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# A Literature Review Comparing Enzymatic and Non-Enzymatic Methods for Adipose-Derived Stem Cell (ADSC) Isolation

**Fillah Muty Syahidah**
Universitas Indonesia, Indonesia
*fillah.muty@ui.ac.id*

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**ABSTRACT**

The isolation of adipose-derived stem cells (ADSCs) is essential in regenerativ medicine and tissue engineering. Two primary approaches, enzymatic and non enzymatic methods, are widely used to obtain ADSCs from adipose tissue.
Objective: This review aimed to compare the efficiency, viability, purity, regulator compliance, and clinical applicability of both methods.
Methods: A comprehensive literature review was conducted by searching PubMed Scopus, and Google Scholar for articles published between 2018 and 2024.
Results: The findings indicated that the enzymatic method yielded a higher number o ADSCs with viability exceeding 90% and greater cell purity. However, the use o collagenase and other proteolytic enzymes posed risks of xenogenic contamination an required strict regulatory approval, as it was classified as "more than minima manipulation" by the FDA. Conversely, the non-enzymatic method, includin mechanical dissociation and explant techniques, was categorized as "minima manipulation", making it more suitable for clinical applications due to fewer regulator constraints. Despite its advantages in cost and safety, this method produced lower cel yields and required longer processing times.
Conclusion: The enzymatic method remained the gold standard for ADSC isolatio due to its high efficiency and purity. However, optimizing non-enzymatic techniques i necessary to enhance their efficiency and broaden their application in stem cell-base therapies.



## INTRODUCTION

Mesenchymal stem cells (MSCs) are a type of multipotent stem cell with significant potential in regenerative medicine and tissue engineering. MSCs can differentiate into various cell types, including osteoblasts, chondroblasts, and adipocytes, making them widely utilized in both research and clinical applications (See Fig.1). MSCs can be isolated from various tissues, including both adult and fetal sources. Among these, adipose tissue is a particularly abundant and accessible source, offering a straightforward isolation procedure. Since their initial isolation by Zuk et al. (2001), research on adipose-derived stem cells (ADSCs) has expanded rapidly. Recent studies have demonstrated that ADSCs exhibit high proliferative capacity and promising immunomodulatory potential for cell-based therapies (Fuoco et al., 2020; Sherman et al., 2019; Zuk et al., 2001).

Research on adipose-derived stem cells (ADSCs) has expanded rapidly. ADSCs demonstrate high proliferative capacity and immunomodulatory potential, contributing to their frequent use in cell-based therapies (Fuoco et al., 2020; Sherman et al., 2019). They exhibit low expression of major histocompatibility complex (MHC) class I molecules and lack MHC class II and key costimulatory molecules such as CD40, CD80 (B7-1), and CD86 (B7-2). This immunophenotypic profile enables ADSCs to evade immune detection, reducing the risk of rejection in allogeneic transplantation settings (Conese et al., 2020). Their immune-privileged status, combined with their ability to secrete bioactive factors that promote angiogenesis, reduce fibrosis, and regulate inflammation, supports their

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application in a broad range of diseases, including wounds, cardiovascular conditions, and neurodegenerative disorders (Zhou et al., 2024).

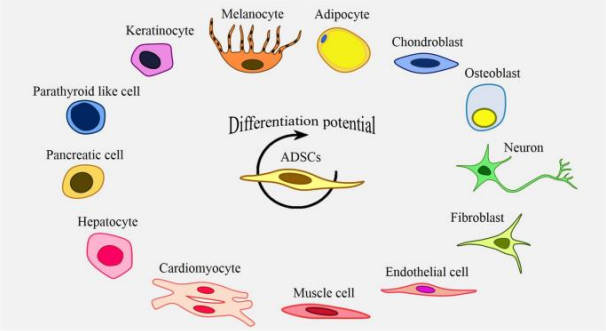
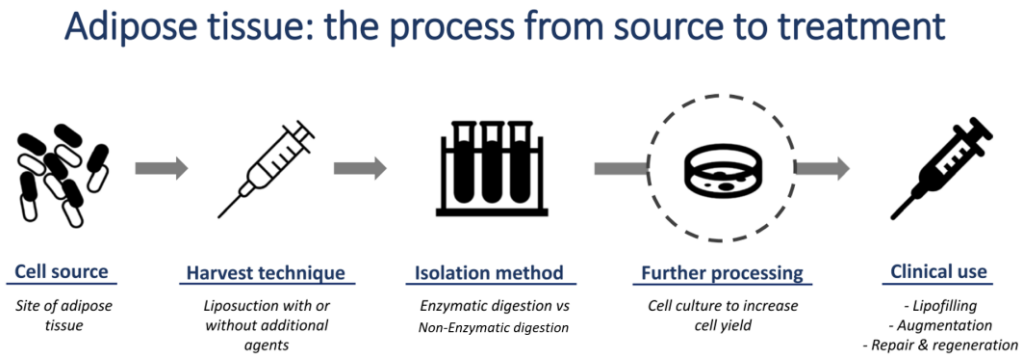



**Figure 1. Differentiation Potential of ADSCs** (Khazaei et al., 2022)

ADSCs exhibit low expression levels of major histocompatibility complex (MHC) class I molecules, while lacking expression of MHC class II and key costimulatory molecules, including CD40, CD80 (B7-1), and CD86 (B7-2). This immunophenotypic profile allows ASCs to evade recognition by the immune system, reducing the risk of immune rejection when used in allogeneic transplantation. Their immune-privileged nature makes them highly suitable for regenerative medicine and cell-based therapies, as they can modulate immune responses while maintaining their differentiation potential (Conese et al., 2020). ADSCs possess broad therapeutic potential, particularly in inflammation modulation and tissue regeneration. They exert their effects primarily through the secretion of bioactive factors that enhance angiogenesis, reduce fibrosis, and suppress inflammation by promoting macrophage polarization toward the M2 phenotype. Several studies have highlighted the effectiveness of ADSCs in wound healing and the treatment of metabolic, cardiovascular, and neurodegenerative diseases, establishing them as a leading candidate for regenerative medicine (Zhou et al., 2024). ADSCs can be obtained through stromal vascular fraction (SVF) isolation or directly from adipose tissue. SVF is a heterogeneous fraction containing various cell types, including endothelial cells, pericytes, fibroblasts, macrophages, pre-adipocytes, and ADSCs (Yaylacı et al., 2023). Currently, two main approaches are widely used for ADSC isolation: enzymatic and non-enzymatic methods. The enzymatic method involves the use of proteolytic enzymes, such as collagenase, to dissociate ADSCs from adipose tissue, whereas the non-enzymatic method utilizes mechanical or explant-based approaches to obtain ADSCs without additional enzymatic digestion (Goulas et al., 2024).

The selection of an appropriate isolation method is a crucial aspect of ADSC application in clinical therapy. The enzymatic method is widely used due to its ability to yield a high number of cells with optimal purity (Fuoco et al., 2020). However, this method is classified as "more than minimal manipulation" by the Food and Drug Administration (FDA) under 21 CFR 1271.3(f) and 21 CFR 1271.3(c), requiring stringent regulatory compliance, including the Investigational New Drug (IND) Application and Biologics License Application (BLA). This classification is based on the significant alteration of the native tissue during the isolation process. In contrast, the non-enzymatic method, such as mechanical or explant-based approaches, is considered "minimal manipulation", as it does not significantly modify the structure or function of the tissue. Therefore, non-enzymatic methods are more suitable for applications that comply with the "homologous use" principle and are subject to more flexible regulatory requirements. Despite advancements in ADSC isolation techniques, strict regulations remain a significant challenge in clinical applications (FDA, 2017). A schematic flowchart illustrating this process, outlining the key stages involved in transforming adipose tissue into a viable cell-based therapeutic product (See Fig.2).




**Figue 2. Schematic of cell-based therapy development from adipose tissue** (Liu et al., 2022)

The enzymatic method is known for its high efficiency in obtaining an optimal number of ADSCs. However, this approach has several limitations, including the risk of xenoprotein and endotoxin contamination due to the use of exogenous enzymes such as collagenase. Additionally, the use of fetal bovine serum (FBS) in ADSC culture raises concerns regarding potential contamination with viruses and xenobiotic agents. As an alternative, xeno-free approaches, such as human platelet lysate (hPL), have been developed to provide a safer option that complies with Good Manufacturing Practice (GMP) standards (Fuoco et al., 2020). Conversely, the non-enzymatic method offers a simpler solution by eliminating the need for exogenous enzymes, making it more compatible with GMP regulations and reducing the risk of contamination. However, this method has certain limitations, including lower isolation efficiency compared to enzymatic methods, as well as the need for larger volumes of lipoaspirate to obtain a sufficient number of ADSCs. Additionally, ADSCs isolated using non-enzymatic techniques tend to have lower viability, which may affect their effectiveness in clinical applications (Uguten et al., 2024).

Although both methods have their respective advantages and disadvantages, research gaps remain regarding the optimal isolation method in terms of efficiency and safety. Further studies are required to evaluate the differences between enzymatic and non-enzymatic isolation techniques across various parameters, including cell yield efficiency, viability and differentiation potential, cell purity, regulatory compliance, clinical safety, cost, and accessibility. Therefore, this review aims to comprehensively analyze both isolation methods, focusing on their effectiveness in obtaining ADSCs that meet the required standards for regenerative therapy. By understanding the advantages and limitations of each method, more efficient isolation strategies can be identified to better align with current clinical needs.

## RESEARCH METHODS

This review was conducted by analyzing literature related to enzymatic and non-enzymatic methods for isolating adipose-derived stem cells (ADSCs). A comprehensive literature search was performed using PubMed, Scopus, and Google Scholar to obtain articles published between 2018 and 2024. The search utilized keyword combinations such as "Adipose-derived stem cells" OR "ADSCs", "Isolation" OR "Enzymatic digestion" OR "Collagenase", and "Non-enzymatic isolation" OR "Mechanical isolation" OR "Explants method", with Boolean operators (AND/OR) applied to refine relevant results.

A total of 214 articles were initially identified through database searches. After removing duplicates (n = 37), 177 articles remained. Titles and abstracts were then screened for relevance, resulting in the exclusion of 109 studies that did not meet the inclusion criteria. The full texts of the remaining 68 articles were assessed in detail. Following this screening, 32 articles were included in the final review. The screening process adhered to PRISMA guidelines and is illustrated in a flow diagram (See Fig. 3).

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The articles included in this review met the following inclusion criteria: (1) peer-reviewed publications, (2) written in English, and (3) studies comparing enzymatic and non-enzymatic ADSC isolation methods, with evaluations of cell viability, proliferation, and differentiation potential. Conversely, articles that were purely literature reviews without experimental data, studies focusing on genetically modified ADSCs, and research that did not use human adipose tissue sources were excluded from the analysis. The selected articles were critically analyzed based on their methodology, findings, and relevance to the comparison of efficiency and clinical applications of enzymatic and non-enzymatic ADSC isolation techniques.

## RESULTS AND DISCUSSION

### Enzymatic Method for ADSC Isolation

The enzymatic method is a widely used approach for isolating ADSCs utilizing proteolytic enzymes to digest the extracellular matrix (ECM) and release cells from adipose tissue. The main procedure in this method involves tissue digestion using enzymes such as collagenase, trypsin, and dispase, followed by filtration and centrifugation to obtain stromal vascular fraction (SVF), which is rich in ADSCs.

The isolation of ADSCs using enzymatic methods has been extensively studied to improve cell recovery efficiency while maintaining optimal viability. A study reported that enzymatic isolation of ADSCs produced more than $1 \times 10^6$ cells per gram of adipose tissue, with a viability rate exceeding 90%. This isolation procedure was carried out by digesting adipose tissue using a combination of collagenase, trypsin, and dispase, followed by centrifugation and filtration to separate SVF from unwanted tissue components. The main advantage of this method lies in its ability to preserve the biological properties of ADSCs, ensuring that the isolated cells retain their proliferation and differentiation capacity. This study also highlighted the role of culture media in supporting ADSC growth and expansion after isolation. Conventionally, fetal bovine serum (FBS) is used as a nutrient source in culture media; however, its application raises concerns regarding xenogenic contamination and the potential to trigger immunogenic responses in clinical applications. Therefore, recent studies have proposed human platelet lysate (HPL) as a safer alternative that is more clinically compatible. HPL is known to contain various growth factors that support cell proliferation more effectively than FBS, without the risk of contamination from animal-derived sources. Several reports have also shown that ADSCs cultured in HPL-based media exhibit faster expansion rates and maintain a more stable differentiation capacity compared to FBS-based cultures (Wang et al., 2024).

Another study evaluated various ADSC isolation approaches from bovine adipose tissue, incorporating different enzymes and incubation conditions. The results showed that incubation with 0.1% Liberase™ for 3 hours produced $30.48 \times 10^6$ to $67.1 \times 10^6$ cells per gram of adipose tissue, with a viability rate exceeding 95%. The technique included filtration using 70 μm cell strainers and centrifugation at 400×g for 5 minutes to obtain an ADSC-rich fraction. Additionally, the isolated cells retained their ability to differentiate into adipogenic, osteogenic, and chondrogenic lineages, demonstrating that their biological quality remained preserved. This study also emphasized that isolation efficiency is significantly influenced by factors such as enzyme type, concentration, and incubation conditions. Compared to other methods, the use of Liberase™ has been shown to be more efficient in ECM digestion, resulting in higher cell yields with optimal viability. Nevertheless, the study also noted potential challenges in clinical applications, particularly the risk of contamination from animal-derived enzymes, which could lead to stricter regulatory requirements for ADSC-based therapies (Heyman et al., 2024).

Although variations in cell yield were observed between the two studies, these differences may be attributed to the type of adipose tissue used (human vs. bovine), the type and concentration of enzymes applied, and differences in incubation conditions. The study by Wang et al. (2024) utilized human adipose tissue with a combination of several enzymes, whereas Heyman et al. (2024) used bovine adipose tissue with a broader range of enzymes, including Liberase™, which is known to be more efficient in ECM digestion. The main advantage of the enzymatic method is its high efficiency

in isolating ADSCs, yielding a greater number of cells compared to the non-enzymatic method. Furthermore, the optimal viability of the isolated cells allows for their use in various applications, particularly in regenerative medicine and tissue engineering.

Nevertheless, a major challenge of this method is the risk of contamination from animal-derived enzymes, which can increase production costs and require stricter regulatory compliance in clinical applications. Heyman et al. (2024) noted that the use of animal-derived enzymes, such as collagenase type I and IV, poses a potential immunogenic risk in human cell-based therapies, necessitating additional steps for purification and clinical validation. To overcome these challenges, several alternatives are currently being developed, including recombinant enzymes and enzyme-free cell isolation protocols. In conclusion, despite some challenges related to regulatory and safety concerns, the enzymatic method remains the gold standard for ADSC isolation due to its ability to produce high cell yields with optimal viability. Therefore, this technique continues to be widely used in both research and clinical applications.

**Non-Enzymatic Method for ADSC Isolation**

The non-enzymatic method enables the isolation of ADSCs without using proteolytic enzymes, instead relying on physical techniques such as filtration, emulsification, centrifugation, cutting/mincing, decantation, and washing. Unlike the enzymatic method, which utilizes enzymes to digest the ECM, the non-enzymatic approach aims to separate ADSCs while preserving the microenvironment of adipose tissue as optimally as possible. This technique has gained increasing attention due to its simpler process, lower cost, and better compliance with minimal manipulation regulations set by the FDA, making it more feasible for clinical applications (Uguten et al., 2024). However, the non-enzymatic method generally yields fewer cells compared to enzymatic digestion. Certain mechanical techniques, such as cutting/mincing, have been shown to enhance cell concentration and viability, with viability rates ranging between 60% and 98%, depending on the processing technique used. Despite these advantages, there is no strong evidence confirming a direct correlation between non-enzymatic ADSC isolation and improved clinical outcomes (Liu et al., 2022).

In the following sections, several non-enzymatic techniques, including mechanical dissociation, emulsification, microfluidic systems, and explant methods, will be discussed in detail, highlighting their advantages, limitations, and clinical relevance.

***Mechanical Dissociation Technique***

Mechanical dissociation techniques for ADSC isolation involve various physical approaches that break down adipose tissue without the use of enzymes. These methods rely on shear stress, cutting forces, and mechanical disruption to release ADSCs while preserving extracellular matrix ECM components. Several mechanical dissociation methods have been developed, including intersyringe emulsification, microblades dissociation, and needle-based dissociation. Each technique offers distinct advantages and limitations, influencing cell yield, viability, and clinical applicability.

One of the mechanical approaches used for ADSC isolation is intersyringe emulsification (See Fig.3). In this method, adipose tissue is cut into small fragments and then transferred back and forth between two 10 mL syringes equipped with a Luer-lock system approximately 30 times to break down the tissue structure. This technique applies controlled mechanical shear forces to fragment the extracellular matrix while preserving cell viability. Studies have shown that the size of the Luer-lock connector significantly impacts the level of mechanical stress applied, which in turn influences the viability and composition of ADSCs. A three-way stopcock with a 2 mm diameter for each hole has been evaluated for its impact on tissue viability, with findings indicating that intersyringe processing up to 30 times does not negatively affect cell viability (Schipper et al., 2023)**.** After the emulsification process, the sample is centrifuged at 558×g for 10 minutes to obtain SVF rich in ADSCs. The isolation results showed that this technique was capable of yielding $59 \times 10^6$ ADSCs from 20 mL of lipoaspirate, with a viability rate of 91%, and 89% of cells expressing CD90 and CD105. Although the yield obtained is lower than that of the enzymatic method, this technique offers several advantages, including a faster procedure (approximately 15–30 minutes), lower risk of contamination, and a more cost-effective approach. Additionally, optimizing connector aperture size can mitigate

excessive shear stress and minimize cellular damage, making intersyringe emulsification a promising mechanical approach for ADSC isolation (Schipper et al., 2023). However, since this method does not use enzymes to digest ECM, the resulting fraction tends to be more heterogeneous, containing various cell types in addition to ADSCs, such as fibroblasts and endothelial cells (Goulas et al., 2024).

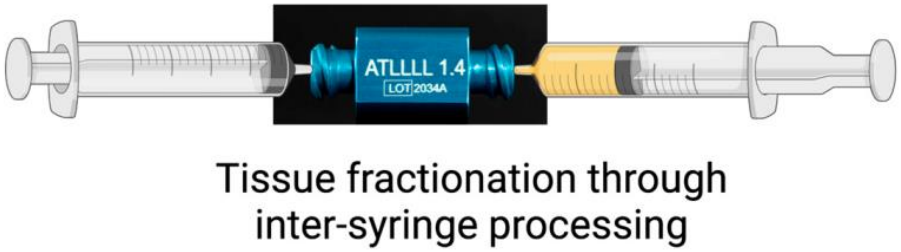


**Figure 3. The intersyringe emulsification technique** (Schipper et al., 2023)**.**

Another mechanical approach for ADSC isolation is using the MycroLizer Method. This technique utilizes a microfluidic system with microblades measuring 2400, 1200, and 600 microns to disrupt adipose tissue without requiring enzymes. The principle of this method is based on the mechanical fragmentation of adipose tissue by passing it through rapidly rotating microblades, which facilitate controlled tissue dissociation while minimizing damage to the cells. After the disruption process, the sample is washed with saline solution to remove residual fat and damaged cells, followed by centrifugation at 400×g for 10 minutes to obtain SVF rich in ADSCs. This technology falls under the closed-system category, similar to Lipogems, allowing cell isolation under sterile conditions with minimal contamination. The cell yield obtained through this method reaches 75% of that achieved with enzymatic methods while maintaining a high viability rate (~90%). One of the main advantages of the MycroLizer Method is its efficiency in preserving the physiological conditions of ADSCs and ensuring compliance with clinical regulations, as it does not involve animal-derived enzymes. Additionally, the retention of extracellular matrix (ECM) components during this process may enhance the regenerative potential of the isolated cells. However, this method has certain limitations, including a lower cell yield compared to enzymatic methods and the potential loss of tissue during microblade processing (Yaylacı et al., 2023).

Adipose tissue can also be disrupted using two L-shaped needles and then centrifuged at 900×g for 10 minutes to obtain SVF. This method relies on repeated mechanical shear forces applied by the L-shaped needles, which fragment the adipose tissue while preserving stromal cells. The repeated passage of tissue through the needles helps to break down the extracellular matrix (ECM), facilitating the release of ADSCs without the need for enzymatic digestion. One key factor influencing the efficiency of this method is the needle gauge, as variations in diameter affect the extent of mechanical disruption and the overall yield of viable cells. The main advantage of this technique is its simplicity, as it does not require specialized equipment such as microfluidic systems or Lipogems. Although the cell yield obtained is lower compared to the enzymatic method ($(6.4 \pm 1.4) \times 10^5$ cells per gram of adipose tissue), the viability of ADSCs remains high (~87.5%). Additionally, the use of HPL in culture media has been shown to enhance ADSC proliferation, even accelerating the population doubling time (PDT) to ~20.69 hours, compared to ~49.75 hours with FBS. Furthermore, the preservation of ECM components in this process may contribute to improved cell-matrix interactions, supporting better engraftment and functional integration in regenerative applications. Thus, this method serves as a more economical and safer alternative to the enzymatic method, although it requires a longer time to reach confluency in culture (Fuoco et al., 2020).

Mechanical dissociation techniques have evolved beyond the use of L-shaped needles, incorporating methods such as mincing (adiponizing) and vibration-based dissociation. These approaches aim to reduce mechanical stress on the cells while maintaining high viability. Unlike repeated emulsification, which may induce shear stress and apoptosis, vibration-based dissociation applies gentler mechanical forces, minimizing cellular damage. Furthermore, mechanical dissociation

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has the advantage of preserving the ECM, which serves as a biological scaffold that supports ADSC adhesion, proliferation, and differentiation. Studies have suggested that ECM retention in SVF may enhance its regenerative potential by providing essential signaling molecules. Recent developments in microfluidics-assisted dissociation and automated mechanical SVF extraction devices also present promising advancements in standardizing mechanical isolation while improving cell yield and purity. These innovations offer potential solutions to address the current limitations of mechanical dissociation, such as lower cell yield compared to enzymatic methods (You et al., 2024).

***Explant Technique***

Another non-enzymatic approach is the explant method, which allows ADSC isolation through natural cell migration from adipose tissue cultured in nutrient-rich media. A study demonstrated that this method can be performed by placing lipoaspirate or abdominoplasty fragments into tissue culture plates containing nutrient-enriched media. ADSCs migrate to the surface of the plate, adhere, and proliferate over 3–5 days, although in some cases, the process may take more than 7 days. The explant technique relies on the inherent ability of ADSCs to detach from tissue fragments and migrate towards a suitable substrate under appropriate culture conditions. This process is primarily driven by chemotactic signals and the interaction between cells and ECM components. Unlike enzymatic digestion, which actively breaks down ECM to release ADSCs, the explant method preserves ECM integrity, allowing gradual cell migration. The adherence of ADSCs to the culture plate is a crucial step, influenced by factors such as substrate coating, media composition, and oxygen levels. Over time, proliferating ADSCs form a monolayer, which can then be expanded for further applications. Since this method does not use enzymes or high-speed centrifugation, the resulting ADSCs are better suited for clinical applications requiring minimal manipulation. Although the initial cell yield is relatively low (~$5–7 \times 10^4$ cells per gram of adipose tissue) and the isolation process takes longer, this method offers advantages such as high cell viability and a lower risk of contamination compared to enzyme-based methods (Sherman et al., 2019).

Another study also reported similar findings in ADSC isolation using the explant method. Using subcutaneous adipose tissue from animal models, researchers found that cell migration occurred within 1–3 days, with optimal growth observed after 4–7 days. Unlike the enzymatic method, which can cause microstructural alterations in the tissue, the explant method preserves the natural ADSC microenvironment, making it more suitable for the minimal manipulation regulations established by the FDA. Additionally, supporting technologies, such as stainless steel mesh, can be used to prevent tissue fragments from floating in the medium, thereby enhancing isolation efficiency. The cell yield obtained through this method is approximately $2.4 \times 10^4$ cells per mg of adipose tissue after 7 days, with high cell viability, although slightly lower than that of enzymatic methods (Li, Jie. Li, Hui; Tian, 2018).

**Comparative Evaluation of Enzymatic and Non-Enzymatic Methods for ADSC Isolation**

Based on the discussion of ADSC isolation, both enzymatic and non-enzymatic methods have advantages and limitations that must be considered across various aspects, including cell yield efficiency, viability and differentiation potential, cell purity, regulatory and clinical safety, as well as cost and accessibility. The selection of the optimal method largely depends on the research needs or targeted clinical applications. To summarize the key differences between the two approaches, a comparative overview is presented in Table 1.

***Cell Yield Efficiency***

Cell yield is a key parameter in the efficiency of ADSC isolation, as it determines the number of cells available for further applications. The enzymatic method generally yields higher cell numbers compared to the non-enzymatic method. The use of collagenase allows for more effective cell separation from the ECM of adipose tissue, with yields reaching $1–5 \times 10^6$ cells/mL, depending on the technique and optimization conditions applied. In contrast, the non-enzymatic method shows more variable results. Mechanical techniques, such as intersyringe emulsification and microfluidic systems, can produce approximately 75% of the yield achieved by enzymatic methods, with relatively high efficiency without requiring enzyme use. However, the explant method has a lower yield, around (5–

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7) × $10^4$ cells per gram of adipose tissue, because cell release relies on natural cell migration from the tissue into the culture medium. This yield difference suggests that the enzymatic method remains the standard for large-scale ADSC production.

***Viability and Differentiation Potential***

Beyond quantity, ADSC quality is also a critical factor, particularly regarding viability and differentiation potential. The enzymatic method produces cell viability >90%, whereas the non-enzymatic method results in slightly lower viability, ranging from 85–91%, depending on the technique used. Interestingly, although the explant method yields fewer cells, the isolated cells retain good differentiation potential, as the natural isolation process better preserves the physiological conditions of the cells. Furthermore, several studies have shown that the use of HPL as a culture medium can enhance ADSC proliferation and accelerate PDT compared to FBS-based media. This strategy can help compensate for the lower yield observed in non-enzymatic methods, particularly in cell therapy applications.

***Cell Purity***

The purity of ADSC populations is a crucial factor in the success of cell-based therapies. The enzymatic method offers a major advantage in this aspect, as collagenase digestion selectively breaks down ECM, resulting in a purer ADSC population with minimal contamination from other cell types, such as fibroblasts, leukocytes, or endothelial cells. In contrast, non-enzymatic methods, particularly mechanical techniques, often produce more heterogeneous SVF fractions. This is because the isolation process does not completely separate ADSCs from other adipose tissue components, increasing the likelihood of contamination with non-mesenchymal cell populations. Therefore, if the goal is to obtain high-purity ADSCs, the enzymatic method remains the preferred choice.

***Regulatory and Clinical Safety***

From a regulatory and clinical safety perspective, the non-enzymatic method offers a significant advantage, as it complies with the minimal manipulation standards set by the FDA and other international regulations. The FDA classifies enzymatic methods as "more than minimal manipulation", as the use of enzymes is considered to significantly alter the biological properties of the tissue. Consequently, enzymatic procedures require additional safety evaluations, including verification that no residual enzymes remain in the final product before clinical use. In contrast, non-enzymatic methods, such as intersyringe emulsification and explant techniques, are more favorable for point-of-care clinical applications, where ADSCs can be isolated and used in a single procedure without requiring further manipulation. Thus, in the context of simplified clinical practices and regulatory compliance, the non-enzymatic method offers greater advantages compared to enzymatic techniques.

***Cost and Accessibility***

In terms of cost and accessibility, the non-enzymatic method is more economical and practical, as it does not require enzymes, which are expensive and require strict quality control to ensure their effectiveness and safety. Mechanical methods, such as intersyringe emulsification and microfluidic techniques, can be performed in a closed system, making them easier to implement in clinical settings without requiring specialized laboratory infrastructure. However, some advanced non-enzymatic techniques, such as MycroLizer or Lipogems, still require additional equipment, leading to relatively high initial costs. In contrast, the enzymatic method demands more complex laboratory infrastructure, as well as additional steps to ensure the safety of the isolated cells before their clinical application, making it more expensive and less accessible in certain healthcare facilities.

**Table 1. Comparison of Enzymatic and Non-Enzymatic Methods for ADSC Isolation**

| Aspect | Enzymatic Method | Non-Enzymatic Method |
|---|---|---|
| Cell Yield Efficiency | 1–5 × $10^6$ cells/mL of adipose tissue | Approximately 75% of the enzymatic method, depending on the technique |

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|---|---|---|
| Viability and Differentiation Potential | Viability >90%, high proliferation, and optimal differentiation | Viability 85–91%, good differentiation potential |
| Cell Purity | Higher purity due to enzymatic separation | More heterogeneous with potential contamination from other cells |
| Regulatory and Clinical Safety | Classified as more than minimal manipulation, requiring strict FDA regulations | Classified as minimal manipulation, making it more suitable for clinical regulations |
| Cost and Accessibility | Expensive due to enzyme usage and additional facilities | More affordable and easier to perform in clinics without specialized facilities |

## CONCLUSION

The fundamental differences between enzymatic and non-enzymatic methods for ADSC isolation lie in efficiency, purity, and compliance with minimal manipulation regulations. The enzymatic method remains the gold standard, as it provides high cell yield with optimal purity, although it requires higher costs and faces stricter regulatory challenges. Conversely, the non-enzymatic method offers a safer approach that is more suitable for clinical applications, particularly in compliance with minimal manipulation standards. However, the primary challenge of this method is its lower cell yield and purity compared to the enzymatic method.

These findings inform best practices by highlighting the need for method selection based on intended application—favoring enzymatic techniques for research and high-purity demands, and non-enzymatic methods for point-of-care or minimally regulated clinical use. Moreover, the comparative data underscore the urgency for harmonized regulatory guidelines that account for the trade-offs between efficacy and manipulation level.

To influence regulatory strategies, future policy frameworks should incorporate technical benchmarks such as minimum cell viability and yield thresholds, validated sterility protocols, and GMP-compliant media alternatives (e.g., HPL) to ensure both safety and functionality. Furthermore, the development of hybrid protocols—combining mechanical dissociation with mild enzymatic assistance—may offer a regulatory middle ground that balances compliance with performance.

Therefore, further advancements in non-enzymatic techniques are required not only to achieve comparable efficiency to enzymatic methods but also to align with evolving regulatory expectations. Such improvements will enable these methods to become widely applicable and acceptable for both basic research and translational ADSC-based therapies.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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