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Stem Cell Identification and Clinical Practice

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Abstract

Stem cells are non-differentiated cells that are capable of proliferation at regular intervals, differentiation into cells in need, self-regenerating and repairing the degenerative tissues in an organism. In this study, we aimed to isolate mesenchymal stem cells from adipose tissue (ADSCs) and induce the differentiation of ADSCs into osteoblast, chondroblast and adipocyte cell types, and to characterize these cells using positive and negative stem cell markers in order to prepare ADSCs for clinical application.
For this purpose, no chemical-based enzymes were used in ADSCs production. ADSCs were isolated from live tissue using a primary cell culture technique and cells were propogated for up to three weeks through third passage. Third passage cells were differentiated into osteoblast, chondroblast and adipocytes. For histological identification of multi-lineage differentiation of cells, staining techniques including Alizarin Red, Alcian Blue and Oil Red O were applied. Before transplantation, ADSCs were immunohistochemically characterized by positive expression of CD 90, CD 105 and negative expression of CD 45, CD11b. For the transplantation of stem cells 3rd passage cells were subcultured by trypsinization. Trypan blue staining method was used for the analysis of living cells. Cells were counted by invert microscope under a 20× objective. For the transplantation of stem cells, the contents of the autologous blood serum and stem cells were transferred into the region of the operation at a dose of 1×10^6 cells / 100 µl by the insulin injector.
In conclusion, we aim to contribute to clinical stem cell studies and literature knowledge by isolating MSCs from adipose tissue followed by differentiation and characterization of these cells using various histological and immunohistochemical techniques, as well as by determining suitable stem cell dose for clinical applications.

Keywords: Adipose Tissue, Mesenchymal Stem cell, Characterization, Transplantation.

INTRODUCTİON

Stem cell-based therapies for repair and regeneration of different tissues are becoming more significant in the treatment of variety of the organ and tissue for human and animals (Tanya et al., 2019). Stem cells are known as unspecified cells that are capable of unlimited divisibility and self-renewal (Baer et al., 2014). Adipose tissue-derived mesenchymal stem cells (ADSCs) that can differentiate into many of the organ and tissue cells. Stem cells can be classified according to their plasticity. Different types of stem cells vary in their degree of plasticity, or developmental versality. Stem cells are categorized into four groups; totipotent, pluripotent, multipotent stem cells (Frencesco et al., 2015). Multipotent mesenchymal stem cells (MSCs) can be isolated from mammalian animals' tissues such as tendon, synovial membrane, bone marrow, liver, dental pulp, adipose tissue, placenta, amniotic fluid and amniotic cord blood (Bourini et al., 2013). The isolation procedure of stem cells from adipose tissue is relatively less invasive compared to bone marrow-derived mesenchymal stem cells (BMSCs) (Dominici et al., 2006). Therefore, many recent studies have assessed the potential of ADSCs for bone engineering (Ying et al., 2012).

MSCs are adhesive and more differentiated stem cells. The types of stem cells are different with many differences existing between each type. Adult MSCs developmental capacity is more limited than embryonic stem cells however they retain a broad differentiation potential (Mannhardt et al., 2016). ADSCs have shown their ability to differentiate into osteogenic, adipogenic, chondrogenic lineage in vitro (Zuk et al., 2002). The use of ADSCs in regenerative medicine has sparked the interest of clinical scientists and surgeons alike given the ease of access, abundance, high proliferative rate, and lower senescence compared with other types of stem cells (Rehman 2004; Ajbog et al. 2010). MSCs are present in tissues or organs from which they exit into the circulation to home into sites of injury or to other tissues. The MSCs are well characterized based on their surface markers and the authors further state that cultured ADSCs are characterized as $CD73^+/CD90^+/CD105^+/CD44^+/CD45^-/CD31^-$ cells (Bourin et al. 2013; Baer et al. 2014). Also MSCs have the ability to regenerate into various cells and tissues (Ghaneialvar et al. 2018). This offers potential for their future application in treating many disorders without fear of rejection possibility.

In this study, we aimed to isolate ADSCs and induce the differentiation of ADSCs into osteoblast, chondroblast and adipocyte cell types, and to characterize these cells using positive and negative stem cell markers in order to prepare ADSCs for clinical application.

MATERIALS AND METHODS

Animal sample for cell isolation

The ethical approval was received from Uludag University Local Ethical Committee of Animal Experiments. All animal handling and surgical procedures were performed in accordance with the Bursa Uludag University Animal Care and Utilization Committee review and institutional guidelines. MSCs were isolated from the fat tissue of 12 Sprague Dawley male rats using an explant culture method.

Isolation of MSCs from adipose tissue

Adipose tissue was collected from rats' (n=6) inguinal and subcutan fat pads . ADSCs were isolated by mincing inguinal adipose tissue into ~3 mm^3 pieces, which were seeded on the T25 flasks and incubated with low–glucose Dulbecco Modified Eagle Medium (DMEM) for 30 min at 37°C in a CO_2 incubator. Mesenchymal stem cell isolation was performed by non-enzymatic process. Upon reaching subconfluency, the cells were harvested, expanded through

3 passages and used for stem cell identification.

Multilineage differentiation of adipose-derived stem cells

Osteogenic differentiation

ADSCs (7×10^4 cells /well) were seeded within 24 well culture plates. Then, MSCgo™ osteogenic differentiation medium was added when cells reached 70 % confluency. On the day of 28th, osteoblast cells were stained with Alizarin Red (Tanya et al. 2019)

Chondrogenic Differentiation

ADSCs (1.5×10^6 cells/well) were seeded within 96 well culture plates. The MSCgo™ chondrogenic differentiation basal medium was added when cells reached 90 % confluency. Cells were incubated within the chondrogenic medium for a month and stained with Alcian Blue (Tanya et al. 2019).

Adipogenic Differentiation

ADSCs (7×10^4 cells/well) were seeded within 24 well plates. The standard medium was replaced with the MSCgo™ adipogenic differentiation basal medium when cells reached 80 % confluency. After two weeks the cells were stained with Oil Red O (Tanya et al. 2019).

Cluster differentiation markers expression analyse

Cells were seeded on plastic cell culture slide and transferred into CO_2 incubator for the cell attachment (under cell culture condition 5 % CO_2, 37° C, 96 % humidity). The cells were fixed with methanol for 15 minutes at – 20 °C. Cells were rinsed with Phosphate Buffer Saline (PBS) four times. Cells were permeabilized with Triton-X 100 for 10 min and rinsed with PBS. After these cells were incubated in hydrogen peroxidase. Blocking step was performed for 5 min at room temperature. The cells were incubated with CD 45, CD 11b, CD 90 and CD 105 stem cell surface primary antibodies were incubated overnight at + 4º C in humidity chamber (Bailey et al., 2010). Other steps of the immunohistochemical staining reagent kits protocols (Cat No: TP-125-HL) were used.

Cell Transplantation

Preparation of cell transplantation; 100µl solution containing MSCs were used. This solution was obtained from rat blood serum. Trypan blue staining was performed to analyze the cell viability. Cells were evaluated by invert microscope under a 20 × **objective.** P3 cells were counted by using inverted microscope to determining the cell preparation dose. We used the insuline injectors for the cell transplantation. The cells were kept on ice until they were implanted. Inhalatory was used for general anesthesia of rats. For the transplantation of stem cells on rat degenerative tissue (n=6), the contents of the autologous blood serum and stem cells were transferred into the region of the operation at a dose of 1×10^6 cells / 100 µl by the insulin syringe.

RESULTS

In-vitro cell culture phase; during P1, P2 and P3 cell proliferation were completed on day 20. Fibroblastoid morphology was seen to be started. The passage third MSCs were observed by inverted microscopy (Figure 1).

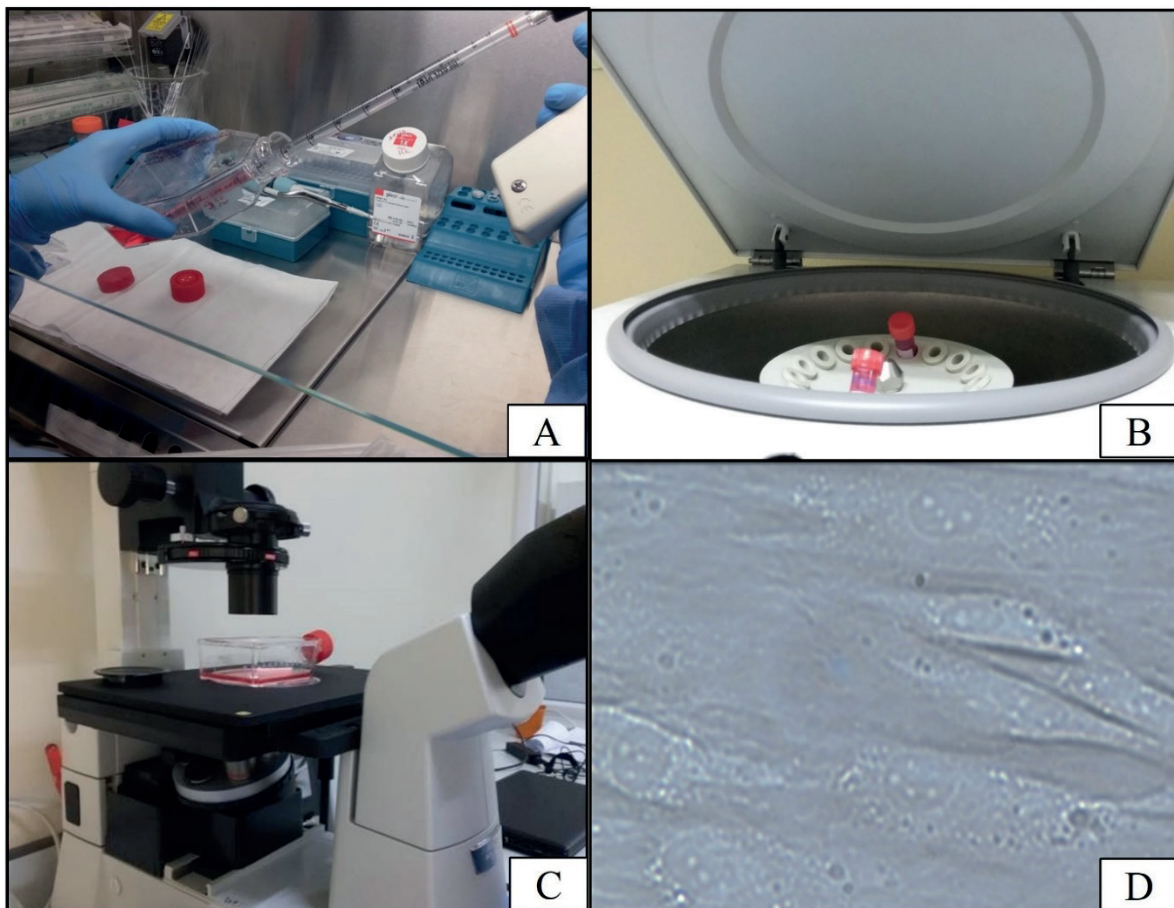

Figure 1 A-D. Subculture period without chemical solution, with cell scraper and centrifuge prosessing (A-B); Inverted microscopic examination at third passage of ADSCs by inverted microscopy like cells (C-D).

In this study, the osteoblastic morphology of ADSCs at P3 was first noticed on the 14th day. Osteoblasts were detected using Alizarin Red staining on day 28.

The chondrogenic morphology of ADSCs at P3 was noticeable started on day 14 in the present study. The alcian blue staining confirmed the presence of chondrogenic cells on day 28.

The differentiation of ADSCs at P3 into adipocytes was confirmed by the presence of prominent lipid droplets. Fat cells were detected by Oil red O staining on day 14.

In our study show that passage third ADSCs were negative for CD 11b and CD 45, while they were positive for CD 90 and CD 105 surface markers as assessed by immunohistochemical analyses.

After the stem cell identification process completed, the clinical transplantation process was performed to rats. During transplantation of stem cells and after injection of stem cells; there was not occured any inflammation or adverse effect on the region of degenerative tissue.

DISCUSSION

Lotfy et al. (2014) and this present study results are the same in according to the attachment of spindle-shaped cells to tissue culture plastic flasks. It was observed after 1 day of culture and confluency was observed on day 7 in ADSCs. In these study the cells can be easily and repeatable harvested by utilizing minimally invasive techniques with low morbidity. The multipotency evaluation of ADSCs showed that the cells could be differentiated into chondrocytes, osteocytes, and adipocytes. The same result was obtained by Hamid et al. (2012) and our results show that ADSCs appeared to have a better ability for differentiation into osteoblastic, chondroblastic and adipocytic cell activity in accordance with the multi-lineage differentaiton potential.

Following the international standard for MSC surface markers declared by ISCT (2013), further assessment of ADSC used in this study showed it complied with those criteria, except lower expression of endoglin marker (CD105) than the standard. But CD105 is recommended only as an alternative or additional marker (Bourin et al. 2013). Thus also; CD90 positivity are used for phenotypic characterization studies (Bourin et al. 2013; Lotfy et al, 2014) and applied the current cell surface antibody for MSCs. In Dominici et al. (2006) and our study results show that the CD 90, CD105 are expressed by MSCs and also CD11b and CD45 are not expressed on MSCs. Therefore, we confirmed that primary cultivated, harvested and growth of ADSCs are complied with MSCs criteria in our study. Additionally; in recent years, literature based on data related to SVF cells and ASCs has augmented considerably: These studies have demonstrated the efficacy and safety of SVF cells and ASCs in vivo in animal models (Francesco et al.2015). In the study by Toma et al. (2002); a volume of 100 μL of cell suspension containing 500.000 or 1 million cells was injected into rat heart tissue. Noteably; in our findings suggest that autologous blood serum was no negative effect during cell transplantation and following days. On the other hand, same cell counts were used by Toma et al. (2002) 1 million cell dose is efficient in order to cell differentiation and cell regeneration on degenerative tissue area. In addidion; in the current study, we were not used chemical solution such as DMEM, D-PBS, HBSS or PBS. These results provide the evidence that cells were not stimulated negatively cellular activation on the region of the degeneration.

CONCLUSION

In conclusion; in the present study, the stem cell identification process and clinical application can be modified other tissue and repeated injections can be performed with in a week interval according to variety of degenerative tissue.

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