

# Adipose-Derived Stem Cells: An Optimized Protocol for Isolation and Proliferation

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## ABSTRACT

**Background and Aims:** Current advances in the researches on the stem cells has opened new approaches for their apply in tissue engineering and clinical trials. The most common sources of stem cells are adult and embryonic stem cells. Due to ethical issues, embryonic stem cells use in research has been hotly debated. Unlike embryonic stem cells, adult stem cell have not ethical problem for clinical and research. The purpose of present study was to stem cells isolation and proliferation from the human dipose tissue.

**Materials and Methods:** In this study, stem cells were successfully isolated from human adipose tissue by digestion with type I collagenase enzymes. In summary, adipose tissue were digested by type I collagenase enzyme. Subsequently, the cell solution was centrifuged for mature adipocytes and debris elimination and obtained sedimentation was cultured in culture medium contains Dulbecco's Modified Eagle's Medium (DMEM) via 1% penicillin/streptomycin antibiotics and 10% fetal bovine serum (FBS) at 37°C, 5% CO<sub>2</sub> and 95% humidity.

**Results:** in this study, ADSCs were successfully isolated and proliferated. Human ADSCs were able to divide in our culture mediums.

**Conclusion:** The results of the current study were suggesting that this adipose-derived stem cell isolation protocol provides an effective and improved method for isolation and proliferation of these cells in order to tissue engineering application.

## INTRODUCTION

Recent studies have shown that the one of the most regenerative system in the body is indicated by stem cells. These cells are available in different part of the body, such as blood, adipose and etc.<sup>1,2</sup> These cells have attractive properties such as multilineage differentiation and self-renewal capability.<sup>3</sup> Adult stem cells, such as adipose-derived stem cells (ADSCs) are one of the most common stem cells because these cells have not ethically challenge.

ADSCs are usually isolated by enzymatic digestion of different tissue such as abdominal subcutaneous adipose.<sup>2,3</sup> Mesenchymal stem cells are well-known by self-renewal capability, to differentiate into different

cell types, to adhere to flask bottom and to expression of variety surface markers.<sup>4,5</sup> MSCs observed and isolated from all post-natal tissue and organ.<sup>6</sup> Recently, the development of stem cell technology has provided significant hope to treat or prevent a disease and tissue damages and finally supplied huge progress in regenerative medicine and tissue engineering.<sup>7-9</sup>

Stem cell technology is an advanced approach to the therapy of diseases and damages that uses suitable cell sources.<sup>10</sup> Studies using this method have been suitable alternative to conventional clinical method.<sup>11</sup> Stem cells are cells with the capability to divide and make a lot of identical stem cells or to specialize and type specific cells of bodily tissues.<sup>12</sup> Recently, the development of stem cell technology has provided significant hope for tissue engineering and regenerative medicine.<sup>13</sup> Try to optimize of isolation and expansion adult stem cells protocols is a critical step in the expansion of regenerative medicine methods for tissue repair or regeneration for the therapy of diseases and damages.<sup>14-28</sup> Adipose-derived stem cells supply a plentiful and suitable source of adult stem cells for regenerative medicine and tissue engineering

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application.<sup>29-32</sup> Several methods have been used during the years to treat a variety of body damages and lesions.<sup>33-35</sup> Among them, recently, the emerging progress in the field of stem cell technology is to treat the diseases and lesions by using different cell sources, which provide an appropriate support for repair of lesions and stimuli for tissue regeneration.<sup>36</sup> This will ensure the availability of autologous ADSCs for research, trial and clinical applications in the future.<sup>37</sup> ADSCs have an appropriate cell source for regenerative medicine.<sup>10,13</sup>

In this study, adipose-derived stem cells were successfully isolated by enzymes digestion.

The purpose of this *in vitro* study was to establish an improved protocol for ADSC isolation and proliferation. We also compared the properties of different protocols in isolation and proliferation of adipose-derived stem cells.

## MATERIALS AND METHODS

Human abdominal subcutaneous adipose sample was obtained from 3 patients undergoing of elective surgery after obtaining written consent from patients (Figure 1). Cell isolation was performed from the adipose tissue samples. Initially, the adipose tissue sample was mechanically segmented and connective tissue and blood vessel were removed carefully from this sample. The obtained pieces were rinsed with phosphate buffered saline (PBS) several times and were digested with type I collagenase (1.5 mg/1g adipose tissue) at 37°C for 45-60 min. Then, the cell suspension was centrifuged at 1800 rpm for 10 min and the cell pellet was cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with penicillin streptomycin and 10% FBS, and incubated at 37°C with 5% CO<sub>2</sub> and 95% humidity. The medium was changed at least twice per week until the cells reach up to 80% confluence. In this method, A large number (approximately 200,000) of adipose-derived stem cells (ADSCs) were obtained per gram of adipose tissue (Figure 2 a-j). Cells were passaged at 80% confluence by washing twice with PBS

and were incubated in trypsin/EDTA at 37°C for 3 min. After the cells reached confluence at the third passage, ADSCs were frozen according to current study protocol. Cold freezing medium contains about 50% fetal bovine serum, 10% dimethyl sulfoxide (DMSO) and 40% DMEM. The viable cells were preserved in a final concentration of up to 1× 10<sup>6</sup> cells/ml at -196°C in liquid nitrogen tank for a long time.

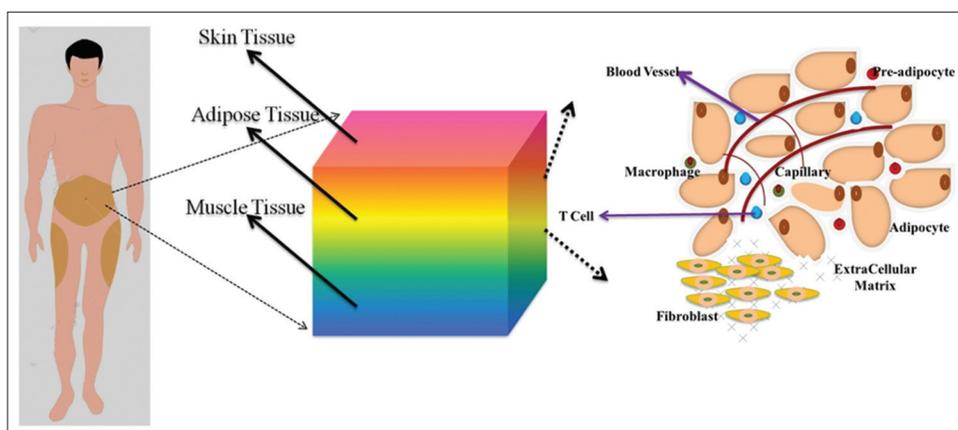
## RESULTS

In the current study, we succeeded in isolating and proliferating stem cells from the subcutaneous adipose tissue using an easy and effective protocol. In this work, cell adhesion observed 2-3 day after initiation of the primary culture. Also, isolated cells having a spindle-shape and gradually they become elongated. At last, these cells showed a typical fibroblast morphology (fibroblast-like shape) under inverted phase-contrast microscope. The cells were started to adhesion and proliferation. Two weeks after cell isolation, ADSCs reached to up to 80% confluent rate. The cells had a higher proliferation rate in the next passages to passage three and obtained at about 90% confluence after the seven days (Figure 3). These cells had a rapid expansion *in vitro* condition. Also, ADSCs displayed positive staining for the mesenchymal surface markers CD44, CD90, CD105 (Figure 4). ADSCs at passage four that showed high-level of CD44, CD90, CD105 expression. More than 70% of ADSCs expressed the mesenchymal-specific markers (CD44, CD90, CD105). In contrast, only a small proportion (less than 5%) of ADSCs expressed the hematopoietic marker CD34 (Figure 5 & Table 1).

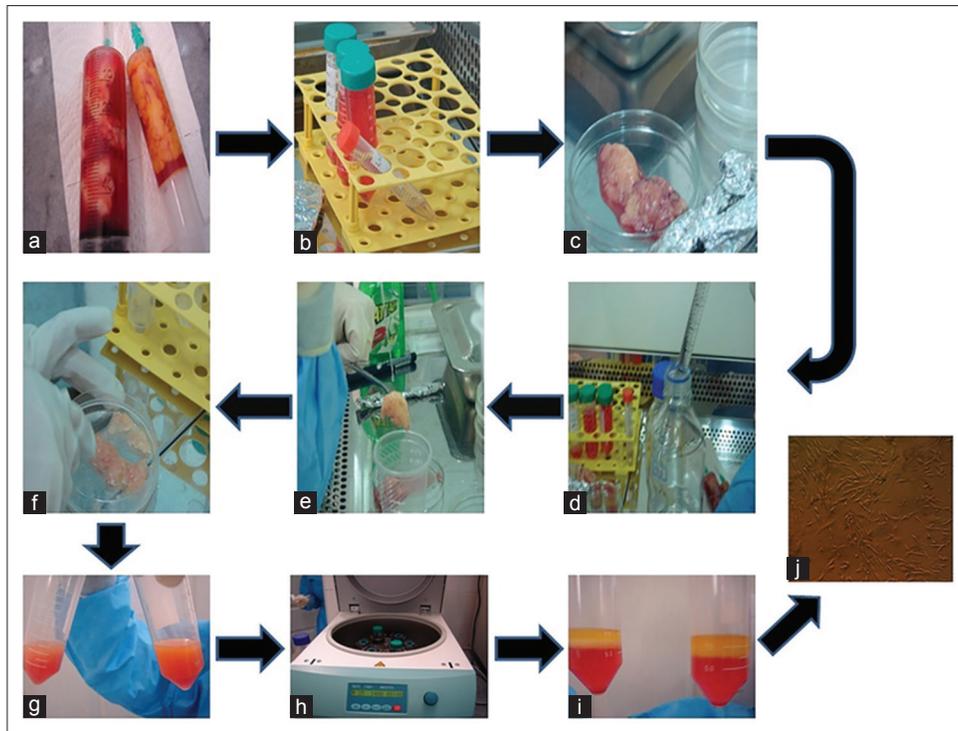
**Table 1: Flow cytometry analysis of adipose-derived stem cells (ADSCs) at passage 4**

| Surface markers | ADSCs at passage 4 |
|-----------------|--------------------|
| CD 105          | 74.9±0.2           |
| CD90            | 72.9±2.5           |
| CD44            | 70.4±0.7           |
| CD34            | 4.4±0.5            |

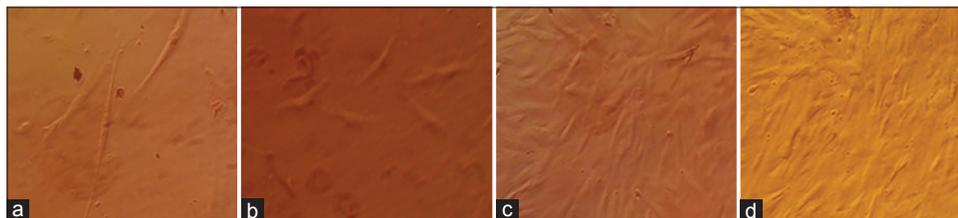
Data are presented as the mean±standard error of mean (SEM), N=5



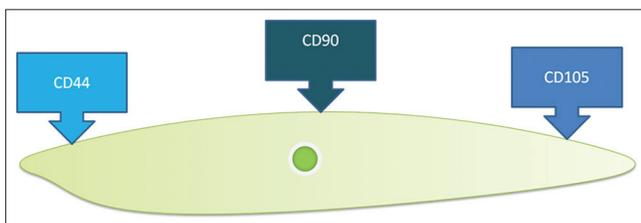
**Figure 1. Schematic image demonstrating of the human abdominal subcutaneous adipose tissue and it's components**



**Figure 2:** different steps of adipose-derived stem cells isolation (a-j). This schematic describes the isolation process of adipose-derived stem cells from harvested whole human adipose tissue



**Figure 3:** Human adipose-derived stem cells at different passages. (a) Passage 0, (b) Passage 1, (c) Passage 2 and (d) Passage 3



**Figure 4:** Schematic image of ADSCs specific surface markers (CD44, CD90, CD105)

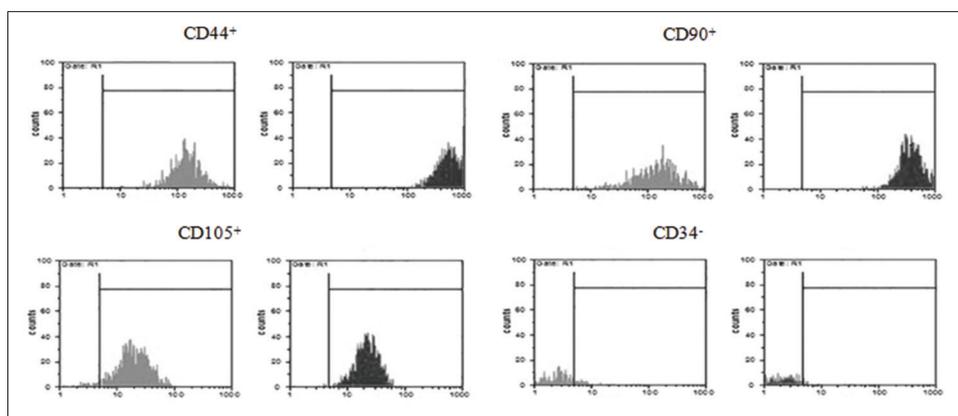
ADSCs were successfully stored more than six months in liquid nitrogen tank (Figure 6).

## DISCUSSION

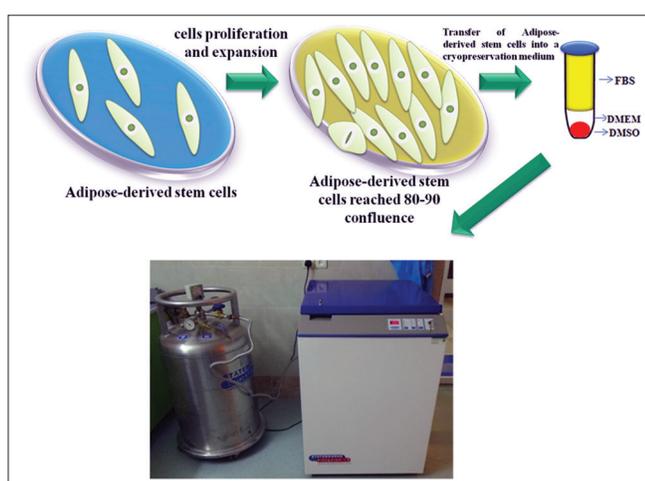
In this study, we showed the potential of this protocol for adipose-derived stem cells isolation. In the present study, in primary isolated cells, more than 70% of the cell population positively expressed mesenchymal surface markers (CD 44, CD90, and CD105). Also, these cell populations negatively expressed hematopoietic

marker (CD34). The characterization of isolated cells was confirmed as ADSC by flow cytometry method. These results were supported by many studies result that reported previously.<sup>2-4,10,38</sup> Our current method was developed by using of a simple protocol of washing adipose tissue. Also, in this method the processing time was modified in comparison with the enzymatic methods. In this work, we succeeded in isolating and proliferating these cells in the try to use them in tissue engineering application in the future.

The stem cells of the adipose tissue can represent a critical source of cells with multipotential properties.<sup>36,39,40</sup> The optimization of the isolation and culture conditions may increase the comparability of the results from different research laboratories.<sup>14</sup> Although, the essential elements in optimal isolation of stem cells are the type of used enzymes and or materials and duration of different steps and the centrifugation process<sup>4,5</sup> but no unique and optimized isolation protocol for ADSCs has been accepted overall.<sup>41</sup>



**Figure 5:** Expression of ADS cells specific markers (CD44, CD90, CD105) on primary culture of ADSCs by flow cytometry method. CD34 antibody was used as negative marker



**Figure 6:** Schematic image demonstration of successful storage of ADSCs in liquid nitrogen tank

The primary techniques to isolate ADSCs were pioneered by rodbell et al utilizing rat adipose tissue.<sup>29</sup> This classic method to isolate human adipocyte progenitors was modified by Van, Roncari, Deslex, Hauner and others.<sup>20,32,42,43</sup>

Different methods were assessed for ADSCs isolation.<sup>8-32</sup> Most Common methods for ADSCs isolation is consisted of washing the adipose tissue, digestion with collagenase and subsequently centrifugation process steps.<sup>31</sup>

The first important step in isolation adipose-derived stem cells is washing process that this process can be affected on ADSCs' heterogeneity.<sup>21</sup>

Different ADSCs isolation protocols were used in variance components such as  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -free Hank's balanced salt solution containing sodium HEPES, Phosphate Saline Serum (PBS) and Normal Saline Serum in washing step.<sup>27,28</sup> In the current study, we used to Phosphate Saline Serum (PBS) for washing the adipose tissue sample.

Katz et al were developed a novel protocol for adipose-derived stem cells isolation with modifying in collagenase digestion and tissue washing steps that were largely corroborated our present study.<sup>36</sup>

Such as our protocol, The recent protocols for human adipose-derived stem cell isolation have been used a collagenase digestion followed by centrifugation process to separate the stromal vascular fraction (SVF) from pre-adipocytes.<sup>22</sup>

In some of the protocols, collagenase enzyme such as 0.075% collagenase type I, 0.3% collagenase type I and 0.6% collagenase type I was replaced with trypsin.<sup>11,12,14</sup> Some protocols such developed protocol by shah et al was used a non-enzymatic method.<sup>21,23,27,44</sup>

For example, in a study, a mechanical strategy was used for isolating adipose-derived stem cells from a conventional liposuction method by raposio et al.<sup>14</sup> In this study, no enzymes such as collagenase and animal-derived materials were used. Also, Developed protocol by baptista et al was reply on mechanical shaking incubation plus to red blood cell lysis solution, simultaneously.<sup>3</sup>

ADSCs proliferation can be highly influenced by basal medium, glucose concentration, Quality of FBS, cell plating, and cell density.<sup>45</sup> Cheng et al were used the KNAC medium culture for growth of human ADSCs with a lower calcium rate.<sup>10</sup> This experiment that use of K-NAC medium for cell growth have been significantly increased in cell proliferation ability.

## CONCLUSION

In conclusion, our findings propose that this protocol can be utilized for the isolation of ADSCs, particularly when purpose of study related to tissue engineering strategies. Also, this protocol can be easier, cheaper, and faster method than other current procedures.

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