutaneous fat depots. This process is essential for accessing regenerative cells such as ADSCs, which are integral to subsequent therapeutic applications. The anesthetic techniques adopted for this procedure vary, with local anesthesia being a preferred method due to its simplicity and direct action.

Local anesthesia involves injecting anesthetic agents into the tissue extraction site and numbing the specific area to be treated. This localized approach offers several advantages - it not only provides effective pain control during the procedure but also allows patients to remain awake, which can be critical for monitoring their well-being throughout the process. This method is associated with a favorable tolerance profile, as it minimizes the patient's exposure to systemic anesthetic agents and the related side effects. Tumescent anesthesia, a derivative of local anesthesia, expands upon its basic principle by infusing a larger volume of a dilute anesthetic solution into the subcutaneous tissue. This solution typically consists of lidocaine, epinephrine, and saline, and serves multiple functions. The lidocaine provides local anesthesia, the epinephrine induces vasoconstriction to minimize bleeding, and the saline facilitates the mechanical process of adipose tissue extraction. This technique is particularly valued in liposuction, a common method for harvesting adipose tissue, and by extension, is often employed in SVF isolation.

General anesthesia is seldom used for SVF isolation alone, primarily due to its comprehensive nature, which renders the patient completely unconscious. This type of anesthesia is generally reserved for procedures that are either too painful or too complex to perform under local or tumescent anesthesia. When SVF isolation is performed in conjunction with other, more invasive surgical procedures, general anesthesia may become necessary to ensure patient comfort and procedural efficiency. The selection of an anesthetic regimen is a multifaceted decision that hinges on several factors. The complexity of the procedure, the anticipated duration, the patient's medical and surgical history, and the presence of comorbidities all play critical roles in this decision-making process. Additionally, patient preference is paramount; some may favor local anesthesia to avoid the risks and recovery associated with general anesthesia, while others may opt for complete sedation due to anxiety or discomfort with being awake during the procedure.

In practice, local or tumescent anesthesia is often the method of choice for SVF isolation, striking a balance between minimizing procedural pain and reducing the risk of systemic complications associated with anesthesia. These approaches ensure that the patient remains comfortable and cooperative, which is especially important in outpatient or office settings where rapid recovery post-procedure is advantageous. The determination of the most suitable anesthetic approach for SVF isolation should be a collaborative endeavor, engaging the patient, the surgeon, and the anesthesiologist. This collaborative approach is essential for aligning the anesthetic plan with the patient's needs, and procedural requirements, and ensuring the highest standards of safety and care. As the field of regenerative medicine continues to evolve, so too will the strategies for anesthesia management, to optimize patient experiences and outcomes in SVF isolation and application.

SITE OF ASPIRATION

The aspiration of SVF is a medical procedure integral to regenerative medicine, wherein subcutaneous adipose tissue is the primary target for harvesting[44]. This layer of fat, located directly beneath the skin's surface, provides a readily

accessible and rich source of adipose tissue, making it ideal for SVF isolation. The process of SVF aspiration is routinely conducted at sites where subcutaneous fat is both abundant and easily accessible (Table 4).

The abdomen, particularly the lower abdominal area, is a prime site for adipose tissue harvesting. It is routinely selected for its accessibility and the substantial presence of subcutaneous fat. This region's predominance of fat deposits not only ensures a sufficient yield for SVF isolation but also contributes to the relative simplicity and lower risk profile of the procedure. Such characteristics render the abdomen an optimal site for various regenerative procedures, including those requiring liposuction or manual aspiration techniques.

The thighs are another common site for SVF aspiration, with both the inner and outer thighs offering ample subcutaneous fat. The choice between these sub-regions is typically informed by the patient's body composition and the quantity of adipose tissue needed. The thighs are comparable to the abdomen in terms of the volume of tissue available and are often used when additional adipose tissue is required or when the abdominal site is less suitable due to previous surgeries, scarring, or patient preference. Flanks are also a favored site for SVF aspiration. The flank area, which extends from the lower back around the side of the body, is another region where adipose tissue tends to accumulate. This site can be particularly beneficial for patients with less adipose deposition in the abdomen or thighs or when a larger quantity of SVF is necessary, as the flanks can provide an additional reservoir of adipose tissue.

In some scenarios, adipose tissue may also be harvested from the buttocks for SVF isolation. The buttocks may be chosen based on the patient's specific anatomical and fat distribution characteristics, and in cases where other sites are not viable due to anatomical constraints, prior procedures, or patient choice. The decision on the precise site for SVF aspiration is multifactorial, taking into account individual patient factors, the desired quantity of SVF, ease of access to the fat deposit, patient comfort, and any concurrent procedures that might be taking place. For example, if adipose tissue is being harvested for aesthetic purposes in addition to SVF isolation, the choice of site might be influenced by the goals of the aesthetic procedure.

Various techniques for SVF aspiration are available, with the choice often depending on the physician's expertise, the volume of tissue needed, and the specifics of the patient's case[23]. Methods can range from manual aspiration, which may be less technically demanding, to more technologically advanced approaches like liposuction, which can facilitate a more significant harvest of adipose tissue. Regardless of the method, the primary aim remains to procure an adequate quantity of viable adipose tissue for SVF isolation while maintaining the highest standards of patient safety and comfort.

Before undertaking SVF isolation, healthcare providers must engage patients in a comprehensive informed consent process. This conversation is vital to ensure that the patient is fully apprised of the procedural details, understands the rationale for site selection, and is aware of any potential risks or complications associated with the procedure. Such informed consent is a cornerstone of ethical medical practice, enabling patients to proceed with a clear understanding of the treatment plan and its implications for their care.

TECHNIQUES OF ADIPOSE TISSUE EXTRACTION

The isolation of SVF from adipose tissue is a critical step in regenerative medicine that requires the precise dissociation of the tissue to harvest a viable population of regenerative cells, including ADSCs and other stromal cells. The adipose tissue lyser, also referred to as a dissociator or homogenizer, is instrumental in this process, applying either mechanical, enzymatic or a combination of forces to efficiently disrupt the adipose tissue matrix[7,45].

The isolation of SVF from adipose tissue employs various techniques, predominantly categorized into enzymatic and mechanical methods. Enzymatic digestion is often considered the gold standard for SVF separation, utilizing collagenase to break down adipose tissue and release stromal cells[26,30,46]. In this approach, adipose tissue is digested with enzymes such as type I and VIII collagenase, followed by centrifugation and washing with phosphate-buffered saline to purify the SVF[27,30]. This method ensures a high yield of viable cells, including ADSCs, pericytes, fibroblasts, and other progenitor cells[28,47]. Mechanical isolation techniques, such as using rotating blades, vibrating shakers, and centrifuges, provide a rapid and cost-effective alternative to enzymatic digestion. These methods involve physically disrupting the adipose tissue to release stromal cells, followed by filtration and centrifugation to obtain the SVF[25,37,46]. Despite yielding lower quantities of cells compared to enzymatic methods, mechanical techniques can still produce clinically acceptable SVF with good viability and regenerative potential[31,46]. Additionally, approaches such as the use of Liberase Blendzyme and ultrasound for cell membrane permeabilization have been explored to enhance SVF yield and efficiency[29].

When selecting an adipose tissue lyser for SVF isolation, several factors must be carefully considered to ensure the optimal release of cells: (1) The volume of adipose tissue being processed is a determinant of the scale and type of lyser required. Larger volumes may necessitate the use of more robust systems capable of processing increased tissue amounts efficiently; (2) The desired level of tissue dissociation is crucial, as different dissociation techniques may yield varying degrees of cell liberation and viability, which in turn can influence the success of subsequent regenerative applications; (3) The throughput requirements of the procedure, including the number of samples to be processed and the time frame for processing, will influence the choice of lyser; (4) The resources available within the laboratory, including equipment and expertise, are also key considerations. Some dissociators may require additional infrastructure or specialized training to operate effectively; and (5) Safety and reproducibility are paramount. The chosen lyser must be reliable and consistent in performance, ensuring patient safety and the highest quality of SVF isolation.

Adhering to the manufacturer's instructions and laboratory best practices is essential when using an adipose tissue lyser. It is this adherence to the protocol that ensures the effective and reproducible dissociation of adipose tissue, which is a cornerstone for obtaining a high yield of viable and functional SVF. Such attention to detail is critical for translating

Table 4 Common sites for stromal vascular fraction aspiration from subcutaneous adipose tissue

Common SVF aspiration sites	Description
Abdomen[44]	Preferred due to accessibility and ample subcutaneous fat; less invasive for adequate tissue collection
Thighs[44]	Inner and outer thighs provide a high concentration of subcutaneous fat suitable for harvesting
Flanks[44]	Often chosen for excess adipose tissue, easily accessible for SVF aspiration
Buttocks[44]	Utilized when a significant amount of adipose tissue is needed, especially in regenerative procedures

SVF: Stromal vascular fraction.

the potential of SVF into successful clinical outcomes in the rapidly advancing field of regenerative medicine.

ADIPOSE TISSUE ASPIRATION CANNULA TYPE

The selection of an appropriate cannula type for the aspiration of adipose tissue during SVF isolation is a decision that hinges on a variety of procedural factors and desired outcomes (Table 5). Clinicians typically choose between two primary instruments: The hypodermic needle and the liposuction cannula (Table 6). Each tool has its advantages and limitations, which must be considered in the context of the SVF isolation process[48].

The hypodermic needle is a ubiquitous and cost-effective tool in medical procedures and is valued for its simplicity and ease of use, especially in manual aspiration techniques. It is available in a range of sizes, allowing for its application across different scales of adipose tissue aspiration. Despite its versatility, the hypodermic needle may not be the optimal choice for larger-scale harvesting or liposuction procedures due to its limited capacity for tissue collection, often necessitating multiple punctures to collect an adequate volume of adipose tissue.

In contrast, the liposuction cannula is specifically designed for the efficient harvest of larger volumes of adipose tissue, making it the instrument of choice for liposuction procedures. Its design minimizes trauma to surrounding tissues during the aspiration process. However, this efficiency comes at the cost of requiring specialized equipment and a higher level of expertise for safe and effective use. Additionally, for smaller-scale or manual aspiration procedures, the use of a liposuction cannula may not be as appropriate. There is a paucity of evidence that explores the variability of the SVF quality based on the choices of liposuction technique, anatomical location, or surgical approach selection.

When determining the most suitable cannula type for SVF isolation, several factors must be evaluated: (1) The volume of adipose tissue required is a decisive factor; larger quantities necessitate the use of a liposuction cannula for practical and efficient extraction; (2) The nature of the procedure is also critical. For manual aspirations, hypodermic needles are typically sufficient, whereas liposuction procedures are best served by cannulas; (3) Patient comfort and procedure tolerance must be considered, as smaller needles can be less invasive and more tolerable during manual aspirations; (4) The expertise of the physician is paramount. The clinician should possess proficiency in the use of the chosen instrument to ensure the procedure's success; and (5) Safety considerations must guide the selection process, ensuring that the chosen needle type aligns with safety protocols to minimize the risk of complications.

Ultimately, the needle type for adipose tissue aspiration should be selected based on a careful assessment of the SVF isolation requirements, the healthcare provider's skill set, and the highest standards of patient care. Adherence to sterile techniques and safety guidelines is essential to secure a successful outcome and the procurement of high-quality SVF for use in regenerative medicine.

CENTRIFUGATION

Centrifugation is a pivotal step in the isolation of SVF from adipose tissue, a process that enriches regenerative cells such as ADSCs by separating them from other cellular components[49]. The process begins with enzymatic digestion of the adipose tissue to break down the ECM and liberate the cells. Following digestion, the tissue is typically mixed with a buffer or saline to create a cell suspension suitable for centrifugation.

The goal of centrifugation in SVF isolation is to leverage the density differences between the various components of the digested adipose tissue to effectively segregate the SVF from adipocytes and blood cells[49-51]. To achieve this, centrifugation parameters are carefully selected based on the specific protocol and the equipment being utilized.

Centrifugation speeds are generally categorized as low, medium, or high, corresponding to the gravitational forces applied to the cell suspension (Table 7): (1) Low-speed centrifugation, ranging from 400 to 600 × *g*, is often employed for a preliminary separation, gently pelleting down the heavier fractions while leaving lighter components in the supernatant; (2) Medium-speed centrifugation, usually between 1200 and 1500 × *g*, can provide a balance between efficiency and cell viability, often used to refine the separation process; and (3) High-speed centrifugation, at 2000 to 2500 × *g*, is utilized to firmly pellet the SVF, ensuring maximal separation from the less dense lipid-rich adipocytes and other cellular debris. Building upon these speed considerations, the duration of centrifugation is equally critical in optimizing SVF isolation.

Table 5 Comparative analysis of adipose tissue harvesting tools: Hypodermic needle *vs* liposuction cannula

Instrument	Advantages	Disadvantages
Hypodermic needle	Widely available and cost-effective. Simple, easy for manual aspiration. Various sizes for different applications	Limited use for large-scale harvesting. Multiple punctures may be necessary
Liposuction cannula	Efficient for large-volume harvesting. Suitable for extensive liposuction needs. Reduces trauma to surrounding tissues	Requires special equipment and training. Less suitable for small-scale procedures

Table 6 Instrument selection criteria: Hypodermic needle *vs* liposuction cannula

Factor	Hypodermic needle	Liposuction cannula
Adipose tissue amount required	Suitable for smaller quantities	Preferred for larger volumes
Procedure type	Ideal for manual aspiration	Designed for liposuction procedures
Patient comfort	Potentially less invasive and more comfortable	Larger cannulas may be more discomforting
Physician expertise	Requires basic proficiency in aspiration techniques	Necessitates expertise in liposuction equipment and methods
Safety	Low-risk tool for manual procedures	Must be used in line with stringent safety protocols

Table 7 Centrifugation speed and time parameters for stromal vascular fraction isolation

Centrifugation parameters	Speed, × *g*	Time in min
Low-speed centrifugation	400-600	5-10
Medium-speed centrifugation	1200-1500	10-15
High-speed centrifugation	2000-2500	15-20

The duration of centrifugation is similarly tiered, with times typically extending from 5 min to 20 min depending on the speed. Shorter times are usually adequate for low-speed centrifugation, while longer periods are reserved for high-speed centrifugation to ensure a complete pellet formation. Some protocols may incorporate a two-step centrifugation process, initially applying a lower speed to remove larger adipocytes and conclude with a higher speed to consolidate the SVF into a pellet. This approach can be particularly beneficial for enhancing the purity and yield of the SVF. It is important to note that centrifugation parameters are not one-size-fits-all and may require optimization based on the volume of the suspension, the centrifuge model, and the intended use of the SVF. Variations in equipment, rotor types, and sample sizes necessitate adjustments to centrifugation speeds and times to achieve the desired outcome.

The key to successful SVF isolation lies in the careful optimization and validation of centrifugation steps to ensure the isolation of a high-quality SVF. This involves following validated protocols, ideally within the framework of Good Laboratory Practices or Good Manufacturing Practices, particularly when SVF isolation is conducted for clinical applications[52,53]. Consulting with experienced laboratory personnel and adhering to established best practices is recommended to achieve the best possible results in SVF isolation for regenerative medicine purposes.

VOLUME OF DELIVERY

Having established the methods for SVF isolation, the next critical consideration is determining the appropriate volume for therapeutic delivery, particularly in the context of treating knee osteoarthritis. The treatment protocol itself is a primary determinant of the volume of SVF to be delivered. Different protocols, which may be based on the latest research findings, expert consensus, or institutional practices, can vary in their recommendations. These protocols consider the concentration of the cellular components within the SVF, the expected volume of the knee joint space, and the therapeutic regimen's design, whether it be a single treatment or part of a series[54-57]. The severity of the OA condition directly impacts the volume of SVF required. A knee joint with extensive cartilage degeneration or significant synovial inflammation may require a higher volume of SVF, as the therapeutic goal is to reach a larger area of damaged tissue and to provide a sufficient number of cells capable of exerting a regenerative effect[57-60]. In establishing the appropriate SVF volume for knee OA, a thorough assessment of the patient's clinical presentation is essential. A careful evaluation of imaging results, such as magnetic resonance imaging or X-rays, can offer insights into the extent of joint damage and the volume of space available for SVF injection.

Individual patient characteristics are pivotal in tailoring the SVF volume. The patient's age can influence the regenerative capacity of their cells, while overall health and comorbidities may affect the tissue's receptivity to the treatment[61-64]. A few studies have reported a positive correlation between body mass index and SVF yield[62,63].

However, other studies have found no significant relationship between body mass index and SVF yield[64,65]. Moreover, the patient's history of responses to previous interventions can inform the likelihood of a favorable response to SVF therapy, potentially adjusting the volume needed to achieve clinical improvement. The intended goals of the treatment also guide the volume decision. For patients seeking relief from pain, a different volume may be employed compared to those whose primary objective is to enhance joint function or induce repair of the cartilage itself. The volume administered may be modulated to prioritize these distinct therapeutic endpoints. Furthermore, considerations of the joint's biomechanical function, previous surgical history, and individual anatomy will also play a role. Having noted suboptimal clinical response in single-dose treatment protocols, multiple dosing has also been implemented to compensate for the reduced primary volume of SVF delivered during the index procedure[66].

Given that SVF therapy for knee OA is still a developing area, it is subject to the dynamic flow of new research and clinical experience. The field is evolving with ongoing studies that seek to optimize dosage, frequency, and delivery methods to enhance the efficacy and safety of SVF treatments. Healthcare providers must therefore remain informed about the latest advancements and incorporate this evolving knowledge into their practice. It is also critical to adhere to high standards of clinical practice, including Good Laboratory Practices or Good Manufacturing Practices, especially when preparing SVF for clinical applications[67,68]. This ensures the quality and safety of the cellular product being administered. The determination of the SVF volume for knee OA treatment is a complex decision that integrates multiple clinical variables. The optimal volume must be carefully calculated to provide the greatest potential for joint repair and symptom alleviation while minimizing the risks of adverse effects. As the field advances, it is anticipated that more standardized protocols will emerge, enhancing the precision and predictability of SVF therapy for knee OA.

HETEROGENEITY OF MESENCHYMAL STEM CELLS

The heterogeneity of mesenchymal stem cells (MSCs) leads to discrepancies in therapeutic efficacies due to differences in donor sources, tissues, and intercellular variations, affecting proliferation, differentiation, and cytokine secretion[69]. MSCs from the same donor can show variability under identical culture conditions, impacting therapeutic outcomes. Age-related changes, such as telomere shortening and DNA damage, further contribute to this variability[69]. Subpopulations like differentiation-resistant MSCs upregulate YAP1, CDH6, and OCT4, promoting tumorigenic properties[70]. Similarly, osteogenesis-resistant MSCs retain stem cell characteristics and upregulate AJUBA, CDH4, and CTNNB1, facilitating head and neck squamous cell carcinoma[71]. This emphasizes the need for standardization to improve MSC-based therapies.

FUTURE PROSPECTS

SVF, with its rich diversity of cells including ADSCs, is lauded for its ability to differentiate into multiple cell types, supporting tissue repair and regeneration across a variety of medical conditions.

Another perspective frequently encountered in the literature pertains to the non-invasive nature of SVF isolation. Research scholars often advocate for the use of adipose tissue as a source of stem cells due to its abundance and accessibility. The minimally invasive methods employed for harvesting adipose tissue, such as liposuction, are considered advantageous over more invasive stem cell extraction techniques, with the potential for reduced patient discomfort and quicker post-procedure recovery.

Ethical and safety considerations also form a pivotal part of the dialogue surrounding SVF isolation. The autologous nature of SVF, derived from the patient's tissue, circumvents many of the ethical dilemmas associated with embryonic stem cells and alleviates concerns regarding immune compatibility and disease transmission. Researchers and clinicians alike may emphasize this aspect, noting the favorable safety profile of SVF therapies.

The clinical applications of SVF are a subject of extensive discussion among clinicians, with many exploring its role in managing OA, enhancing wound healing, and facilitating tissue repair. There is a keen interest in the anti-inflammatory and angiogenic properties of SVF and its utility in a range of therapeutic contexts. Additionally, the potential of SVF in novel medical applications continues to be a vibrant area of research, with research experts advocating for continued exploration into its full clinical potential.

Standardization and quality control in SVF isolation are points of emphasis for some researchers, who argue for the necessity of consistent and reproducible protocols. This perspective is grounded in the importance of ensuring patient safety and treatment efficacy, with a call for rigorous standards and practices to be established and maintained across the field.

The evolving nature of SVF research is a recurring theme in scholarly discussions. The research scientists acknowledge that while the therapeutic promise of SVF is considerable, there remains a need for further studies to elucidate its mechanisms of action and to refine its application in clinical practice. The consensus is that ongoing research and clinical trials will be critical in advancing the field and expanding the therapeutic repertoire of SVF. It is essential to recognize that the views expressed by researchers are influenced by their respective research focuses their interdisciplinary backgrounds, and the evolving body of evidence. As such, the perspectives on SVF isolation are as diverse as the scientific community itself, unified by a common pursuit of advancing regenerative medicine through robust evidence, well-designed clinical trials, and stringent adherence to ethical and regulatory standards (Table 8).

Table 8 Perspectives on stromal vascular fraction isolation in regenerative medicine

Perspective	Description
Regenerative potential	Highlights the diverse cells in SVF, like adipose derived stem cells, for tissue repair and potential in regenerative medicine
Non-invasive approach	Stresses minimally invasive adipose tissue harvest, leading to less discomfort and quicker recovery
Ethical and safety considerations	Notes the ethical advantage of autologous use and lower risk of rejection or disease transmission
Clinical applications	Discusses the role of SVF in osteoarthritis, wound healing, and other regenerative areas
Standardization and quality control	Urges for standardized protocols to ensure consistent results and safety in SVF therapies
Evolving research	Emphasizes the need for ongoing research to expand understanding and clinical use of SVF

SVF: Stromal vascular fraction.

CONCLUSION

SVF isolation and application represents a promising frontier in regenerative medicine, offering a rich source of ADSCs and regenerative factors. The field grapples with the complexities of autologous *versus* allogeneic sourcing, each presenting unique advantages and challenges. Isolation techniques, ranging from enzymatic to mechanical methods, require optimization to balance efficiency and cellular viability. The potential of SVF in treating conditions like osteoarthritis is significant, yet it demands rigorous standardization and quality control measures. Moving forward, large-scale clinical trials are essential to evaluate long-term efficacy and safety. Researchers must elucidate the mechanisms of action, explore combination therapies, and develop standardized protocols. Establishing robust regulatory frameworks is crucial for clinical translation. While SVF shows great promise, ongoing research is vital to fully realize its therapeutic potential and establish it as a mainstream regenerative medicine approach. The field's advancement hinges on addressing these multifaceted challenges.

FOOTNOTES

Author contributions: Jeyaraman N designed the research; Jeyaraman N and Nallakumarasamy A analyzed the articles for performing the review and wrote the manuscript; Shrivastava S, Ravi VR, Pundkar A, and Jeyaraman M finalized the manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: India

ORCID number: Naveen Jeyaraman 0000-0002-4362-3326; VR Ravi 0009-0006-7303-8474; Arulkumar Nallakumarasamy 0000-0002-2445-2883; Madhan Jeyaraman 0000-0002-9045-9493.

S-Editor: Wang JJ
L-Editor: Filipodia
P-Editor: Che XX

REFERENCES

1 **Ramakrishnan VM**, Boyd NL. The Adipose Stromal Vascular Fraction as a Complex Cellular Source for Tissue Engineering Applications. *Tissue Eng Part B Rev* 2018; **24**: 289-299 [PMID: 28316259 DOI: 10.1089/ten.TEB.2017.0061]
2 **Iyyanki T**, Hubenak J, Liu J, Chang EI, Beahm EK, Zhang Q. Harvesting technique affects adipose-derived stem cell yield. *Aesthet Surg J* 2015; **35**: 467-476 [PMID: 25791999 DOI: 10.1093/asj/sju055]
3 **Jeyaraman M**, Jeyaraman N, Jayakumar T, Ramasubramanian S, Ranjan R, Jha SK, Gupta A. Efficacy of stromal vascular fraction for knee osteoarthritis: A prospective, single-centre, non-randomized study with 2 years follow-up. *World J Orthop* 2024; **15**: 457-468 [PMID: 38835682 DOI: 10.5312/wjo.v15.i5.457]
4 **Jeyaraman M**, Maffulli N, Gupta A. Stromal Vascular Fraction in Osteoarthritis of the Knee. *Biomedicines* 2023; **11** [PMID: 37239130 DOI: 10.3390/biomedicines11051460]

5 **Yu NH**, Chun SY, Ha YS, Kim HT, Kim DH, Kim J, Chung JW, Lee JN, Song PH, Yoo ES, Kim BS, Kwon TG. Optimal Stem Cell Transporting Conditions to Maintain Cell Viability and Characteristics. *Tissue Eng Regen Med* 2018; **15**: 639-647 [PMID: 30603585 DOI: 10.1007/s13770-018-0133-y]

6 **Lee SJ**, Lee CR, Kim KJ, Ryu YH, Kim E, Han YN, Moon SH, Rhie JW. Optimal Condition of Isolation from an Adipose Tissue-Derived Stromal Vascular Fraction for the Development of Automated Systems. *Tissue Eng Regen Med* 2020; **17**: 203-208 [PMID: 31997256 DOI: 10.1007/s13770-019-00238-3]

7 **Gentile P**, Calabrese C, De Angelis B, Pizzicannella J, Kothari A, Garcovich S. Impact of the Different Preparation Methods to Obtain Human Adipose-Derived Stromal Vascular Fraction Cells (AD-SVFs) and Human Adipose-Derived Mesenchymal Stem Cells (AD-MSCs): Enzymatic Digestion Versus Mechanical Centrifugation. *Int J Mol Sci* 2019; **20** [PMID: 31684107 DOI: 10.3390/ijms20215471]

8 **Trombi L**, Danti S, Savelli S, Moscato S, D'Alessandro D, Ricci C, Giannotti S, Petrini M. Mesenchymal Stromal Cell Culture and Delivery in Autologous Conditions: A Smart Approach for Orthopedic Applications. *J Vis Exp* 2016 [PMID: 28060333 DOI: 10.3791/54845]

9 **Avelar-Freitas BA**, Almeida VG, Pinto MC, Mourão FA, Massensini AR, Martins-Filho OA, Rocha-Vieira E, Brito-Melo GE. Trypan blue exclusion assay by flow cytometry. *Braz J Med Biol Res* 2014; **47**: 307-315 [PMID: 24652322 DOI: 10.1590/1414-431X20143437]

10 **Mastrolia I**, Foppiani EM, Murgia A, Candini O, Samarelli AV, Grisendi G, Veronesi E, Horwitz EM, Dominici M. Challenges in Clinical Development of Mesenchymal Stromal/Stem Cells: Concise Review. *Stem Cells Transl Med* 2019; **8**: 1135-1148 [PMID: 31313507 DOI: 10.1002/sctm.19-0044]

11 **Church C**, Horowitz M, Rodeheffer M. WAT is a functional adipocyte? *Adipocyte* 2012; **1**: 38-45 [PMID: 23700509 DOI: 10.4161/adip.19132]

12 **Richard AJ**, White U, Elks CM, Stephens JM. Adipose Tissue: Physiology to Metabolic Dysfunction. 2020 Apr 4. In: Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000– [PMID: 32255578]

13 **de Sousa Neto IV**, Durigan JLQ, da Silva ASR, de Cássia Marqueti R. Adipose Tissue Extracellular Matrix Remodeling in Response to Dietary Patterns and Exercise: Molecular Landscape, Mechanistic Insights, and Therapeutic Approaches. *Biology (Basel)* 2022; **11** [PMID: 35625493 DOI: 10.3390/biology11050765]

14 **Coelho M**, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. *Arch Med Sci* 2013; **9**: 191-200 [PMID: 23671428 DOI: 10.5114/aoms.2013.33181]

15 **Tsuji W**, Rubin JP, Marra KG. Adipose-derived stem cells: Implications in tissue regeneration. *World J Stem Cells* 2014; **6**: 312-321 [PMID: 25126381 DOI: 10.4252/wjsc.v6.i3.312]

16 **Miana VV**, González EAP. Adipose tissue stem cells in regenerative medicine. *Ecancermedicalscience* 2018; **12**: 822 [PMID: 29662535 DOI: 10.3332/ecancer.2018.822]

17 **Pharoun J**, Berro J, Sobh J, Abou-Younes MM, Nasr L, Majed A, Khalil A, Joseph, Stephan, Faour WH. Mesenchymal stem cells biological and biotechnological advances: Implications for clinical applications. *Eur J Pharmacol* 2024; **977**: 176719 [PMID: 38849038 DOI: 10.1016/j.ejphar.2024.176719]

18 **Nguyen A**, Guo J, Banyard DA, Fadavi D, Toranto JD, Wirth GA, Paydar KZ, Evans GR, Widgerow AD. Stromal vascular fraction: A regenerative reality? Part 1: Current concepts and review of the literature. *J Plast Reconstr Aesthet Surg* 2016; **69**: 170-179 [PMID: 26565755 DOI: 10.1016/j.bjps.2015.10.015]

19 **Yang S**, Sun Y, Yan C. Recent advances in the use of extracellular vesicles from adipose-derived stem cells for regenerative medical therapeutics. *J Nanobiotechnology* 2024; **22**: 316 [PMID: 38844939 DOI: 10.1186/s12951-024-02603-4]

20 **Mladenović D**, Vesković M, Šutulović N, Hrnčić D, Stanojlović O, Radić L, Macut JB, Macut D. Adipose-derived extracellular vesicles - a novel cross-talk mechanism in insulin resistance, non-alcoholic fatty liver disease, and polycystic ovary syndrome. *Endocrine* 2024 [PMID: 38285412 DOI: 10.1007/s12020-024-03702-w]

21 **He Q**, Liao Y, Zhang H, Sun W, Zhou W, Lin J, Zhang T, Xie S, Wu H, Han J, Zhang Y, Wei W, Li C, Hong Y, Shen W, Ouyang H. Gel microspheres enhance the stemness of ADSCs by regulating cell-ECM interaction. *Biomaterials* 2024; **309**: 122616 [PMID: 38776592 DOI: 10.1016/j.biomaterials.2024.122616]

22 **Hajimortezayi Z**, Daei N, Gholizadeh N, Zakeri M, Alhili F, Hasanzadeh S, Hormozi A, Ebrahimi F, Zamani M. Fat transplant: Amazing growth and regeneration of cells and rebirth with the miracle of fat cells. *J Cosmet Dermatol* 2024; **23**: 1141-1149 [PMID: 38010992 DOI: 10.1111/jocd.16103]

23 **Dai R**, Wang Z, Samanipour R, Koo KI, Kim K. Adipose-Derived Stem Cells for Tissue Engineering and Regenerative Medicine Applications. *Stem Cells Int* 2016; **2016**: 6737345 [PMID: 27057174 DOI: 10.1155/2016/6737345]

24 **Sharma S**, Muthu S, Jeyaraman M, Ranjan R, Jha SK. Translational products of adipose tissue-derived mesenchymal stem cells: Bench to bedside applications. *World J Stem Cells* 2021; **13**: 1360-1381 [PMID: 34786149 DOI: 10.4252/wjsc.v13.i10.1360]

25 **Bora P**, Majumdar AS. Adipose tissue-derived stromal vascular fraction in regenerative medicine: a brief review on biology and translation. *Stem Cell Res Ther* 2017; **8**: 145 [PMID: 28619097 DOI: 10.1186/s13287-017-0598-y]

26 **Agarwal N**, Mak C, Bojanic C, To K, Khan W. Meta-Analysis of Adipose Tissue Derived Cell-Based Therapy for the Treatment of Knee Osteoarthritis. *Cells* 2021; **10** [PMID: 34206010 DOI: 10.3390/cells10061365]

27 **Sadri B**, Hassanzadeh M, Bagherifard A, Mohammadi J, Alikhani M, Moeinabadi-Bidgoli K, Madani H, Diaz-Solano D, Karimi S, Mehrazmay M, Mohammadpour M, Vosough M. Cartilage regeneration and inflammation modulation in knee osteoarthritis following injection of allogeneic adipose-derived mesenchymal stromal cells: a phase II, triple-blinded, placebo controlled, randomized trial. *Stem Cell Res Ther* 2023; **14**: 162 [PMID: 37316949 DOI: 10.1186/s13287-023-03359-8]

28 **Damia E**, Chicharro D, Lopez S, Cuervo B, Rubio M, Sopena JJ, Vilar JM, Carrillo JM. Adipose-Derived Mesenchymal Stem Cells: Are They a Good Therapeutic Strategy for Osteoarthritis? *Int J Mol Sci* 2018; **19** [PMID: 29966351 DOI: 10.3390/ijms19071926]

29 **Jurgens WJ**, Kroeze RJ, Zandieh-Doulabi B, van Dijk A, Renders GA, Smit TH, van Milligen FJ, Ritt MJ, Helder MN. One-step surgical procedure for the treatment of osteochondral defects with adipose-derived stem cells in a caprine knee defect: a pilot study. *Biores Open Access* 2013; **2**: 315-325 [PMID: 23914338 DOI: 10.1089/biores.2013.0024]

30 **Huang SJ**, Fu RH, Shyu WC, Liu SP, Jong GP, Chiu YW, Wu HS, Tsou YA, Cheng CW, Lin SZ. Adipose-derived stem cells: isolation, characterization, and differentiation potential. *Cell Transplant* 2013; **22**: 701-709 [PMID: 23068312 DOI: 10.3727/096368912X655127]

31 **De Francesco F**, Gravina P, Busato A, Farinelli L, Soranzo C, Vidal L, Zingaretti N, Zavan B, Sbarbati A, Riccio M, Gigante A. Stem Cells in Autologous Microfragmented Adipose Tissue: Current Perspectives in Osteoarthritis Disease. *Int J Mol Sci* 2021; **22** [PMID: 34638538 DOI: 10.3390/ijms221910197]

32 **Kim YS**, Suh DS, Tak DH, Kwon YB, Koh YG. Adipose-Derived Stromal Vascular Fractions Are Comparable With Allogenic Human

Umbilical Cord Blood-Derived Mesenchymal Stem Cells as a Supplementary Strategy of High Tibial Osteotomy for Varus Knee Osteoarthritis. *Arthrosc Sports Med Rehabil* 2023; **5**: e751-e764 [PMID: 37388880 DOI: 10.1016/j.asmr.2023.04.002]

33 **Doyle LM**, Wang MZ. Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. *Cells* 2019; **8** [PMID: 31311206 DOI: 10.3390/cells8070727]

34 **Ouchi N**, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol* 2011; **11**: 85-97 [PMID: 21252989 DOI: 10.1038/nri2921]

35 **Zhang X**, Chen X, Hong H, Hu R, Liu J, Liu C. Decellularized extracellular matrix scaffolds: Recent trends and emerging strategies in tissue engineering. *Bioact Mater* 2022; **10**: 15-31 [PMID: 34901526 DOI: 10.1016/j.bioactmat.2021.09.014]

36 **Jeyaraman M**, Muthu S, Sharma S, Ganta C, Ranjan R, Jha SK. Nanofat: A therapeutic paradigm in regenerative medicine. *World J Stem Cells* 2021; **13**: 1733-1746 [PMID: 34909120 DOI: 10.4252/wjsc.v13.i11.1733]

37 **Simonacci F**, Bertozzi N, Grieco MP, Raposio E. From liposuction to adipose-derived stem cells: indications and technique. *Acta Biomed* 2019; **90**: 197-208 [PMID: 31124996 DOI: 10.23750/abm.v90i2.6619]

38 **Gentile P**, Sterodimas A, Pizzicannella J, Dionisi L, De Fazio D, Calabrese C, Garcovich S. Systematic Review: Allogenic Use of Stromal Vascular Fraction (SVF) and Decellularized Extracellular Matrices (ECM) as Advanced Therapy Medicinal Products (ATMP) in Tissue Regeneration. *Int J Mol Sci* 2020; **21** [PMID: 32679697 DOI: 10.3390/ijms21144982]

39 **Copcu HE**. Indication-based protocols with different solutions for mechanical stromal-cell transfer. *Scars Burn Heal* 2022; **8**: 20595131211047830 [PMID: 35003762 DOI: 10.1177/20595131211047830]

40 **Goldman JJ**, Wang WZ, Fang XH, Williams SJ, Baynosa RC. Tumescent Liposuction without Lidocaine. *Plast Reconstr Surg Glob Open* 2016; **4**: e829 [PMID: 27622097 DOI: 10.1097/GOX.0000000000000830]

41 **Desando G**, Bartolotti I, Cattini L, Tschon M, Martini L, Fini M, Schiavinato A, Soranzo C, Grigolo B. Prospects on the Potential In Vitro Regenerative Features of Mechanically Treated-Adipose Tissue for Osteoarthritis Care. *Stem Cell Rev Rep* 2021; **17**: 1362-1373 [PMID: 33469783 DOI: 10.1007/s12015-020-10099-2]

42 **Fodor PB**, Paulseth SG. Adipose Derived Stromal Cell (ADSC) Injections for Pain Management of Osteoarthritis in the Human Knee Joint. *Aesthet Surg J* 2016; **36**: 229-236 [PMID: 26238455 DOI: 10.1093/asj/sjv135]

43 **Tsubosaka M**, Matsumoto T, Sobajima S, Matsushita T, Iwaguro H, Kuroda R. The influence of adipose-derived stromal vascular fraction cells on the treatment of knee osteoarthritis. *BMC Musculoskelet Disord* 2020; **21**: 207 [PMID: 32252731 DOI: 10.1186/s12891-020-03231-3]

44 **Fontes T**, Brandão I, Negrão R, Martins MJ, Monteiro R. Autologous fat grafting: Harvesting techniques. *Ann Med Surg (Lond)* 2018; **36**: 212-218 [PMID: 30505441 DOI: 10.1016/j.amsu.2018.11.005]

45 **Ong WK**, Chakraborty S, Sugii S. Adipose Tissue: Understanding the Heterogeneity of Stem Cells for Regenerative Medicine. *Biomolecules* 2021; **11** [PMID: 34206204 DOI: 10.3390/biom11070918]

46 **Solodeev I**, Meilik B, Gur E, Shani N. A Closed-system Technology for Mechanical Isolation of High Quantities of Stromal Vascular Fraction from Fat for Immediate Clinical Use. *Plast Reconstr Surg Glob Open* 2023; **11**: e5096 [PMID: 37361510 DOI: 10.1097/GOX.0000000000005096]

47 **Borić I**, Hudetz D, Rod E, Jeleč Ž, Vrdoljak T, Skelin A, Polašek O, Plečko M, Trbojević-Akmačić I, Lauc G, Primorac D. A 24-Month Follow-Up Study of the Effect of Intra-Articular Injection of Autologous Microfragmented Fat Tissue on Proteoglycan Synthesis in Patients with Knee Osteoarthritis. *Genes (Basel)* 2019; **10** [PMID: 31861180 DOI: 10.3390/genes10121051]

48 **Vazquez OA**, Markowitz MI, Becker H. Fat Graft Size: Relationship Between Cannula and Needle Diameters. *Cureus* 2020; **12**: e7598 [PMID: 32399332 DOI: 10.7759/cureus.7598]

49 **Yaylacı S**, Kaçaroğlu D, Hürkal Ö, Ulaşlı AM. An enzyme-free technique enables the isolation of a large number of adipose-derived stem cells at the bedside. *Sci Rep* 2023; **13**: 8005 [PMID: 37198228 DOI: 10.1038/s41598-023-34915-0]

50 **Aronowitz JA**, Lockhart RA, Hakakian CS. Mechanical versus enzymatic isolation of stromal vascular fraction cells from adipose tissue. *Springerplus* 2015; **4**: 713 [PMID: 26636001 DOI: 10.1186/s40064-015-1509-2]

51 **Lamblet H**, Ferreira LM. Fat obtained from plastic surgery procedures-stem cells derived from adipose tissue and their potential in technological innovation: a narrative literature review and perspective on dissociative methods. *Eur J Plast Surg* 2022; **45**: 701-731 [PMID: 35308897 DOI: 10.1007/s00238-022-01951-4]

52 **Cho KH**, Kim JS, Jeon MS, Lee K, Chung MK, Song CW. Basic Principles of the Validation for Good Laboratory Practice Institutes. *Toxicol Res* 2009; **25**: 1-8 [PMID: 32038812 DOI: 10.5487/TR.2009.25.1.001]

53 **Ntege EH**, Sunami H, Shimizu Y. Advances in regenerative therapy: A review of the literature and future directions. *Regen Ther* 2020; **14**: 136-153 [PMID: 32110683 DOI: 10.1016/j.reth.2020.01.004]

54 **Tantuway V**, Thomas W, Parikh MB, Sharma R, Jeyaraman N, Jeyaraman M. Clinical Outcome of Minimally Manipulated, Mechanically Isolated Autologous Adipose Tissue-Derived Stromal Vascular Fraction (Sahaj Therapy®) in Knee Osteoarthritis-Randomized Controlled Trial. *Indian J Orthop* 2023; **57**: 1646-1658 [PMID: 37766954 DOI: 10.1007/s43465-023-00981-9]

55 **Lapuente JP**, Dos-Anjos S, Blázquez-Martínez A. Intra-articular infiltration of adipose-derived stromal vascular fraction cells slows the clinical progression of moderate-severe knee osteoarthritis: hypothesis on the regulatory role of intra-articular adipose tissue. *J Orthop Surg Res* 2020; **15**: 137 [PMID: 32272946 DOI: 10.1186/s13018-020-01664-z]

56 **Tang Q**, Zhao XS, Guo A, Cui RT, Song HL, Qi ZY, Pan Y, Yang Y, Zhang FF, Jin L. Therapeutic applications of adipose-derived stromal vascular fractions in osteoarthritis. *World J Stem Cells* 2022; **14**: 744-755 [PMID: 36337155 DOI: 10.4252/wjsc.v14.i10.744]

57 **Garza JR**, Campbell RE, Tjoumakaris FP, Freedman KB, Miller LS, Santa Maria D, Tucker BS. Clinical Efficacy of Intra-articular Mesenchymal Stromal Cells for the Treatment of Knee Osteoarthritis: A Double-Blinded Prospective Randomized Controlled Clinical Trial. *Am J Sports Med* 2020; **48**: 588-598 [PMID: 32109160 DOI: 10.1177/0363546519899923]

58 **Kim YS**, Oh SM, Suh DS, Tak DH, Kwon YB, Koh YG. Cartilage lesion size and number of stromal vascular fraction (SVF) cells strongly influenced the SVF implantation outcomes in patients with knee osteoarthritis. *J Exp Orthop* 2023; **10**: 28 [PMID: 36918463 DOI: 10.1186/s40634-023-00592-1]

59 **Kim YS**, Choi YJ, Koh YG. Mesenchymal stem cell implantation in knee osteoarthritis: an assessment of the factors influencing clinical outcomes. *Am J Sports Med* 2015; **43**: 2293-2301 [PMID: 26113522 DOI: 10.1177/0363546515588317]

60 **Koh YG**, Choi YJ, Kwon OR, Kim YS. Second-Look Arthroscopic Evaluation of Cartilage Lesions After Mesenchymal Stem Cell Implantation in Osteoarthritic Knees. *Am J Sports Med* 2014; **42**: 1628-1637 [PMID: 24743139 DOI: 10.1177/0363546514529641]

61 **Dos-Anjos Vilaboa S**, Navarro-Palou M, Llull R. Age influence on stromal vascular fraction cell yield obtained from human lipoaspirates.

Cytotherapy 2014; **16**: 1092-1097 [PMID: 24726656 DOI: 10.1016/j.jcyt.2014.02.007]

62 **Alaaeddine N**, El Atat O, Saliba N, Feghali Z, Nasr M, Tarabey B, Hilal G, Hashim H. Effect of age and body mass index on the yield of stromal vascular fraction. *J Cosmet Dermatol* 2018; **17**: 1233-1239 [PMID: 29222844 DOI: 10.1111/jocd.12458]

63 **Yu G**, Wu X, Dietrich MA, Polk P, Scott LK, Ptitsyn AA, Gimble JM. Yield and characterization of subcutaneous human adipose-derived stem cells by flow cytometric and adipogenic mRNA analyzes. *Cytotherapy* 2010; **12**: 538-546 [PMID: 20380539 DOI: 10.3109/14653241003649528]

64 **Buschmann J**, Gao S, Härter L, Hemmi S, Welti M, Werner CM, Calcagni M, Cinelli P, Wanner GA. Yield and proliferation rate of adipose-derived stromal cells as a function of age, body mass index and harvest site-increasing the yield by use of adherent and supernatant fractions? *Cytotherapy* 2013; **15**: 1098-1105 [PMID: 23800730 DOI: 10.1016/j.jcyt.2013.04.009]

65 **Faustini M**, Bucco M, Chlapanidas T, Lucconi G, Marazzi M, Tosca MC, Gaetani P, Klinger M, Villani S, Ferretti VV, Vigo D, Torre ML. Nonexpanded mesenchymal stem cells for regenerative medicine: yield in stromal vascular fraction from adipose tissues. *Tissue Eng Part C Methods* 2010; **16**: 1515-1521 [PMID: 20486782 DOI: 10.1089/ten.TEC.2010.0214]

66 **Holzbauer M**, Priglinger E, Kølle ST, Prantl L, Stadler C, Winkler PW, Gotterbarm T, Duscher D. Intra-Articular Application of Autologous, Fat-Derived Orthobiologics in the Treatment of Knee Osteoarthritis: A Systematic Review. *Cells* 2024; **13** [PMID: 38727286 DOI: 10.3390/cells13090750]

67 **Ezzelle J**, Rodriguez-Chavez IR, Darden JM, Stirewalt M, Kunwar N, Hitchcock R, Walter T, D'Souza MP. Guidelines on good clinical laboratory practice: bridging operations between research and clinical research laboratories. *J Pharm Biomed Anal* 2008; **46**: 18-29 [PMID: 18037599 DOI: 10.1016/j.jpba.2007.10.010]

68 **Mazini L**, Ezzoubi M, Malka G. Overview of current adipose-derived stem cell (ADSCs) processing involved in therapeutic advancements: flow chart and regulation updates before and after COVID-19. *Stem Cell Res Ther* 2021; **12**: 1 [PMID: 33397467 DOI: 10.1186/s13287-020-02006-w]

69 **Li J**, Wu Z, Zhao L, Liu Y, Su Y, Gong X, Liu F, Zhang L. The heterogeneity of mesenchymal stem cells: an important issue to be addressed in cell therapy. *Stem Cell Res Ther* 2023; **14**: 381 [PMID: 38124129 DOI: 10.1186/s13287-023-03587-y]

70 **Stucky A**, Gao L, Li SC, Tu L, Luo J, Huang X, Chen X, Li X, Park TH, Cai J, Kabeer MH, Plant AS, Sun L, Zhang X, Zhong JF. Molecular Characterization of Differentiated-Resistance MSC Subclones by Single-Cell Transcriptomes. *Front Cell Dev Biol* 2022; **10**: 699144 [PMID: 35356283 DOI: 10.3389/fcell.2022.699144]

71 **Stucky A**, Gao L, Sun L, Li SC, Chen X, Park TH, Cai J, Kabeer MH, Zhang X, Sinha UK, Zhong JF. Evidence for AJUBA-catenin-CDH4-linked differentiation resistance of mesenchymal stem cells implies tumorigenesis and progression of head and neck squamous cell carcinoma: a single-cell transcriptome approach. *Blood Genom* 2021; **5**: 29-39 [PMID: 34368804 DOI: 10.46701/BG.2021012021106]

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