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SYSTEMATIC REVIEWS

Non-enzymatic methods for isolation of stromal vascular fraction and adipose-derived stem cells: A systematic review

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Abstract

BACKGROUND

Adipose-derived stem cells (ADSCs) and the stromal vascular fraction (SVF) have garnered substantial interest in regenerative medicine due to their potential to treat a wide range of conditions. Traditional enzymatic methods for isolating these cells face challenges such as high costs, lengthy processing time, and regulatory complexities.

AIM

This systematic review aimed to assess the efficacy and practicality of non-enzymatic, mechanical methods for isolating SVF and ADSCs, comparing these to

conventional enzymatic approaches.

METHODS
Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines, a comprehensive literature search was conducted across multiple databases. Studies were selected based on inclusion criteria focused on non-enzymatic isolation methods for SVF and ADSCs from adipose tissue. The risk of bias was assessed, and a qualitative synthesis of findings was performed due to the methodological heterogeneity of the included studies.

RESULTS
Nineteen studies met the inclusion criteria, highlighting various mechanical techniques such as centrifugation, vortexing, and ultrasonic cavitation. The review identified significant variability in cell yield and viability, and the integrity of isolated cells across different non-enzymatic methods compared to enzymatic procedures. Despite some advantages of mechanical methods, including reduced processing time and avoidance of enzymatic reagents, the evidence suggests a need for optimization to match the cell quality and therapeutic efficacy achievable with enzymatic isolation.

CONCLUSION
Non-enzymatic, mechanical methods offer a promising alternative to enzymatic isolation of SVF and ADSCs, potentially simplifying the isolation process and reducing regulatory hurdles. However, further research is necessary to standardize these techniques and ensure consistent, high-quality cell yields for clinical applications. The development of efficient, safe, and reproducible non-enzymatic isolation methods could significantly advance the field of regenerative medicine.

Key Words: Adipose-derived stem cells; Stromal vascular fraction; Regenerative medicine; Non-enzymatic isolation; Mechanical separation techniques

Core Tip: This study highlights the superiority of non-enzymatic methods as alternatives for the isolation of stromal vascular fraction from adipose tissue. It emphasizes the necessity of standardizing these methods to ensure the procurement of consistent and high-quality cell yields suitable for a range of clinical applications.

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INTRODUCTION

Regenerative medicine has emerged as a pivotal area of interest across multiple medical specialties, driven by an increasing volume of literature on the potential of regenerative cells for a myriad of indications. Among various sources, adipose tissue is recognized for its significant role beyond shock absorption, thermoregulation, and energy storage; it stands out as the largest and most crucial reservoir for adipose-derived stem or stromal cells (ADSCs). These cells are predominantly found within the perivascular region of the stroma, an area characterized by a loose connective tissue matrix that houses a diverse array of cells including immune cells, erythrocytes, mesenchymal stem cells (MSCs), and other stromal components[1-4]. The ease of collection through liposuction, a minimally invasive procedure performed under local anesthesia, further underscores the accessibility of adipose tissue for regenerative therapies. Historically, the therapeutic potential of adipose tissue dates back to World War I, when Morestin first utilized fatty tissue injections to enhance wound healing in soldiers. This early application laid the groundwork for the field, which gained substantial momentum following the work of Zuk *et al*[5], who highlighted adipose tissue as a prime source of MSCs[5]. Recent studies have delved into the capabilities of ADSCs, particularly those within the stromal vascular fraction (SVF), focusing on their role in tissue regeneration for injuries and chronic conditions[6,7]. The SVF's rich secretome and the multipotent nature of its cellular constituents underscore its therapeutic potential[8,9].

However, the conventional method of isolating ADSCs from adipose tissue, primarily through enzymatic dissociation, poses significant challenges, including operational complexity and the need for specialized equipment, rendering it impractical for immediate surgical application[10,11]. This enzymatic process, despite its efficacy in isolating SVFs, disrupts the stem cell niche and necessitates compliance with good manufacturing practice standards, as defined by regulatory authorities[12,13]. Such limitations have catalyzed interest in mechanical stromal-cell separation techniques,

exemplified by the development of nanofat by Tonnard *et al*[2], which offers a non-enzymatic alternative for cell isolation. Despite the advent of intraoperative isolation techniques that promise to circumvent the challenges of enzymatic methods, there remains a paucity of research comparing the efficacy, cell yield, and phenotype of cells isolated through these novel mechanical methods to the traditional enzymatic approach[11,14]. This knowledge gap is particularly significant given the logistical and operational constraints faced by peripheral hospitals, which often lack the resources for the labor-intensive enzymatic isolation of ADSCs[15].

This systematic review aimed to critically assess the therapeutic potential of non-enzymatic methods for producing SVF, comparing these newer mechanical isolation techniques against the established enzymatic method. By evaluating the quality and quantity of SVF obtained through non-enzymatic methods, this review seeks to address a critical gap in the literature and validate the feasibility of these approaches for regenerative medicine applications.

MATERIALS AND METHODS

This systematic review was meticulously designed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, ensuring a structured and transparent methodology. The PRISMA flow diagram, illustrating the search and selection process, is presented in Figure 1[16]. The foundation of our search strategy was the well-established Population, Intervention, Comparison, and Outcome framework[17], which facilitated a focused and comprehensive literature search. It is noteworthy to mention that this investigation did not undergo formal registration, as it was developed primarily for academic purposes, specifically as part of a master's thesis project.

Eligibility criteria

The selection of studies for inclusion in this systematic review was governed by the precise inclusion and exclusion criteria tailored to the objectives of our investigation.

Inclusion criteria: Our review targeted studies that discussed non-enzymatic isolation procedures for the isolation of the SVF from adipose tissue. We included studies that utilized the adipose fraction obtained from lipoaspirate and those that evaluated the effectiveness of centrifugation forces, sonication, or red blood cell (RBC) lysis buffer. Studies were also considered if they compared non-enzymatic isolation techniques directly with enzymatic methods.

Exclusion criteria: We excluded studies published before the year 2000 and those not written in English to maintain a contemporary focus and ensure comprehension across the research team. Articles that solely utilized enzymatic isolation techniques or combined enzymatic with mechanical methods for SVF extraction were not considered. Furthermore, case studies, case series, and reviews focusing exclusively on adipose tissue processing techniques for fat grafting purposes were disregarded to maintain a clear focus on SVF isolation methodologies.

Information sources and search strategy

A comprehensive search was conducted across several databases, including The Cochrane Central Register of Controlled Trials, Embase (OvidSP), and PubMed, to identify relevant studies. The search strategy was meticulously crafted, combining keywords and phrases related to the population of interest (adipose stromal cells, ADSCs, adipose stem cells, stem cells, and SVF) with terms associated with the intervention (cell separation, isolation, dissociation, and isolation system) and comparison elements (non-enzymatic, mechanical, vibration, and sonic). This approach ensured a broad yet focused retrieval of pertinent literature.

Study selection and data collection process

Given the nature of this investigation as a master's thesis, the article selection and data collection processes were undertaken by a single author. This involved screening the identified records based on the predefined eligibility criteria, followed by a thorough examination of the full texts of potentially relevant studies. This approach, while somewhat limited by the capacity of a single researcher, ensured a consistent and focused evaluation of the literature.

Risk of bias across studies

To address the potential risk of bias across the included studies, several measures were implemented. The variability in SVF analysis result variables and the methodological heterogeneity inherent in the investigated isolation techniques necessitated a qualitative synthesis rather than a quantitative meta-analysis. To this end, the Modified IFATS/ISCT Index Score was utilized to provide a comprehensive overview of the outcome measures reported in each study. Additionally, the potential for publication bias, particularly in studies where authors may have conflicts of interest, was carefully considered. Disclosure agreements and funding sources were examined for each study to assess the risk of bias and ensure transparency in the reported findings. The Office of Health Assessment and Translation (OHAT) Risk of Bias Tool for Human and Animal Studies was used to assess the risk of bias and internal validity[18]. Six questions from the tool relating to cross-sectional research were assessed for each study. Each question required a score that reflected the risk of bias: As per the original tool, '++' reflects a low risk of bias, '+' reflects probably a low risk of bias, '-' reflects probably a high risk of bias, and '- -' (double negative) reflects a high risk of bias.

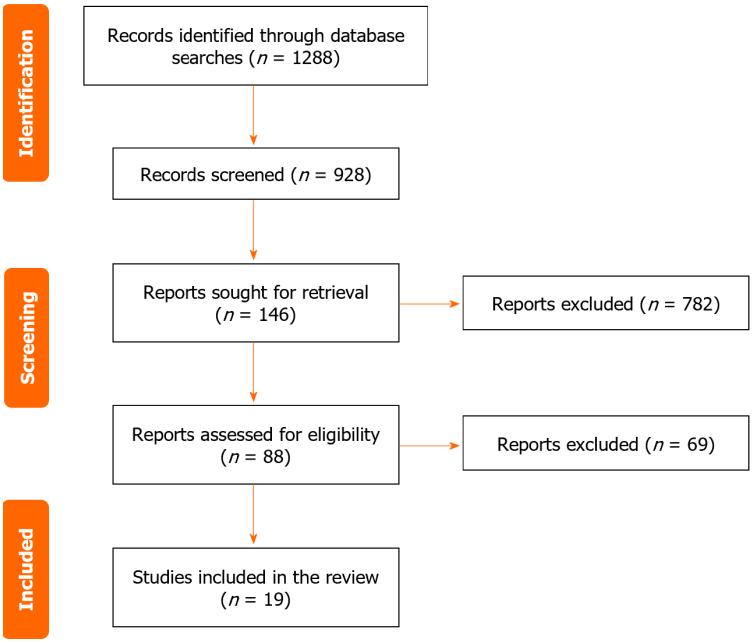

Figure 1 Preferred reporting items for systematic reviews and meta-analyses flow diagram of the included studies.

RESULTS

The outcomes of this systematic review, synthesized in a Prisma flowchart in Figure 1, highlight the rigorous methodology adopted from the initial literature search to the final inclusion of studies. The review was initiated with an exhaustive search across multiple databases, yielding a preliminary tally of 1288 articles. This was supplemented by five additional studies identified from alternative sources, bringing the total to 1293 articles considered for inclusion. The elimination of duplicates pared down this number to 928 unique articles, indicating the extensive nature of the initial search and the importance of reducing redundancy to streamline the review process.

A critical screening of titles further narrowed the pool to 146 articles, with 782 being excluded due to their lack of alignment with the review's stringent preliminary criteria. This step is essential in ensuring that only articles with the most relevant content proceed to the next stage, thereby maintaining the precision and focus of the review. The in-depth evaluation of 88 full-text articles for eligibility resulted in the exclusion of 58 articles. The reasons for these exclusions were varied but primarily related to a divergence from the review's central theme or failure to meet the established inclusion criteria, emphasizing the review's commitment to methodological rigor and thematic relevance. Particular attention was given to the types of studies considered, with a focus on excluding reviews (systematic reviews and literature reviews), surveys, case reports, and other research designs not directly contributing to the review's objectives. This led to the exclusion of 12 studies[11,14,19-28] due to factors such as thematic inconsistency and methodological flaws, highlighting the critical evaluation process in maintaining the integrity of the review. Furthermore, the research designs of two additional studies[29,30] resulted in their exclusion, underscoring the stringent adherence to the review's methodological standards.

Upon meticulous consideration, 19 articles were selected for inclusion, each offering significant insights into the research question through diverse methodological approaches. This selection underscores the necessity of a systematic and objective evaluation to identify studies that significantly contribute to understanding the topic. These studies collectively span a broad range of methodologies, from quantitative analyses to qualitative assessments, reflecting the complexity of the research field and the evolving nature of its investigative methods. Table 1 presents a detailed comparison of cell separation techniques, from mechanical to enzymatic methods, and their impact on cell yield, viability, and efficiency[31-47]. Techniques such as the vibrating shaker and centrifugation, as employed by Raposio *et al*[31], alongside innovative tools like the LipocubeNano and Tulip NanoTransfer Kit by Cohen *et al*[32], are highlighted for their procedural variability and outcomes in terms of cell viability and counts. The pioneering concept of nanofat by Tonnard *et al*[2] and the comparison of mechanical disaggregation *vs* enzymatic dissociation by Sesé *et al*[33] are notable for demonstrating significant differences in cell yields. Additionally, the table reviews procedural innovations, such as the use of the Lipogems system reported by Bianchi *et al*[34], which indicated a higher percentage of mature pericytes and MSCs, showcasing the critical role of methodology in optimizing cell isolation and viability for therapeutic purposes. The inclusion of data on processing techniques by Bright *et al*[35] and a comparison of cell yields across different systems by Gentile *et al*[36] provide essential insights into the efficiency and effectiveness of various separation methods. Table 2 represents the risk of bias in the included studies based on the OHAT criteria. This comprehensive analysis underscores the methodological nuances that influence the advancement of regenerative medicine and cell-based therapies, serving as a pivotal reference in understanding the landscape of SVF and ADSC separation techniques.

Table 1 Summary of the included studies in the systematic review

Ref.	Method of separation	No. of samples	Open/closed	Time taken	Cell counts	Significance
Raposio *et al*[31]	Vibrating shaker at 6000 rpm for 6 min followed by centrifugation at 1600 rpm for 6 min	-	Open	15 min	125000 nucleated cells per cc of lipoaspirate, but only about 5% of these were found to be progenitor cells	A pellet is formed at the end
Cohen *et al* [32]	LipocubeNano decanted for 3 min in a syringe: First, Port 1 is used to pass the fat graft once, resulting in 1 mm parcel sizes. After that, the fat is transferred 10 times back and forth between Ports 2 and 3, smoothing and homogenizing the fat tissue. Finally, to produce the final product, Nanofat, the fat was transferred once from Port 3 to Port 4 *via* a 500-micron single filter. Tulip NanoTransfer Kit. After decantation for 3 min, transfer millifat from Port 1 to 2. Flush the fat between Ports 2 and 3 for 10 times. Collect the final product by transferring fat from Ports 3 to 4 in a single stroke	10 patients	Closed	Not mentioned (Approximately less than 10 min for both methods)	LipocubeNano resulted in relatively high cell counts (2.24×10^6/mL) and cell viability (96.75%), whereas Tulip's NanoTransfer method resulted in a lower cell count of 1.44×10^6/mL and cell viability of 96.75%	-
Tonnard *et al*[2]	Lipoaspirate is washed and rinsed followed by 30 passes done between two 10 mL syringes connected by leur lock and the resultant whitish fluid is filtered over a sterile nylon cloth	67 cases	Open	Not mentioned (Approximately less than 10 min)	1975000 cells per 100 mL of lipoaspirate	Introduced the concept of nanofat
Sesé *et al* [33]	Enzymatic dissociation using GID stromal vascular fraction protocol. Mechanical dissociation using Tulip. NanoTransfer Protocol	20 for enzymatic and 6 for mechanical	Closed	Not available	Enzymatically dissociated stromal vascular fraction resulted in 0.68 million cells/g lipoaspirate, whereas mechanically disaggregated nanofat resulted in 6.63 million cells/g lipoaspirate	-
Bianchi *et al*[34]	Lipogems	-	Closed	Less than 20 min	The significantly higher percentage of mature pericytes and MSCs, and lower number of hematopoietic elements, than enzymatically digested lipoaspirates	-
Bright *et al* [35]	Centrifugation for 2 min at 200 *g* of lipoaspirate. Followed by an ultrasonic cavitation device probe using Hielschler UP200s set at 50% amplitude and cycle of 0.4 for 1 min with the probe lowered and 30 s at the top of the tube. The resultant fluid is subjected to centrifugation for 5 min at 300 *g* with a temperature not rising above 43 degrees and preferably not over 37 degrees	-	Open	-	169 million cells were injected intraartic-ularly for a patient with anterior cruciate tear mentioned but not specified the volume	Described as a patented procedure. Different modific-ations of this technique also have been described based on the indication and site of therapeutic application
Gentile *et al*[36]	Mystem system – washing and filtration. Fastem- Filtration and centrifugation for 10 min at 1700 rpm	10 for Fastem and 10 for Mystem	Closed for both systems	Not available	Cell yield achieved with Mystem is less than that with Fastem and Cytori (enzymatic isolation technique)	Improved contour seen after breast reconstruction with fat grafts enriched with ADSCs from Fastem (equal to Cytori) greater than Mystem
van Dongen *et al*[22]	Centrifuged at 300 rpm for 2.5 min followed by non-enzymatic dissociation performed by pushing the lipoaspirate to and through a fractionator 30 times. The resultant fluid is centrifuged for 2.5 min at 3000 rpm	-	Open	10 min	2.7×10^6/mL	-
Chaput *et al*[37]	Vortexing and centrifuging. Vibrating shaker for 6 min at 3200 rpm followed by	21	Open	22 min	The percentage of ADSCs in SVF	Final pellet for vortexing and

	centrifugation for 6 min at 558 g followed by 100 micrometre sieves followed by centrifugation for 10 min Dissociation by inter-syringe processing 30 passes through leur lock connected syringes passed through 100-micrometre sieve followed by centrifugation for 10 min		Open	11 mins	extracted by vortexing and centrifugation, dissociation by inter-syringe process, and enzymatic isolation techniques are 5.81 ± 1.3, 38.11 ± 5.1, and 21.45 ± 2.52, respectively	centrifuging Final pellet for dissociation by inter-syringe processing
Copcu *et al* [38]	Centrifuged at 500 *g* for 2 min. Adinizing was first performed with a 4000-micron Adinizer; after approximately 25 passes, the cutting process was continued with the next-smaller diameter disk followed by centrifugation for 6 min at 1600 *g*	24 patients	Open	Not mentioned	93% mean viability and cell counts of 28.66 to 88.88 × 10^6 from 100 mL of condensed fat	Volumes ranging from 3-12 mL can be produced depending on the indication
Rose *et al* [39]	Sedimentation for 1 h, centrifugation at 3000 rpm for 3 min or washing with normal saline combined with 3 min of centrifugation at 3000 rpm	24 fat samples	Open	-	The mean cell count per high-powered field of histologically intact adipocytes was 27.1 for specimens processed by sedimentation, 14.2 for centrifuging, and 11.8 for washing	-
Amirkhani *et al*[40]	Dissected for 10 s using a blender mixer followed by sonic cavitation for 2 min at 18 MHz followed by centrifugation for 10 min at 900 *g* followed by suspension with 150 mM ammonium chloride for 5 min and centrifugation for 5 min at 400 g. The pellets are then resuspended in DMEM supplemented with 10% FBS and then seeded into a T25 culture flask. After 24 h, the adherent cells were used for further confirmation tests. The SVFs harvested by both methods were suspended in PBS and then incubated for 30 min at 4 °C with the antibodies conjugated with FITC against CD34, CD44, CD73, CD90, and CD105 biomarkers	-	Open	Less than 30 min	Viable cells 2.6 × 10^5 cells from 1 mL of fat tissue	-
Victor[41]	Ultrasonic cavitation performed using a 200 W generator (SONIC 200) for a range of 10 to 20 min at a frequency of about 20-30 kHz	-	Open	-	From 2 million up to 22 million stromal vascular cells per mL of adipose tissue	Described as a patented procedure
Domenis *et al*[42]	Fastem - automated system performing filtration and centrifugation for 10 min at 1700 rpm	6	Closed	Not available	Only mentioned that cell yield from Fastem was less than Lipokit and less than Cytori	Enriched grafting has greater subcutaneous thickness
Condé-Green *et al* [43]	Centrifugation followed by vortexing for 3 min. Centrifugation followed by RBC lysis	9	Open	Not available	cSVF of 1.2 × 10^4 per mL for the first method and 2.3 × 10^4 per mL for the second method	Mechanical methods have greater cells positive for CD14 than with enzymatic process which is a marker for monocytes and macrophages
Markarian *et al*[44]	The first method involved RBC lysis of lipoaspirate and then centrifugation for 10 min at 600 *g*. The second and third techniques each included an additional initial stage of centrifugation at 800 *g* and 1280 *g* for 15 min, respectively	10	Open	Not available	The cell yield obtained from collagenase was greater than that of mechanical and trypsin. The second and third methods produced viable cells that had not prolif-erated even after 14 d	-
Shah *et al* [45]	Rigorous washing in PBS with handshaking followed by centrifugation for 15 min at 900 *g*	13	Open	1 h for mechanical and almost 3 h for isolation with collagenase	The mechanical method produced 19 times fewer cells compared to the enzymatic extraction technique	-
Condé-Green *et al*	Lipoaspirate is subjected to RBC lysis followed by 15 min of centrifugation at	10	Open	Not available	The highest concen-tration of ADSCs was	-

[46]	900 *g*				found in the pellet found at the bottom after centrifugation	
Baptista *et al*[47]	The procedure followed in the same sequence. RBC lysis, centrifugation for 15 min at 900 g, resuspension in fetal bovine serum plus dimethyl sulfoxide, cryopreservation at –196 degrees centigrade	13	Open	Mechanical processing required less time	Cell yield was less with mechanical compared to enzymatic processing	Adherent cells were positive for CD44, CD90, CD105, and CD34 and negative for CD45 and CD73

MSCs: Mesenchymal stem cells; ADSCs: Adipose-derived stem cells; SVF: Stromal vascular fraction; FBS: Foetal bovine serum; RBC: Red blood cell; cSVF: Cellular stromal vascular fraction; PBS: Phosphate buffered saline.

Table 2 Risk of bias in the included studies based on office of health assessment and translation criteria

Ref.	Did the selection of study participants appropriate?	Did the study account for confounding and modifying variables?	Were the outcome data complete without attrition bias?	Can we be confident in exposure characterization?	Can we be confident in outcome assessment?	Were all measured outcomes reported?
Raposio *et al* [31]	++	+	++	++	++	++
Chaput *et al* [37]	++	++	+	+	++	+
Cohen *et al* [32]	++	+	+	- (NR)	+	+
Copcu *et al* [38]	++	++	++	+	+	++
Tonnard *et al*[2]	++	+	+	+	+	+
Sesé *et al*[33]	++	+	+	+	+	+
Rose *et al* [39]	++	+	++	++	++	++
Bianchi *et al* [34]	++	++	++	++	++	++
van Dongen *et al*[22]	++	+	+	- (NR)	+	+
Amirkhani *et al*[40]	++	++	++	++	++	++
Victor[41]	++	++	++	++	++	++
Bright *et al* [35]	++	-	+	+	+	+
Domenis *et al*[42]	++	++	+	- (NR)	+	+
Gentile *et al* [36]	++	+	+	+	+	+
Condé-Green *et al* [43]	++	++	++	++	++	++
Markarian *et al*.[44]	++	++	+	++	++	++
Shah *et al* [45]	++	++	++	++	++	++
Condé-Green *et al* [46]	++	++	++	- (NR)	+	+
Baptista *et al*	++	+	+	++	++	++

[47]

NR: Not reported; ++: Reflects a low risk of bias; +: Reflects probably a low risk of bias; -: Reflects probably a high risk of bias.

DISCUSSION

In the evolving landscape of regenerative medicine, the utilization of autologous cellular SVF (cSVF) for therapeutic applications represents a significant advancement. This discussion systematically reviews the efficacy, challenges, and clinical implications of mechanical *vs* enzymatic isolation techniques of cSVF, with a focus on their application in osteoarthritis, chronic wounds, bone and cartilage disorders, and Crohn's disease, and as vectors for drug delivery to malignancies[48-51]. The traditional enzymatic digestion method, while effective, faces several limitations including extensive processing time, high costs, and stringent regulatory challenges as outlined by the United States Food and Drug Administration[52,53].

The advent of mechanical cell separation techniques introduces a promising alternative, offering reduced processing time and potentially lower regulatory hurdles. Techniques such as centrifugation, vortexing, and manual shaking have been developed, yet their clinical applicability remains underexplored due to limited published data[31,36,42,45,54]. This gap underscores the necessity for further empirical evidence to validate the reliability and usefulness of these mechanical methods in clinical settings. Mechanical isolation techniques, including innovative automated systems like Fastem, Mystem, and Lipogems, have shown the potential to enhance outcomes in fat grafting procedures. These systems promise a streamlined isolation process within a single device, potentially mitigating risks of contamination and improving volume retention in breast reconstruction surgeries[36,42]. However, the efficiency of these mechanical methods, especially in terms of cell yield and viability, needs thorough evaluation when compared to traditional enzymatic digestion, which is known for its higher cSVF output.

A critical aspect of mechanical separation is its product outcome. Techniques developed by researchers such as Tonnard *et al*[2] and Bianchi *et al*[34] focus on producing a fat-grafting material rich in viable MSCs rather than isolating cSVF as a standalone product. This approach highlights the variability in mechanical isolation outcomes and their implications for clinical practice, emphasizing the need to delineate between methods aimed at enriching fat grafts *vs* those isolating cSVF for broader therapeutic applications. The time efficiency of mechanical methods presents a significant advantage over enzymatic procedures, with some requiring as brief as 30 s for processing[55]. However, the variability in cell yield, survival, and composition of the SVF obtained through mechanical means raises questions about their efficacy and the potential impact on therapeutic outcomes. Furthermore, the effects of mechanical manipulation on cell integrity and the proliferative potential of ADSCs warrant careful consideration, as repetitive processing may compromise cell yield and increase the risk of contamination[42,44,45,56].

The role of ADSCs, characterized by their immunomodulatory, angiogenic, and multipotent properties, is crucial in the context of fat graft maintenance and overall therapeutic efficacy[11,31,45,47,57,58]. The potential adverse effects of mechanical *vs* enzymatic isolation on these cell populations and their functional capabilities remain a pivotal area for further investigation. This exploration is essential to determine whether the differences in cell output and population composition observed with enzymatic methods translate to superior clinical outcomes, justifying their longer processing time and higher associated costs. Considering the therapeutic potential of enriching autologous adipose tissue transfers with ADSCs, the exploration of mechanical processing techniques becomes imperative. These methods offer a promising avenue for enhancing the outcomes of reconstructive and cosmetic procedures by potentially providing a safer and more efficient alternative to enzymatic digestion[25,59,60]. Nonetheless, the challenge of achieving consistent and replicable results due to the heterogeneous nature of mechanically processed SVF highlights the necessity for standardized procedures and rigorous quality control measures.

The primary limitation of this review lies in the novelty of the mechanical isolation techniques and the corresponding scarcity of comprehensive, large-scale comparative studies. The existing literature, characterized by a diversity of methods, small sample sizes, and a lack of randomized control trials, hampers the ability to draw definitive conclusions about the efficacy and safety of mechanical *vs* enzymatic isolation techniques. This variability and methodological heterogeneity limit the strength of the evidence base, underscoring the need for further research. Specifically, well-designed studies comparing mechanical and enzymatic isolation methods are critical to establishing standardized, efficient, and safe practices that can be broadly implemented in clinical settings. The journey toward optimizing cSVF isolation techniques for clinical application is complex and requires a multifaceted approach to research and development. As the field of regenerative medicine continues to evolve, the quest for effective, efficient, and safe methods of cell isolation remains at the forefront of scientific inquiry. The potential of cSVF to revolutionize the treatment of a wide range of conditions is immense, yet realizing this potential hinges on overcoming the current limitations and advancing our understanding of the best practices for cell isolation and application.

CONCLUSION

This systematic review meticulously evaluates the non-enzymatic methods for isolating the SVF and ADSCs from adipose tissue, offering a comprehensive comparison to the traditional enzymatic approaches. The findings underscore the promise of mechanical isolation techniques in addressing the limitations of enzymatic methods, including reducing

processing time, mitigating regulatory hurdles, and potentially enhancing the safety and efficacy of cell-based regenerative therapies. Despite the demonstrated advantages of mechanical methods, such as increased procedural simplicity and the avoidance of enzymatic reagents, this review also highlights the variability in cell yield, viability, and functional integrity of the isolated cells. The current evidence suggests that while non-enzymatic methods hold significant potential for clinical application, their outcomes are varied and require further investigation to optimize cell quality and therapeutic efficacy. The scarcity of large-scale, randomized controlled trials comparing mechanical and enzymatic isolation methods signifies a crucial gap in the literature, emphasizing the need for standardized methodologies and rigorous research to establish evidence-based practices in the field of regenerative medicine. As the field advances, the development and refinement of non-enzymatic isolation techniques will be critical in realizing the full therapeutic potential of SVF and ADSCs, offering promising avenues for enhancing patient outcomes across a broad spectrum of medical conditions.

FOOTNOTES

Author contributions: Naidu M contributed to conceptualization; Mundluru VK, Mundluru RT, and Jeyaraman N contributed to data collection; Mundluru VK, Jeyaraman M, and Ramasubramanian S contributed to manuscript writing; Muthu S contributed to manuscript revision; Muthu S and Jeyaraman M contributed to proofreading; Jeyaraman M contributed to administration.

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