Original Article

The Convenient and Economical Method to Collect Adipose Mesenchymal Stem Cells

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Abstract

Background: Enzymatic digestion is an essential stage for culturing Mesenchymal Stem Cells (MSCs) and their therapeutic application. Several factors such as being cost-benefit, efficiency, safety, yield and amount of produced cells are determinant for choosing the appropriate enzyme. Collagenase is a conventional enzyme commonly used for enzymatic digestion. However, other enzymes like trypsin and even combination of these enzymes can be used as an alternative strategy in different situations. **Materials and Methods:** Abdominal subcutaneous adipose tissue was obtained from male BALB/c mice and digested under three different enzymatic processes: collagenase and collagenase/trypsin and trypsin. Cell culture process was performed under standard condition and MSCs at 3rd passage were used for further characterization by flow cytometry. **Results:** In this study, two different enzymatic methods for digestion of adipose-derived MSCs (ADSCs) of BALB/c mice were investigated. The morphology of cells was pretty different and was more homogenous in collagenase group. Also the yield of cells was varied among groups. Furthermore, the obtained data from flow cytometry revealed that ADSCs were positive for CD90 (70%), CD29 (98%), CD105 (52%) and negative for CD45 (<2%).**Conclusion:** Application of different enzymes depends on various conditions. Altogether, these data indicate that although use of trypsin in isolation protocol is cost-benefit, it can be used as an alternative method whenever limited number of cells will be needed. However, collagenase as a well-known and conventional method can be used for isolation of larger quantity of cells with several applications.

Keywords: Mesenchymal Stem Cell, Adipose, Collagenase, Trypsin

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Introduction

Different enzymatic methods are used to breakdown tissues in cell culture and other approaches. All these different methods are commonly followed by special steps such as washing, centrifugation and incubation. However, the most important differences are costs, efficiency and safety issue of different enzymes for obtaining applicable cells (1, 2). Considering that recently the MSCs have been used as a therapeutic agent, an appropriate method that is not only cost-effective but also is convenient and efficient for isolation of MSCs can be favorable (3, 4).

There are a group of multipotent cells known as MSCs. These multipotent cells can be isolated from different tissues such as bone marrow, amniotic fluid, umbilical cord blood, adipose tissue, placental tissue and dental pulp (5, 6). MSCs are capable to

differentiate into adipocyte, osteoblast and chondrocyte lineages and also appealing candidate for clinical practice because of their immunomodulatory, self-renewal and regenerative properties (7-10). Having low immunogenicity, lack of immunostimulating factors expression and their easy to access, make MSCs appealing candidates for cell transplantation (11, 12). Moreover, anti-inflammatory/immunomodulatory properties altogether with potential of gene modifications make MSCs as a novel vehicle for delivery of different therapeutic agents (13). As adipose tissue is available abundantly and its access is non-invasive, adipose-derived MSCs are one of desirable source of MSCs for therapeutic application (8, 14).

Expression of cell surface markers like CD90, CD29, CD105, CD73, CD44 and lack of CD45 and CD31 are beneficial for identification and characterization of MSCs (15-17).

As collagen network is the main structure of the extracellular matrix (ECM), using of some special enzymes such as trypsin, collagenase and their combination are necessary for tissue digestion (18, 19). The pro-enzyme, trypsinogen, is inactive form of trypsin which convert to trypsin and cleave the carboxyl terminal of lysine and arginine amino acids. Collagenase is another digestive enzyme. This enzyme alone or in combination with other enzymes (especially trypsin) could digest all types of collagen by its proteolytic ability (19-21). As all enzymatic methods are used for isolation and digestion of MSCs, in this study we used collagenase and combination of trypsin/collagenase to assess and compare their cost and impact of their digestive power on proliferation, viability and stability of MSCs.

Methods

Isolation and culture of BALB/c mice MSCs. Abdominal subcutaneous adipose tissue was obtained from male BALB/c mice (6-8 weeks old and 25g weight). To acquire MSCs, the adipose tissue must be digested under enzymatic process. For this purpose, two different digestive enzymes were used to assess their cost-benefitness.

Collagenase. The isolated adipose tissue was placed within the falcon tube which filled up with 500µl cell culture medium (Dulbecco's Modified Eagle Medium/F12-Atocel). 500µl collagenase was then added to the mixture immediately. The falcon tube was incubated under condition of 5 % (v/v) CO2 and 37°C for 20 minutes with at least vortex every 10 minutes in order to digest the pellet completely. The resulting mixture was centrifuged at 1200rpm for 5 minutes. Supernatant was removed and the pellet was re-suspended in 1ml media with 10% Fetal Bovine Serum (FBS) and transferred to the T75 flask with a final volume of 15ml.

Trypsin/Collagenase. Similar to collagenase method, the whole abdominal adipose tissue was suspended in the 500µl cell culture medium. Then collagenase and trypsin were added in an equal volume up to 500µl. The mixture was incubated for more than 20 minutes with vortex every 5 minutes. Other steps were performed as same as collagenase method. Resultant cells were then seeded in cell culture flasks and incubated in an standard cell culture condition.

Trypsin. Similar to two previous enzymatic methods, the abdominal adipose tissue of BALB/c mice was isolated. In this method after putting whole abdominal adipose tissue in the 500µl cell culture medium, 500µl trypsin was added to the falcon tube. The rest of protocol was as same as two previous methods.

Cell growth assessment. Cells were assessed daily and after reaching up to 85-90% confluency, MSCs were passaged (around 3 days after cell isolation). MSCs were counted at every passage and split into two individual flasks. This process was continued to the 3rd passage when cells grew up and were still in undifferentiated stage (Fig 1).

Cell viability testing. The cell viability was calculated using hemocytometer and trypan blue staining in three different digestion methods, based on following formula:

$$\%\ \text{Viability} = \frac{\text{Live cells}}{\text{Total cells}} \times 100$$

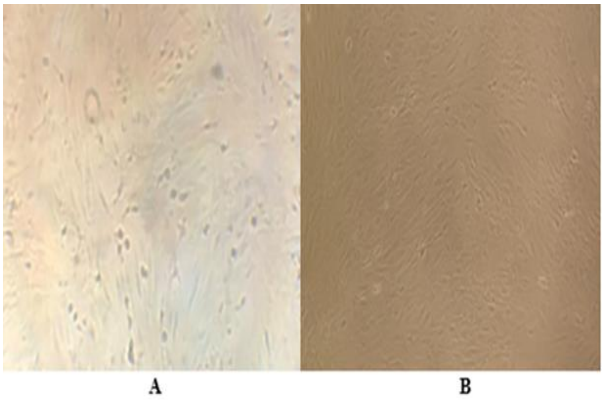

Fig 1. Mesenchymal Stem Cells, the 3rd passage. (A): digestion with trypsin (B): digestion with collagenase.

Immunophenotyping. To identify and characterize the adipose derived MSCs, cells were prepared to analyze by flow cytometry. According to the protocol, trypsin was used to detach the cells and centrifugation was done consecutively. After cell counting, they were distributed into the double sets of tubes due to the analysis with three different digestive methods on MSCs. Isolated cells were added to tubes and conjugated antibodies were then added. Phycoerythrin (PE) –labeled anti-mouse CD29 and CD90, APC-labeled anti-mouse CD105 and fluorescein isothiocyanate (FITC)-labeled anti-mouse CD45 were added as markers to tubes and they were kept in 4°C for 30 minutes. Finally, cell mixtures were analyzed by the flow cytometry.

Results

Isolation and culture of adipose tissue, cell viability and morphology. In this study, we have investigated three different enzymatic methods to digest adipose tissues of BALB/c mice. The cultures were performed under the same conditions. In other word, total number of isolated cells was equal in the initial step of three methods.

The morphology of ADSCs was pretty different. In trypsin and trypsin/collagenase-based method, there were some heterogeneous cells in the initial step which were removed after the second passage, while cells were more homogenous in collagenase group.

In the collagenase treated group, the cells were obviously more populated indicative of higher cell proliferation. Also doubling time of cells was almost the same prior to second and third passages.

We also compared cell viability and cell counting in these three different digestive methods. Collagenase-treated group, cell number was obviously higher compared with trypsin/collagenase-treated group (1.8x106 and 7x105 cells/ml, respectively). Different ratios of trypsin/collagenase (3:1, 1/2:1/2) were assayed and an inverse correlation between the amount of cell number and trypsin concentration was observed. It is notable that the number of cells in the trypsin-based method was unfavorable.

Flow cytometry. Flow cytometric analyses revealed that ADSCs were positive for CD90 (70%), CD29 (98%), CD105 (52%) and negative for CD45 (>2%) (Fig2).

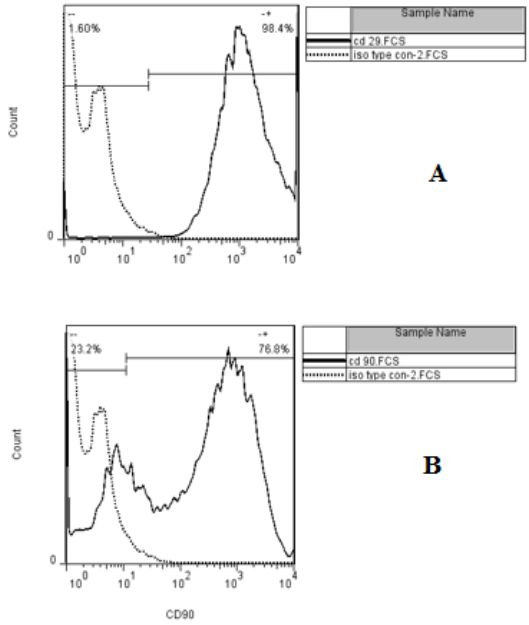

Fig 2. Flow cytometry results. (A): CD29, (B): CD90

Discussion

Two major methods including (i.e. mechanical and enzymatic methods) are commonly used for isolation of MSCs from various tissues. There various forms of enzymatic method (1, 22). As some different enzymatic systems exist for isolation of ADSCs, several factors such as cell viability and stability, time of proliferation, cost-benefit ratio should be considered for their application (1, 4).

In the current study, we compared three different enzymatic systems including collagenase-

based digestion, trypsin-based digestion and collagenase/trypsin-based digestion, to isolate MSCs from adipose tissue. We observed that cell doubling-time and time interval between passages were the same in these three methods. Markarian et al. have carried out the study on 9 different culture groups, they have found also that cell doubling-time is similar in all cultures (3).

Salehinejad and colleagues also demonstrated the unfavorable effect of trypsin on isolation and establishment of human umbilical cord and placenta mesenchymal stem cell culture. However, they found that collagenase/trypsin-based protocol was superior to trypsin alone (23).

Conclusion

In the current study we found although collagenase and collagenase/trypsin have the same capacity in the time of proliferation, but once some cells may not be resistant to trypsin and because of cellular damages, it is better to isolate cells in the absence of trypsin. Nevertheless, the application of different enzymes is based on the condition. Albeit use of trypsin in isolation protocol is not expensive but it can be considered as an alternative method whenever if limited number of cells are required. However, considering the yield of MSCs and its cost-effectiveness, it seems that collagenase-based method is an ideal digestive protocol especially where large amount of cells for various purposes are required.

Conflicts of Interest

The authors declare that they have no conflict of interest exist.

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References

1. Lockhart R, Hakakian C, Aronowitz J. Tissue dissociation enzymes for adipose stromal vascular fraction cell isolation: a review. J Stem Cell Res Ther. 2015;5(12):321-28.
2. Oberbauer E, Steffenhagen C, Wurzer C, Gabriel C, Redl H, Wolbank S. Enzymatic and non-enzymatic isolation systems for adipose tissue-derived cells: current state of the art. Cell Regen. 2015;4(1):7-20.
3. Markarian CF, Frey GZ, Silveira MD, Milani AR, Ely PB, Horn AP, et al. Isolation of adipose-derived stem cells: a comparison among different methods. Biotechnol lett. 2014;36(4):693-702.
4. Larijani B, Arjmand B, Ahmadbeigi N, Falahzadeh K, Soleimani M, Sayahpour FA, et al. A simple and cost-effective method for isolation and expansion of human fetal pancreas derived mesenchymal stem cells. Arch Iran Med. 2015;18(11):770-5.
5. Rodriguez VR, Llop M, Poddubnyy D. Hematopoietic and mesenchymal stem cells: a promising new therapy for spondyloarthritis? Immunotherapy. 2017;9(11):899-911.
6. Rizvanov AA, Persson J, Şahin F, Bellusci S, Oliveira PJ. Hematopoietic and Mesenchymal Stem Cells in Biomedical and Clinical Applications. Stem cells int. 2016;2016:1-3.
7. Hosseini S, Taghiyar L, Safari F, Eslaminejad MB. Regenerative Medicine Applications of Mesenchymal Stem Cells. Adv Exp Med Biol. 2018;2:115-41.
8. Han I, Kwon B-S, Park H-K, Kim KS. Differentiation potential of mesenchymal stem cells is related to their intrinsic mechanical properties. Int neurourol j. 2017;21:24-31.
9. Yang Y-HK, Ogando CR, See CW, Chang T-Y, Barabino GA. Changes in phenotype and differentiation potential of human mesenchymal stem cells aging in vitro. Stem cell res ther. 2018;9(1):131-44.
10. Ridge SM, Sullivan FJ, Glynn SA. Mesenchymal stem cells: key players in cancer progression. Mol cancer. 2017;16(1):31-40.

11. Calabrese G, Giuffrida R, Forte S, Fabbi C, Figallo E, Salvatorelli L, et al. Human adipose-derived mesenchymal stem cells seeded into a collagen-hydroxyapatite scaffold promote bone augmentation after implantation in the mouse. Sci Rep. 2017;7(1):7110-20.
12. Chulpanova DS, Kitaeva KV, Tazetdinova LG, James V, Rizvanov AA, Solovyeva VV. Application of mesenchymal stem cells for therapeutic agent delivery in anti-tumor treatment. Front pharmacol. 2018;9:259-68.
13. Kerrigan BCP, Shimizu Y, Andreeff M, Lang FF. Mesenchymal stromal cells for the delivery of oncolytic viruses in gliomas. Cytotherapy. 2017;19(4):445-57.
14. Liu M, Lei H, Dong P, Fu X, Yang Z, Yang Y, et al. Adipose-derived mesenchymal stem cells from the elderly exhibit decreased migration and differentiation abilities with senescent properties. Cell transplan. 2017;26(9):1505-19.
15. Camilleri ET, Gustafson MP, Dudakovic A, Riester SM, Garces CG, Paradise CR, et al. Identification and validation of multiple cell surface markers of clinical-grade adipose-derived mesenchymal stromal cells as novel release criteria for good manufacturing practice-compliant production. Stem cell res ther. 2016;7(1):107-22.
16. Carvalho AdM, Yamada ALM, Golim M, Álvarez L, Jorge L, Conceição M, et al. Characterization of mesenchymal stem cells derived from equine adipose tissue. Arq Bras Med Vet Zoo. 2013;65(4):939-45.
17. Johal KS, Lees VC, Reid AJ. Adipose-derived stem cells: selecting for translational success. Regen med. 2015;10(1):79-96.
18. Eikenes L, Tufto I, Schnell EA, Bjørkøy A, Davies CDL. Effect of collagenase and hyaluronidase on free and anomalous diffusion in multicellular spheroids and xenografts. Anticancer res. 2010;30(2):359-68.
19. Miersch C, Stange K, Röntgen M. Effects of trypsinization and of a combined trypsin, collagenase, and DNase digestion on liberation and in vitro function of satellite cells isolated from juvenile porcine muscles. In Vitro Cell Dev-An. 2018;54(6):406-12.
20. Taghizadeh RR, Cetrulo KJ, Cetrulo CL. Collagenase Impacts the Quantity and Quality of Native Mesenchymal Stem/Stromal Cells Derived during Processing of Umbilical Cord Tissue. Cell transplant. 2018;27(1):181-93.
21. Shi L, Ermis R, Garcia A, Telgenhoff D, Aust D. Degradation of human collagen isoforms by Clostridium collagenase and the effects of degradation products on cell migration. Int wound j. 2010;7(2):87-95.
22. Han Y-F, Tao R, Sun T-J, Chai J-K, Xu G, Liu J. Optimization of human umbilical cord mesenchymal stem cell isolation and culture methods. Cytotechnology. 2013;65(5):819-27.
23. Salehinejad P, Alitheen NB, Ali AM, Omar AR, Mohit M, Janzamin E, et al. Comparison of different methods for the isolation of mesenchymal stem cells from human umbilical cord Wharton's jelly. In Vitro Cell Dev-An. 2012;48(2):75-83.