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Better isolation, proliferation and differentiation of human adipose-derived mesenchymal stem cells using human serum

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Abstract

Mesenchymal stem cells have many applications in medicine. Attention to the proliferation and differentiation of stem cell differentiation is an important issue. The aim of this study was to investigate the possibility of optimal isolation, proliferation, and differentiation of adipose tissue-derived mesenchymal stem cells (ADSCs) using human serum. Human serum (HS) was obtained from the venous blood of eight healthy individuals. The rate of proliferation and differentiation of ADSCs and expression of surface markers was assessed by flow cytometry. Bone differentiation was assessed using Alizarin Red staining. Data were analyzed using statistical software. Over time, HS showed more proliferation than fetal bovine serum (FBS) - enriched cells ($p < 0.05$). Differentiation of ADSCs cells ls in HS-enriched medium is faster and more pronounced than differentiation in the control group. The expression of surface markers in the medium containing HS was the same as the medium containing FBS where the expression levels of CD105 and CD95 were found to be positive and the expression of CD34 and CD45 was negative. Due to the better proliferation of adipose tissue-derived mesenchymal cells in the medium containing HS than FBS, it is suggested that human serum be used in future clinical studies. Also, HS is healthier, safer, more accessible, and more affordable than FBS.

Key Words: Replication; differentiation; mesenchymal stem cells; human serum; human adipose-derived stem cells (ADSCs).

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So far, many research studies have been conducted in the field of medicine, most of which are in the field of common and infectious diseases. For instance, various studies have been conducted in the field of cancer,[1-5] and in recent years, with the outbreak of coronavirus,[6-8] studies have mainly gone in this direction. However, the treatment of these diseases has always been very significant. Additionally, much research has been conducted regarding medicine and health statistics to prove the importance of the healthcare system.[9-12] One of these methods is cell therapy using stem cells and extracellular vesicles derived from these cells. Stem cells have many applications in medicine.[13,14] Mesenchymal stem cells are commercially used for three reasons: first, they can be located in areas of inflammation and tissue damage, such as wounds or tumors. Second, mesenchymal stem cells have immunosuppressive properties and can modulate innate and adaptive immune responses. Third, these cells have the ability to support other cells with paracrine factors.[13-15] Among these, mesenchymal stem cells are of great interest.

Mesenchymal stem cells have the power to differentiate into different cells and are also able to become undifferentiated cells similar to their own. These cells have the ability to differentiate into connective tissue lineages, including adipocytes, chondrocytes, and osteocytes. Mesenchymal stem cells are capable of regenerating their divisions for a long time and at the same time maintaining their differentiation power.[16,17]
The clinical application of stem cells in the treatment of brain injury, Parkinson's, myocardial infarction, incomplete osteogenesis, fracture healing, and tendon rupture, as well as treatments of joint, liver and other disorders have been well demonstrated.[18,19]
One of important sources of potent mesenchymal stem cells is adipose tissue, which is considered a very suitable source of stem cells.[20,21] Adipose tissue-derived stem cells (ADSCs) are easily harvested and multiplied, are effective and non-invasive, and have a high capacity for multiplication and proliferation. These types of autologous sstem cells significantly reduce donation complications.[22,23]
On the other hand, litterature findings suggest that use of animal serum in clinical cases of cell therapy is not a completely safe choice. Indeed, animal serum may cause immune reactions and infections.[24-26] Providing the safest and most cost-effective serum with optimal properties and greater safety for cell therapy is an important issue.[27]
Human serum (HS) seems to be a suitable alternative, because of to its high availability, safety and rapid collection. Human serum contains many useful proteins, including growth factorsand immunoglobulins, inducing a wide variety of positive effects on cell culture.[28]
Studies on ADSC have found that HS can be used to produce and proliferate fat stem cells, reporting that human ADSC proliferation in growth medium containing HS is higher than in culture medium containing fetal bovine serum (FBS). In fact, proteins and other nutrient factors in human serum affect the expression of inflammatory agents in stem cells.[30-32]
Quantitative studies have been performed on various aspects of the advantage of human serum over animal serum, so the aim of this study was to confirm or not the effects of human serum for better isolation, proliferation and differentiation of ADSC in cell culture medium.

Materials and Methods

Preparation of medium and HS serum

A total of 100 cc of venous blood was taken from 8 healthy adult donors with written consent. The prepared samples were centrifuged at 3000 rpm for 8 minutes and the serum was separated. The isolated serum was incubated for 56 minutes at 56 ° C to inactivate. It was then placed at -80 ° C for later use.

Cell extraction and culture

ADSCs were prepared from liposuctioned human adipose tissue. 60 g of adipose tissue sample was transferred to the culture room in a sterile container. In order to remove blood cells, fat samples were washed several times with Phosphate-Buffered Saline (PBS) solution (Shellmax) and penicillin and streptomycin (Gibco) antibiotics were added. The adipose tissue was then divided into small pieces and mixed with 3 ml of type I collagenase enzyme (0.075%) (Gibco) dissolved in PBS. After digestion, the small pieces were placed in a

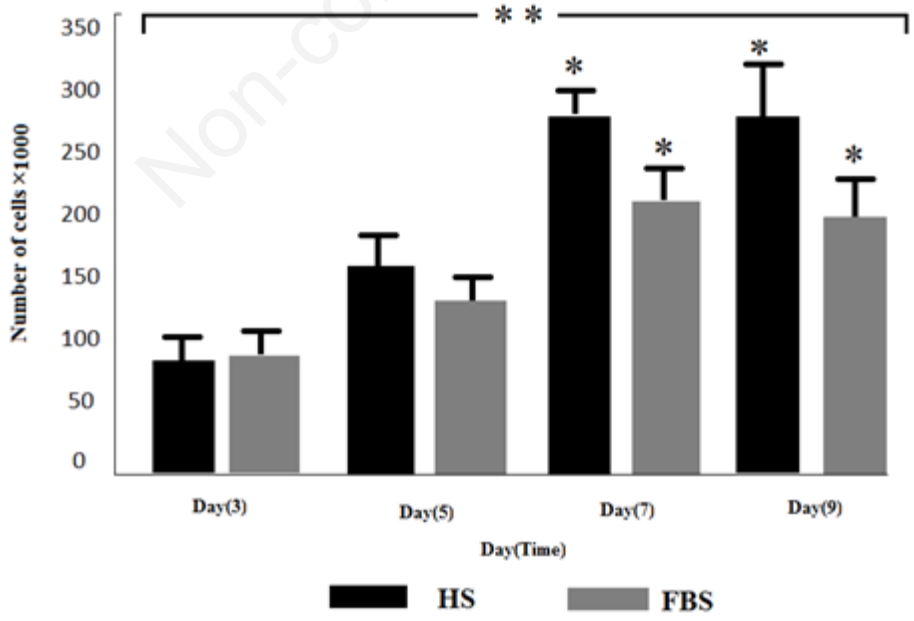

Fig 1. *Difference in the number of cells on consecutive days of culture in both mediums enriched with HS and FBS*

37-degree incubator for 40 minutes, during which time the sample was shaken every 8 minutes for further digestion of the tissue.
The sample was then divided into two parts, one was added to DMEM (Gibco) medium enriched with 10% HS (human serum) and the other to DMEM (Gibco) medium enriched with 10% FBS (animal serum). The cells were then centrifuged at 1200 rpm for 6 minutes. After the addition of penicillin and streptomycin antibiotics to the solution containing adipose tissue mesenchymal cells. Finally, cultures containing HS and FBS were incubated at 37 ° C with 5% CO2 and 90% humidity.
It is noteworthy that adipose tissue was collected after receiving written consent from the volunteer of lipolysis and this operation was performed by a surgeon in the operating room under completely sterile conditions.

Cell proliferation of ADSCs

The culture medium of ADSCs containing HS or FBS was replaced with a fresh medium every three days after incubation with the mentioned conditions. After cell density reached 90-70% by trypsinization (trypsin / EDTA; Sigma) of the culture plate, the isolated cells were used for cell passage or subsequent analysis.[33]
Stem cell proliferation rates in both samples containing HS or FBS were assessed after the second passage. Some cells with a density of 103 were selected and cultured in 4-well cell culture plates according to the conditions of the cultured cells. Cells were examined for proliferation on days 3, 5, 7, and 9. Cell passage
As the cells grew in the culture flask, the supernatant was discarded and the cells were washed with PBS. The cells were then added to the trypsin flask and incubated in a 37 ° C incubator for 3 minutes. After separating the cells from the flask, DMEM containing HS or FBS was added with trypsin to inactivate them. The solution containing the cells was then centrifuged at 1000 rpm for 6 minutes. In order to count the cells, 10 ml of homogeneous medium was cultured in 4-well cell culture plates 3 times each time in each cell passage.
In each passage, their number was counted using a *Neobar* lam and an electronic reed counter (Japan, Cis Max) in the capillary position.

Differentiation of adipocytes and cellular osteocytes

To evaluate the ability to differentiate ADSC in HS in the adipogenic differentiation, cells were incubated in DMEM supplemented with dexamethasone Mμ 1, insulin M μ 10, indomethacin M μ 200, and isobutyrate methylxanthine M μ 0.5 in either medium supplemented with HS or FBS. About two weeks later, ADSC was fixed in 10% formalin neutral buffer for 25 minutes and stained with Oil red O. The dye reaction was examined under an inverted microscope.

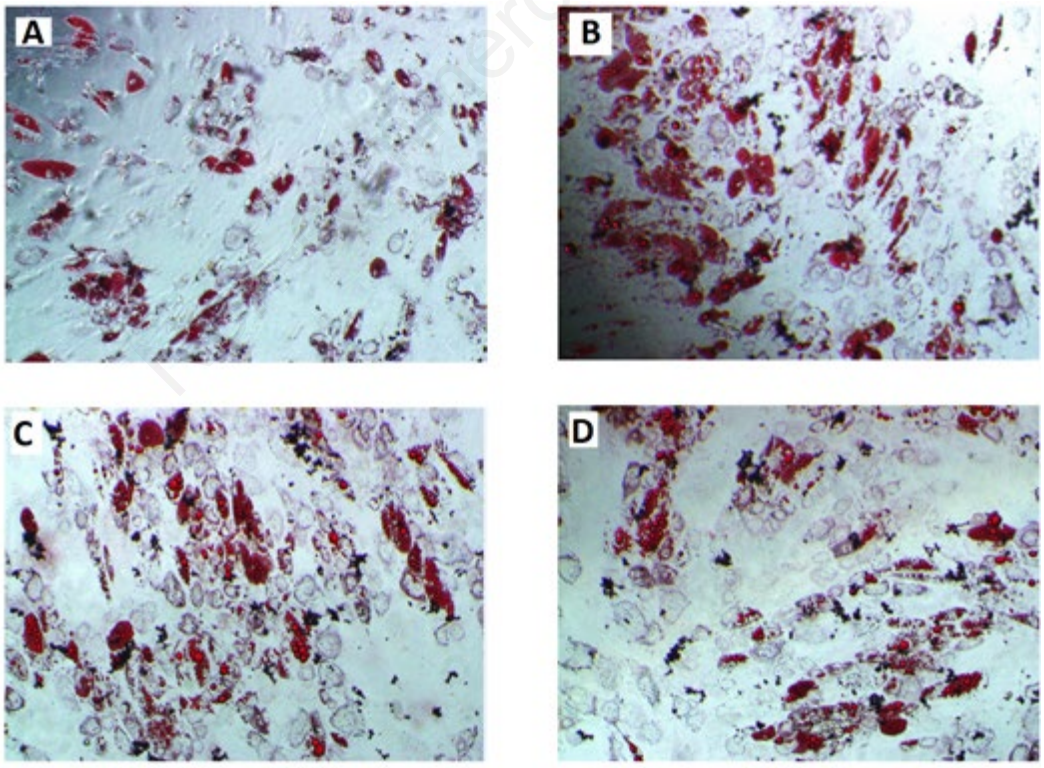

Fig 2. *Specific staining of cultured cells containing medium enriched with HS and FBS. A. Control group (FBS), b) The first group of human serum (day 9), c) The second group of human serum (day 12), d) The third group of human serum (15th day), (10x magnification).*

To differentiate the bone class, ADSC was cultured on culture plates. First, the primary culture medium was discarded. Cells were then incubated in DMEM differential medium containing 7-10 M dexamethasone, 0.2 M ascorbic acid, and 10 mM beta-glycerol phosphate in either medium supplemented with HS or FBS for 21 days. The existing culture medium was changed based on ocular vision or using a microscope every 3 days. Differentiation was assessed by Alizarin Red staining to detect calcium stores.

Data analysis

Frequency and percentage were used to express qualitative variables. T-test was used to evaluate the variables. SPSS software version 25 was used for analysis. A p value less than 0.05 was considered to be statistically significant.

Results

Cell proliferation of ADSCs

Cell proliferation findings showed that ADSCs in HS-enriched medium in the early days were not significantly different from FBS-enriched cells. However, serum containing HS showed significantly more proliferation over time than FBS-enriched cells ($p < 0.05$; Figure 1). The difference between the seventh and ninth days was statistically significant, where the groups had a significant difference on different days ($p<0.05$).

Ability to differentiate into cellular osteocytes

From the tenth day, calcium deposition, which was a characteristic of bone differentiation, was observed in the culture medium and the differentiation reached the desired level on the 15th day. According to Figure 2, it was observed that the differentiation of ADSCs cells in the medium enriched with HS is faster and more pronounced than the differentiation in the control group.

Expression of cell surface markers

In flow cytometric analysis performed on cells harvested from the fourth passage, the expression pattern of surface markers in proliferating cells in media enriched with HS and FBS was observed. The expression of surface markers in the medium containing HS was the same as the medium containing FBS where the expression levels of CD105 and CD95 were seen and the expression of CD 34 and CD45 was negative (Figure 2).

Ability to differentiate cellular adipocytes

Cells cultured with HS and FBS-enriched media that were affected by adipocyte differentiation had changed from a spindle to a spherical shape in both groups and contained fat vacuoles (Figure 3).

Discussion

Much research has been done on the differentiation of mesenchymal stem cells. However, limited studies have been performed so far on the use of HS for better proliferation of ADSCs. The present study seeks to optimize the proliferation and differentiation of mesenchymal stem cells using human serum. The findings of the present study showed that the proliferation of ADSCs in the medium containing HS is better and more efficient than in the medium containing FBS. Studies by Lindroos et al.[29] and Bieback et al. also stated that the optimal percentage of human ADSC proliferation in a human serum-containing growth medium was higher than that of an FBS culture medium.[30]

Josh et al. showed that human ADSC growth in HS-supplemented medium was faster than that of FBS-supplemented medium.[33] Another study found that the use of self-derived serum was less dangerous than the use of animal-derived serum and reduced the harms of using FBS for ADSC proliferation.[34]

In a study by Bahn et al.,[35] it was reported that ADSC cells proliferated and grew faster in culture medium containing human serum than in culture medium containing FBS. Thus, human serum can potentially be used instead of FBS in ADSC medium. de Paula et al. (2013)[36] stated that human serum is a promising supplement for the proliferation of ADSC cells in the laboratory.

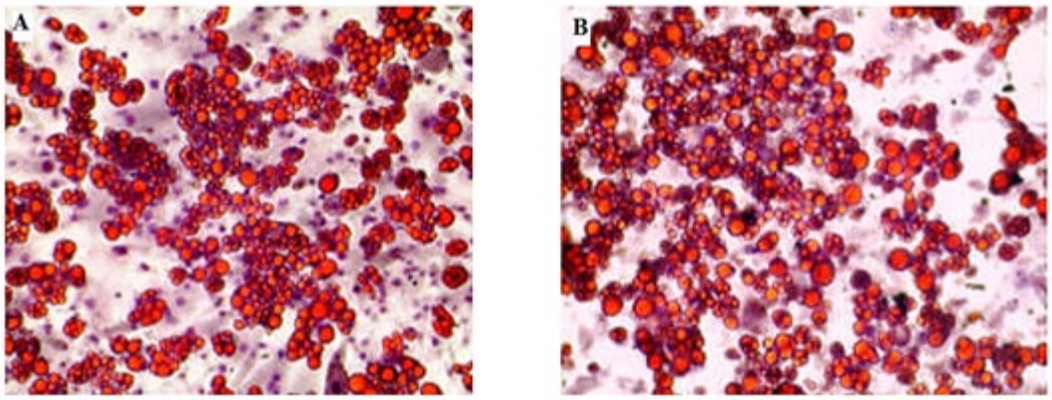

Fig 3. *Specific oil red O staining on cultured cells containing media enriched with HS and FBS. A) Control group (FBS), b) Human serum group.*

A study by Koellensperger et al.[37] showed that culturing ADSC cells in human serum is a viable alternative to FBS. In another study in 2021, the effect of FBS, human serum and human platelets on mesenchymal stem cells was investigated by examining the behavior of mesenchymal stem cells (metabolic activity, proliferation, morphology, and cytokine production). The results of this study showed that human serum (autologous and allogeneic) and released platelet lysates are promising and relatively good alternatives for MSC culture.[38] This study confirms that differentiation of ADSCs cells in HS-enriched medium was faster and more pronounced than the differentiation in the control group. Studies by Dreher et al. (2013)[39] showed that the more differentiating effects of HS on ADSC cell culture were greater than those of FBS. In general, similar studies have focused little on this issue. Given the role of differentiation in stem cell function, this is a very important issue.

Of course, one of the limitations that has been raised in various studies related to human serum is the condition of donors in terms of age, immune system, and serum quality. Some studies have suggested that serum donor conditions affect mesenchymal cell metabolism. Some factors including different serum quality (the content of growth factors and immune elements), serum obtained from adult donors (exposure of the immune system to various stimuli throughout life), and serum extracted from younger people (more naive immune system) have been mentioned to be capable of affecting the mesenchymal cell metabolism.[40,41]

To overcome this limitations, in the present study, the serum of 8 people was used and donors were selected from healthy adults.

In conclusion, our study confirm that mesenchymal cell proliferation and bone differentiation were higher in HS medium than in FBS. Due to better proliferation of adipose tissue-derived mesenchymal cells in the medium containing HS than FBS, it is suggested that human serum can be used in future clinical studies. Furthermore, study of compounds in human serum can be effectively used for better optimization of culture media.

List of acronyms

ADSCs - adipose tissue-derived mesenchymal stem cells
CD34 - Cluster of Differentiation 34
CD45 - Cluster of Differentiation 45
CD105 - Cluster of Differentiation 105
FBS - fetal bovine serum
HS - Human serum

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Publication Statement

I confirm that I have read the Journal's position on ethical publication issues and affirm that this report is consistent with those guidelines.

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