Comparison of Human Mesenchymal Stem Cells Derived from Various Compartments of Human Adipose Tissue and Tunica Adventitia Layer of the Arteries Subsequent to Organ Donation

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ABSTRACT

Background: Mesenchymal stem cells are one of the most interesting cell sources used in regenerative medicine.

Objective: In the present study, we isolated and characterized the mesenchymal stem cells from various compartments of human adipose tissue and tunica adventitia layer of the arteries.

Methods: Tissue explant culture was done from various compartments of the human adipose tissue and tunica adventitia layer of the arteries, including adipose tissue far from the vessels, perivascular tissues that are completely attached to the vessels, and tunica adventitia layer of the arteries. After the cell culture, characterization of the cells was determined at 3rd–5th passages. Flow cytometry was performed for antigen expression analysis of CD34, CD45, CD44, CD90, CD29, CD73, and CD105. For the evaluation of cell differentiation potential, adipogenic and osteogenic differentiation was conducted under appropriate protocols.

Results: The cells were positive for CD44, CD90, CD29, and CD73 and negative for CD34, CD45, and CD105. Adipogenic and osteogenic differentiation potentials were different among the cells from various compartments. The cells derived from perivascular tissue demonstrated better adipogenic and osteogenic differentiation.

Conclusion: It is essential to characterize the cells from different tissues and compartments for different purposes in regenerative medicine.

KEYWORDS: Human; Mesenchymal stem cells; Adipose tissue; Adventitia; Organ transplantation

INTRODUCTION

Mesenchymal stem cells (MSCs) have been considered to be used in cell-based therapies, because of their features and potentiality. These cells show a fibroblast-like shape and are involved in differentiation processes. Moreover, they secrete several cytokines and growth factors that play roles in immunomodulatory, angiogenic and tissue regeneration pathways [1]. On the other hand, although in vivo studies have demonstrated safety in the application of MSCs, they could not show similar functionality. Hayashi, *et al*, reported that the bone volume of the bone-marrow-derived mesenchymal stem cells (BM-MSCs) composites was more than that of the adipose tissue-derived mesenchymal stem cells (AT-MSCs) composites in syngeneic rats [2]; this suggests that BM-MSCs can be the treatment of choice for clinical bone tissue engineering. However, in another study, Wen, *et al*, found that there were no differences between human BM-MSCs and AT-MSCs in the bone regeneration of cranial defect in rat models [3].

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Table 1: Characteristics of the studied donors

Donor No.	Sex	Age (yrs)	Cause of death
1	Female	53	Intra-ventricular hemorrhage
2	Male	32	Intra-ventricular hemorrhage
3	Female	23	Head trauma

MSCs have been isolated from different sources. Some studies have shown that these cells are able to present different characteristics when they are isolated from different tissues [4-6]. Isobe, *et al*, compared the stem cells derived from the bone marrow, synovial fluid, adult dental pulp, and exfoliated deciduous teeth and found that BM-MSCs and synovial fluid-derived cells were involved in the highest levels of osteogenesis. Moreover, lipid vesicles were observed in all of them subsequent to adipogenic differentiation induction. Therefore, they suggested selection of the cell type depending on the therapeutic purposes [6]. Overall, it seems essential to focus on the source of the cells and their heterogeneity that impact future clinical application.

On the other hand, researchers reported that MSCs derived from different compartments of a specific tissue may represent different features. MSCs can be obtained from different compartments of the umbilical cord including the UC lining, Wharton's jelly, subendothelial layer, and perivascular zone. The superiority of one compartment of the umbilical cord over another part is still controversial [7]. Researchers demonstrated that protein and antigen profile expression might be influenced by different regions of this tissue [4, 8].

The application of available and ethically acceptable stem cells sources such as umbilical cord, placenta, and discarded parts of a tissue during organ transplantation is valuable because they are rich and from unusable tissues. Therefore, in the present study, we isolated and characterized MSCs derived from the human adipose tissue and tunica adventitia layer of the arteries, from the discarded parts of tissues during organ transplantation, to compare their phenotype and differentiation potential to be considered in future regenerative medicine applications.

MATERIALS AND METHODS

Isolation and Culture of Cells

In accordance with the Ethics Committee of Shiraz University of Medical Sciences for brain-dead donors, informed written consent was obtained for clinical and research purposes. Human adipose tissue removed from the epigastric region of the abdominal cavity (n = 3) was transferred to the cell isolation lab. The donors' information is shown in Table 1. Tissue explant cultures were done from various compartments of the human adipose tissue and tunica adventitia layer of the arteries, including adipose tissue far from the vessels (FV), perivascular tissue (PV) that were completely attached to the vessels, and tunica adventitia layer of the arteries (TA) (Fig 1). The mentioned vessels were arteries in the range of small ones (0.1–10 mm in diameter). The

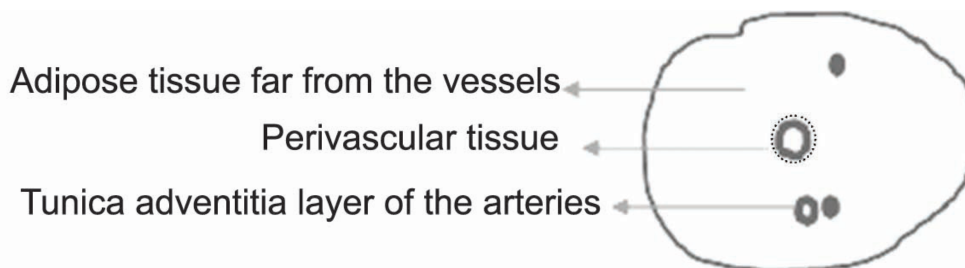

Figure 1: Schematic cross-sectional view of the compartments within a human adipose tissue. Three separate regions have been considered to isolate MSCs: 1) adipose tissue far from the vessels (FV); 2) perivascular tissue (PV); and 3) tunica adventitia layer of the arteries (TA).

tissue explants were cultured in DMEM-F12 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. After seven days, the cells started to exit from around the tissues. They were cultured and characterized at the 3rd–5th passages.

Characterization of the Isolated Cells

Flow cytometry

The cells from FV, PV, and TA regions at the 4th passage were recruited for antigen expression analysis of CD34 and CD45, as hematopoietic lineage markers; and CD44, CD90, CD29, CD73, and CD105, as stromal surface markers, using flow cytometry. First, the cells were washed and incubated with antibodies against the mentioned markers and their isotypes. After washing the residual antibodies, we did flow cytometry analysis with BD FACS Calibur flow cytometer.

Evaluation of the Differentiation Potential of the Cells

For the evaluation of cell differentiation potential, adipogenesis and osteogenesis procedures were conducted under appropriate differentiation media that were purchased from Stem Cell Technology Research Center, Tehran, Iran. After 14 and 21 days from the beginning of adipogenic and osteogenic differentiation, the samples were stained with Oil Red O (Merck, Germany) and Alizarin Red S staining (Sigma-Aldrich, China), respectively, to confirm the differentiation potential.

The quantification of mineralization was done using Gregory, *et al.*'s protocol with some modifications [9]. After Alizarin Red S staining, 200 µL acetic acid was added to each well of a 24-well plate. The plate was then incubated on a shaker at room temperature for 30 min. The cells were detached by a cell scraper and transferred to microcentrifuge tubes. The slurry was heated at 85 °C for 10 min. The tubes were then centrifuged at 20,000 g for 15 min. Finally, 100 µL of the supernatant was removed to new tubes and neutralized with 10 µL of 10% (v/v) sodium hydroxide. At this step, the pH was kept between 4.1 and 4.5. The absorbance was read at 405 nm/630 nm in 96-well plates.

To compare the adipogenic differentiation between different groups, we counted the cells containing lipid droplets in five random fields under 400× magnification. The ratio of the differentiated cells to the total cells was considered the adipogenic differentiation rate [10]. Moreover, relative Oil Red O accumulation was evaluated by spectrophotometry. In this regard, after staining and washing of the cells, isopropanol was added to each well of the plate to elute the dye. The plate was shaken for 15 min and the absorbance was read at 490 nm/570 nm.

Statistical Analysis

We used χ^2 and Kruskal-Wallis tests to compare three groups. A p value <0.05 was considered statistically significant. Mann-Whitney U test with Bonferroni adjustment was used to compare two groups.

RESULTS

Isolation and the Cell Cultures

The cells were observed from around the explanted tissues 7–9 days after initiating the culture. They were fibroblast-like. Colony formations were observed in different groups (Fig 2).

Characterization of the Isolated Cells

Antigen expression analysis showed that these cells expressed CD44 (p=0.92), CD90 (p=0.93), CD29 (p=0.98), and CD73 (p=0.21) but did not express CD34 (p=0.99), CD45 (p=0.98), and CD105 (p=0.99) (Fig 3). There was no significant difference in the markers' expression rate between different groups.

On the other hand, the adipogenic and osteogenic differentiation potential was different among the cells from various compartments. The quantification of mineralization using the spectrophotometer showed that the cells derived from PV region had a better differentiation than the other compartments. These differences were significant between the PV group and other groups (Fig 4). In adipogenic differentiation, the PV region showed a better differentiation than the cells taken of other

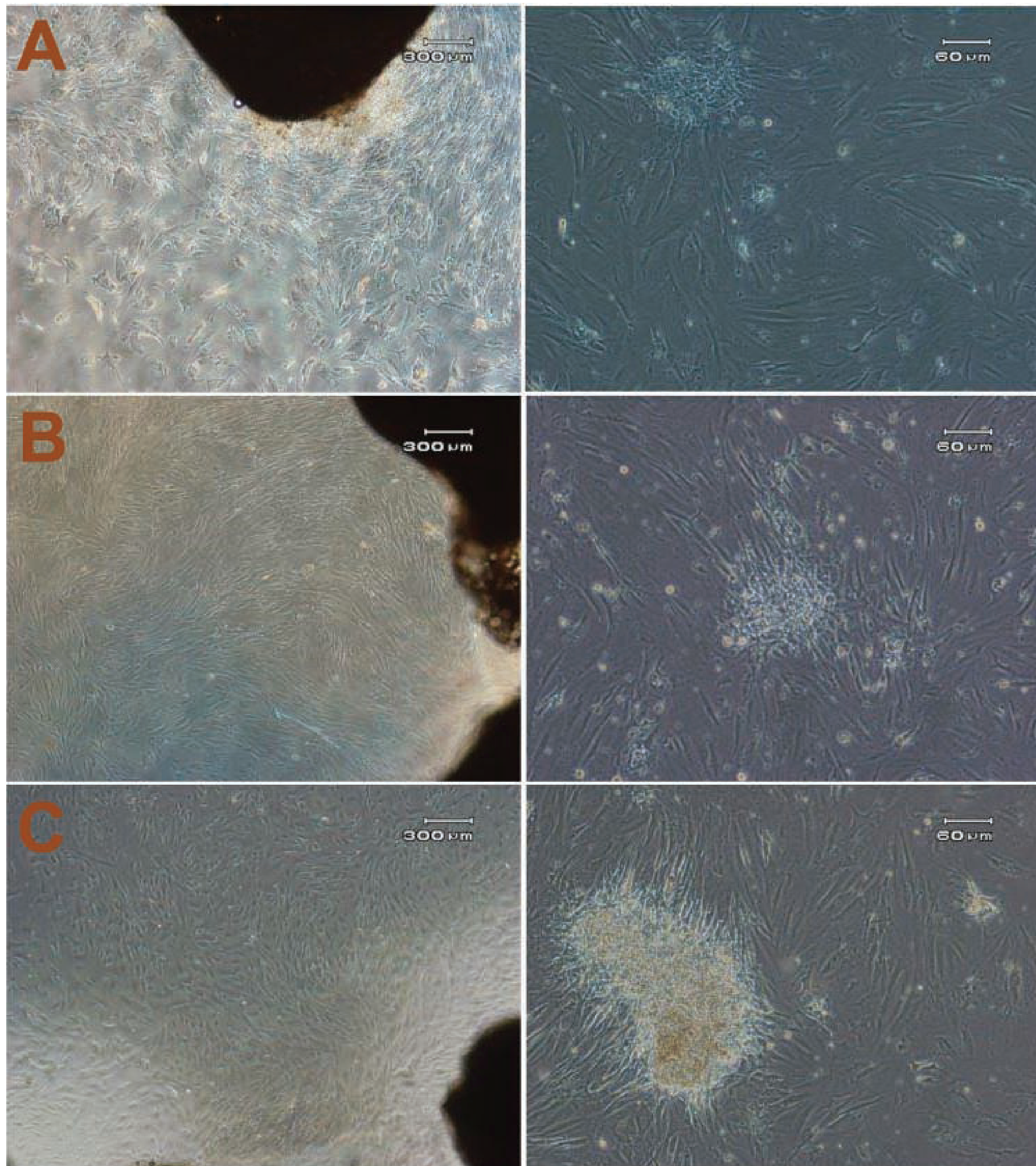

Figure 2: Cell morphology (Left, 40×) and colony formation (Right, 100×) in explant culture of various compartments of human adipose tissue and tunica adventitia layer of the arteries including the cells from FV (A), PV (B), and TA (C) regions.

compartments; however, the difference was not significant (Fig 5).

DISCUSSION

The results of this study suggested that MSCs taken from various compartments of a tissue may provide different capacities. We found that MSCs from various compartments of human adipose tissue and tunica adventitia layer of the arteries can express the same surface antigens. The mentioned groups including the cells derived from FV, PV, and TA regions ex-

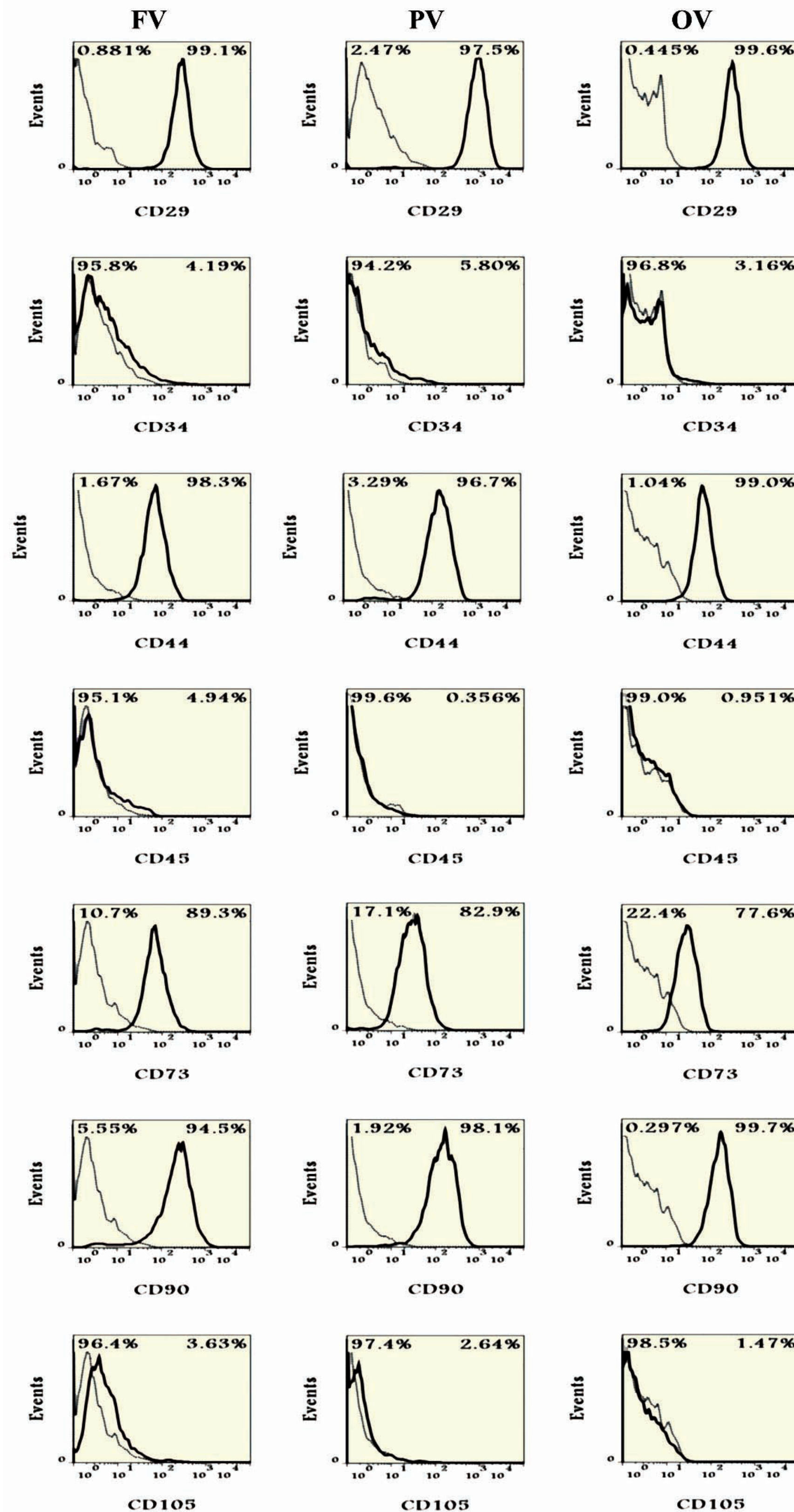

Figure 3: Flow cytometry analysis of the cells within various compartments of the human adipose tissue and tunica adventitia layer of the arteries. The dotted gray histograms show the isotype control-stained cells and the black histograms show the antibody-stained cells.

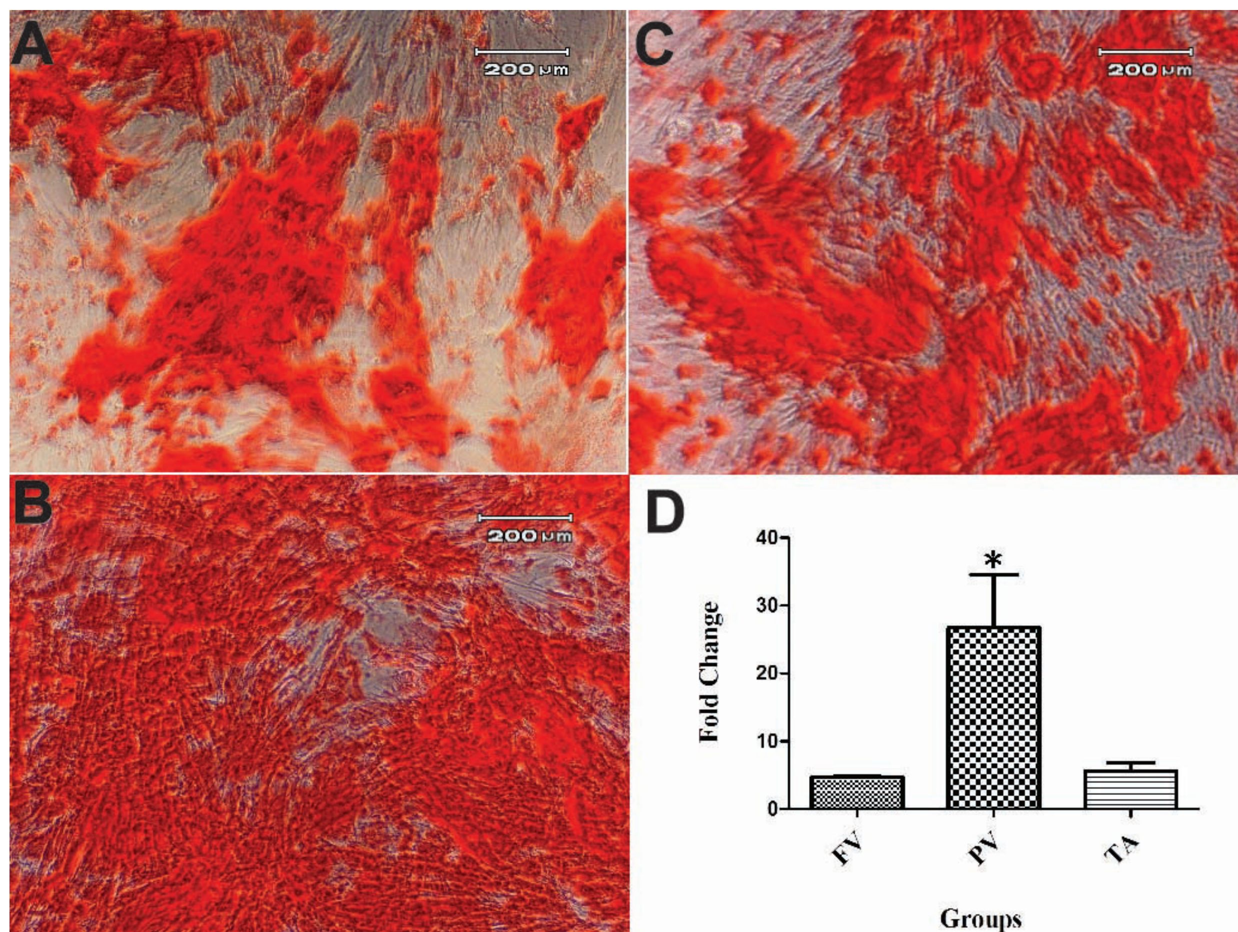

Figure 4: Osteogenic differentiation potential of the cells within various compartments of the human adipose tissue and tunica adventitia layer of the arteries, including FV (A), PV (B), and TA (C). Quantification of the mineralization using the spectrophotometer was done. The results are presented as mean±SD of the absorbance of Alizarin Red S staining and reported as the fold change compared with the non-differentiation culture media (D). Adipose tissue far from the vessels (FV); perivascular tissue (PV); and tunica adventitia layer of the arteries (TA). *Significant differences (p=0.01) in comparison with FV and TA groups.

pressed CD44, CD90, CD29 and CD73 positively, and CD105, CD34 and CD45 negatively.

Although MSCs derived from various sources presented similar phenotypic profile, some differences existed. Researchers reported the expression of surface markers in MSCs [11, 12]. Lee, *et al*, compared MSCs from the human bone-marrow and adipose tissue and demonstrated that both of them express CD29, CD44, CD90, and CD105 similarly [13]. Friedman, *et al*, have shown the expression of CD49b in UC-MSCs, rather than in BM-MSCs [7]. Different surface antigen biomarkers are mentioned in previous research studies on MSCs of various compartments of UC. UC-lining membrane and UC-WJ-derived MSCs showed similarity in the expression of CD29, CD44, CD90, CD73, CD105, CD146, CD13, and CD10 and lack of expression of CD34 and CD45. On the other hand, CD14 is only expressed in UC-lining membrane cells [14-16]. Researchers also reported the expression of CD117 in the whole UC cells not in the UC-WJ cells [17, 18]. In a recent study, MSCs were isolated and characterized from different layers of full-term placenta including amniotic epithelium (AE), amniotic membrane (AM), chorionic membrane (CM), chorionic villi (CV), chorionic trophoblast without villi (CT-V), decidua (DC), and whole placenta (Pla). This study showed that CD44, CD73, CD90, and CD105 are strongly expressed in different layers. However, CD34 and CD45 are not expressed in them [19]. According to the mentioned studies, characteristics of MSCs can be different in various tis-

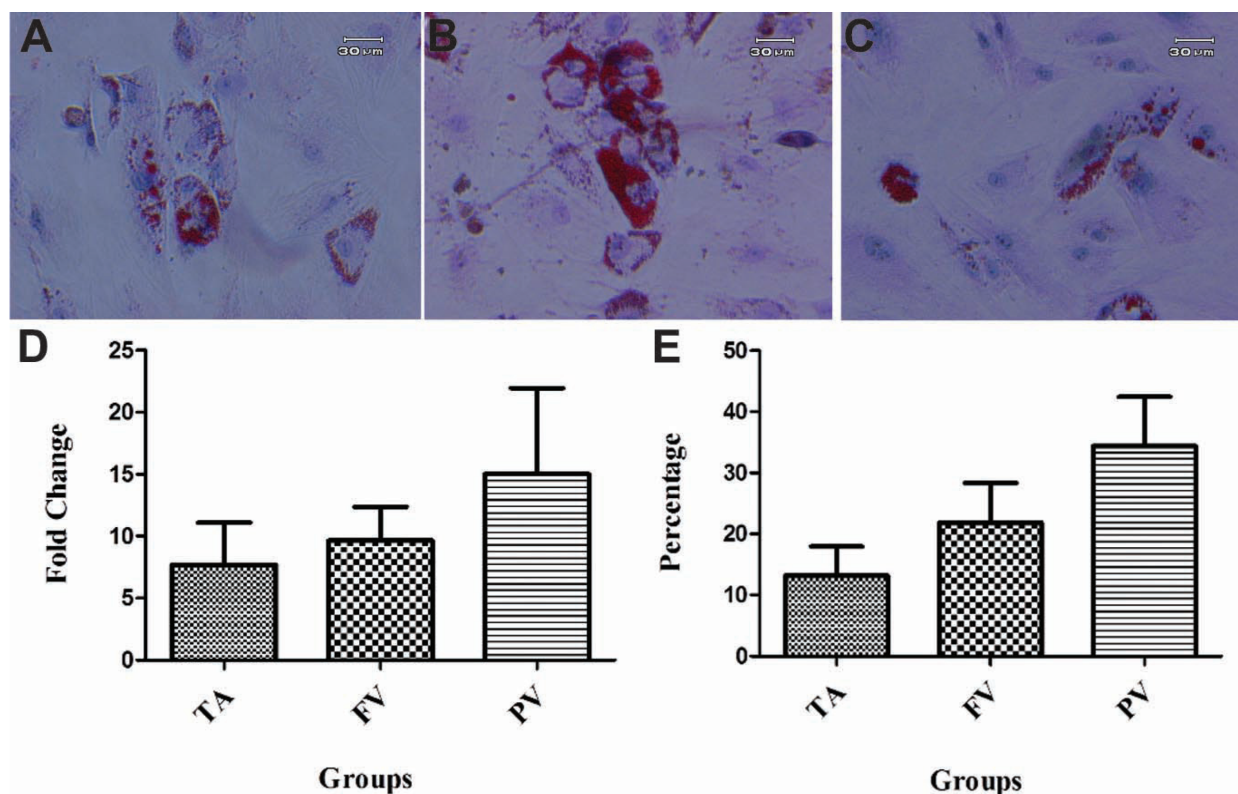

Figure 5: Adipogenic differentiation potential of the cells within various compartments of the human adipose tissue and tunica adventitia layer of the arteries, including FV (A), PV (B), and TA (C). The fold change of the relative Oil Red O accumulation compared with the non-differentiation culture media (D) and image analysis results (F) are observed. The results are presented as mean±SD. Adipose tissue far from the vessels (FV); perivascular tissue (PV); and tunica adventitia layer of the arteries (TA). (p=0.981 and 0.139 for fold change and differentiation percentage analysis, respectively)

sues and different compartments of a specific tissue. The expression of surface markers in different sources of MSCs is summarized in Table 2.

We found that the adipogenic and osteogenic differentiation potentials were different among the cells from various compartments of the studied tissues. Image analysis and relative Oil Red O accumulation assay after adipogenic differentiation showed a greater potential in the cells derived from PV region than the ones obtained from FV and TA regions. However, the differences among these groups were not statistically significant. We observed that the osteogenic potential of the PV region was significantly better than that in the other groups.

Studies suggest that MSCs from various sources can possess different multi-lineage differentiation potential. Shafiee, *et al*, compared osteogenic differentiation potential of the human unrestricted somatic stem cells and MSCs derived from the bone marrow and adipose tissue. They concluded that after osteogenic induction BM-MSCs have the highest and AT-MSCs have the lowest mineralization [20]. Moreover, the comparison between human MSCs from the bone marrow, umbilical cord blood, or adipose tissue indicates that BM- and AT-MSCs possess osteogenic and adipogenic differentiation, whereas the umbilical cord blood cannot undergo mesodermal lineage differentiation [21]. The comparison of multi-lineage differentiation potential of human MSCs derived from the umbilical cord and bone marrow shows a higher rate of osteogenic differentiation in human umbilical cord's perivascular cells. Therefore, this study supports the application of these cells as a candidate in regenerative medicine [16].

Researchers have isolated and characterized MSCs derived from different regions of

Table 2: The expression of surface markers in different sources of MSCs

MSCs Sources	The Expressed Surface Markers	Ref.
Human bone marrow	CD29, CD44, CD90, CD105	13
Human adipose tissue	CD29, CD44, CD90, CD105	13
Human umbilical cord		
Lining membrane	CD29, CD44, CD90, CD105, CD73, CD146, CD13, CD10, CD14	14-16
Wharton's Jelly	CD29, CD44, CD90, CD105, CD73, CD146, CD13, CD10	

the umbilical cord. Recent studies showed a greater differentiation potential in WJ compared with other parts of the umbilical cord, including the cells from the subendothelial layer, the perivascular zone, and the umbilical cord lining [4]. A recent study on the characterization of MSCs derived from different layers of full-term placenta has shown that MSCs are involved in all layers and possess different characteristics. These cells show different differentiation potentials according to the situation of the layer. In this regard, adipogenic and osteogenic induction results in higher differentiation in MSCs derived from CM, CT-V, CV, and DC. Therefore, they suggest that these compartments may have potential applications in cell-based therapies [21].

According to the present study results and other reports, it is essential to focus on MSCs in different tissues and different compartments of a specific tissue and to characterize them separately. Therefore, it would be possible to access more reproducibility and reliability in the application of these cells in regenerative medicine.

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