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ORIGINAL ARTICLE

Differentiating between Stem Cells attained from the Omentum of the adult rat from the NMRI breed comparing to Heart cells using the essence of an Immature rat`s heart

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ABSTRACT
Stem cells are undifferentiated cells which can becomes advantageous for various cells, tissues, and organs and have a significant role in cell therapy. One of the resources which has found its way into graphical medicine are the MSCs of omentum tissue which have found fame among many researchers. Purpose of this study is differentiating between stem cells attained from omentum of an adultrat from NMRI breed and heart cells of using the essence of the an immature rat`s heart. MSCs of the NMRI breed ratomentum tissue are cultured in filtered flasks and thereafter are induced toward heart cells using the essence of heart tissue for 30-35 days. To prove the difference, mRNA statement of heart`s specific genes such as ANF was investigated using RT-PCR method in cells treated by heart essence. Omentum MSCs after differentiation exits its spindly morphology and finds many similarities with heart sells. Highlighted and lengthy appearance along with their ovate core is the clear characteristics of these cells. The difference in day 21 and 30 was confirmed by the mRNA statement related to specific heart genes in differentiated cells. From this study it can be concluded that MSCs of omentum tissue, induced by heart essence and in the absence of cell external matrix, is differentiated form the heart cells in lab conditions.
Keywords: *Omentum MSCs, heart tissue essence, Cardiomyocytes differentiation*

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INTRODUCTION

Stem cells are a kind of undifferentiated somatic cell which have two unique characteristics: 1) self-renewal: which can continuously replicate in culturing environment and maintain their replication and reconstruction ability for infinite cycles. 2) Differentiation: which under certain conditions can transform into specified cells and differentiate from the different types of cells constructing a living?These characteristics of stem cells have made their use in cell and finally different tissues of a living body a possibility. Hence, stem cells researches create hopes for a better treatment of various dangerous illnesses such as Type 1 diabetes, chronic liver diseases, Alzheimer, Parkinson, multiple sclerosis, heart diseases, spinal cord damages and even caner though cell therapy. We can point to MSCs as the most important mature stem cells which today have attracted the attention of the majority of researches. They exist in certain regions of each tissue. It seems that their task is substitution of tissue cells which are old and depreciated or have died due to being old or erosion. It seems mature stem cells are inactive most f the times and remain in the static or undistributed form for many years and in fact these cells are activated in response to a disease pr damage. One of the known sources in mature stem cells field is the MSCs resulting from omentum which are considered as the fat layer protecting visceral organs from external strikes. These cells are graphical tools for correction and substitution of damaged cells in living tissues. Currently research on features and culturing of MSCs attained from omentum proves that it

guarantees its multi-differentiation and replication capability. This capability is a restorative treatment source [4].

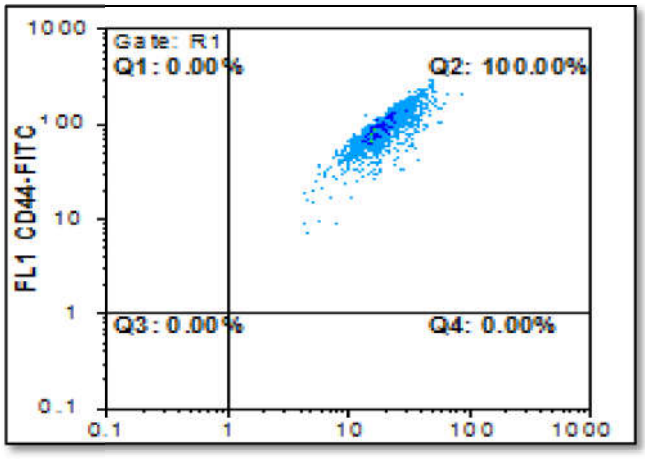
MATERIAL AD METHODS

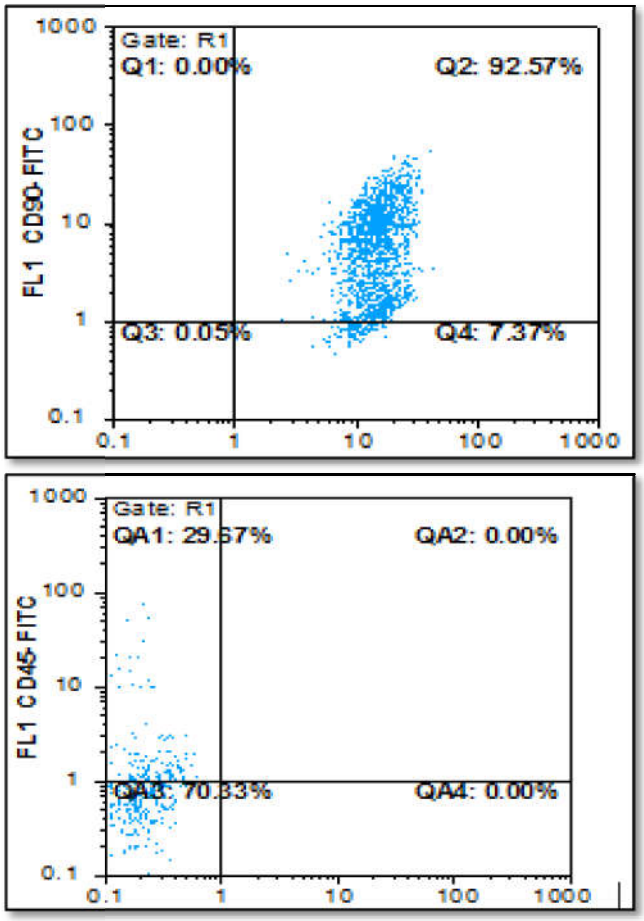
Best option for extracting omentum tissue for extrapolating MSCs is NMRI mature cells, since our purpose is not extracting MSCs from omentum`s fat tissue but from the omentum tissue itself. In this test we extracted omentum tissue from NMRI breed rat with 25-36 Gs weight, mice` spinal cord were separately injured and were laid on their back on the sheet and their legs were pinned with pins. Then stomach was disinfected with 70% alcohol and then using scissors and sterile forceps stomach skin was cut and the skin was opened and peritoneum slowly cut. When peritoneum hole was completely opened, omentum tissue which like a narrow wrinkle runs through stomach`s big curve carefully separated from the body of the animal using sterile forceps and was transferred inside a sterile petri dish containing PBS with dry ice under the hood. After passing culturing stages of MSCs, filtered flasks containing culturing environment and cells were held in incubator. Usually every 4 days cells culturing dilution were completely extracted under the hood by the sampler and washed with cool PBS washing dilution. Then fresh DMEM dilution with serum was added to cells culturing environment and transferred to the incubator. When stem cells are replicated NMRI breed`s heart essence was added to the dilution plates for 35 days, every 4 days.

RESULTS

Cell culturing, was daily observed by the phase contrast microscope. Based on these observations. In the primary culturing after 19 hours of cell culturing and elimination of non -sticky cells, first cells with spindly morphology was seen like separate colonies. Number of these cells was little in the beginning, however, with passage of time cells grew in a way that 5 days after the beginning colonies increased gradually and with the rapid replication of these colonies all of the flask`s floor was filled after a week (primary culturing reached inflation after a week.) Therefore a single layered culture of spindly cells was formed and showed a wavy view which is a characteristic of MSCs. Due to inflation of the cells and for purification, cells became data passage in a way that cells growth speed was increased during cell passage. Omentum MSCs were replicated by forming 4 consecutive passages. These cells maintained their fibroblast morphology during culturing and third passage was used as upcoming stages of the test which is differentiation. Also important point is that mentioned cells stick to the culturing dishes following melting after freezing stage, and their growth speed is reduced, however, they maintain their spindly morphology and their distinctive potential. Therefore, maintaining these cells in freezing conditions for long periods is possible and can be used in future researches such as gene transfer, simulation and etc. To proof cells being mesenchymal, in addition to their sticking to culturing dishes characteristic one of the ways for the identification is use of cell surface markers which after passaging and separating of MSCs stem cells from other cells a couple of times are investigated through flowcytometry method. Using this analysis MSCs stem cells specific markers expression include CD90. CD44, and hematopoietic cells specific markers lack of expression such as CD45 were confirmed in cells separated from tissue. Hence, CD90 anit-body 92/5% and CD44 100% in analyzed cells were expressed (in other words, 92/5 percent of cells possessed CD90 phenotypes and 100 percent of cells CD44 phenotypes.) Whole CD45 antibody was not expressed in any of the cells.

Fig 1: CD44, CD90, ,CD45 antibodies analysis in Flowcytometr test





Investigating the statistical results flowcytometr test in omentum stem cells

In omentum stem cells CD_{+90} marker is expressed as 92/57 percent and CD_{+44} is expressed as 100 percent. However we have CD_{+45} lack of expression which is the specific marker for fat.

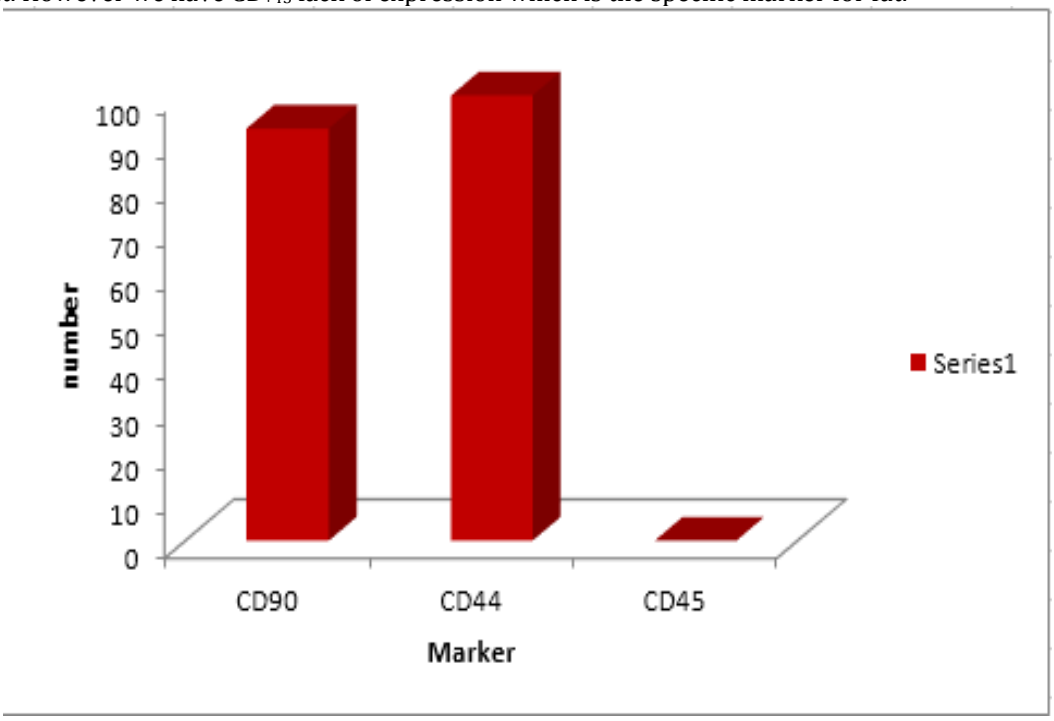


Fig 2: CD44, CD90, ,CD45 antibodies analysis in Flowcytometr test

In this study MSCs stem cells were treated with heart essence with doses of 10λ,40 λ, and 50 λ every four days after extraction from omentum and inflation in cell culturing plates. For investigating and proving the results RT-PCR test was used. After the treatment of the cells with diluted heart essence cells went through a period of 35-40 processing. Until 21st day the effects of the essence was not seen. However, from the 21st to the 30th changes were gradually seen on the surface of the cells. And on the 34th day cells morphologically had a significant change toward heart cells and with RT-PCR expression test of heart genes it was confirmed that indication of firm differentiation of MSCs cells is compared to heart`s semi-muscle cells. Treated MSCs cells with 10λ essence dose showed the least changes and with increasing the dose heart essence effect was gradually increased in the amount of induced MSCs cells till in 40λ dose it reached its highest amount of induction in MSCs cells. However, with MSCs cells treatment with 50λ dose cells of the cell culturing plates were completely polluted and faced apoptosis. Experimental test of a cell culturing plate containing MSCs was considered as a control sample and did not undergo the heart essence treatment. As a result after RT-PCR test MSCs un-differentiation was seen. In the current study, RNA samples were extracted from cells under treatment of heart essence in the 30th day differentiating omentum MSCs of NMRI rat. After qualitative and quantitative examination of extracted RNA, inverted transcription reaction and following it PCR reaction was conducted using designed primers for each gene. As a result ANF heart gene`s expression in flasks treated with 10λ and 40λ essence were examined. ANF gene is expressed with the amount of 380bp and obtained images show that flask treated by 40λ essence expression of ANF gene is higher than the flask treated by the 10λ essence.

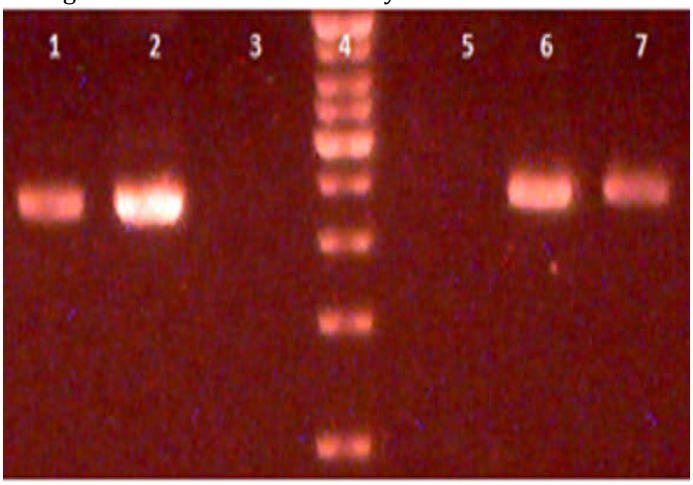


Fig3:Investigating ANF gene expression in differentiated cells
Column 1: Normal cell, Column 2: Positive control, Column 3: Negative control, Column 4: 100bp marker, Column 5: Negative control cell, Column 6: Cells under treatment with 40λ heart essence, Column 7: cells under treatment with 10λ heart essence.

DISCUSSION AND CONCLUSION

Majority of the congenital diseases such as heart failure and sever damages of heart, spinal cord, pancreas and etc organ transplant is not successful in majority of the cases due to its many problems. One of the diseases that today men are mostly facing is heart diseases from the birth time or in midlife [1-5]. Heart`s capability as a blood pumper body organs leads to the maintenance of the physiological function of organ and heart insufficiencies and damages inflict severe damages on other organs specially the brain. Since mature cardiomyocytes are not capable of self - renewal and restoration, the only effective treatment for heart patients is heart transplant which itself faces many problems such as low heart donator and transplant rejection. Makino *et. al.*, [6] For the first time in 1999 showed that bone marrow MSCs of a rat under the treatment of azacitidine-5 have the ability to differentiate pulsating cardiomyocytes. Xu *et. al* [7] reported that Human`s MSCs form heart under the induction of growth factors and stated the differentiation factors such as desmin, T cardiac troponin, alpha-cardiac actin. Hollowic *et al* [8] in 2011 using oxytocin and azacitidine-5 were able to differentiate between human umbilical cord MSCs and semi-cardiac cells and show the coloring of Immunocytochemistry expression of heart`s specific proteins such as cardiac actin and myosin`s heavy chain and cardiac troponin. Study of Orlic *et al* [9], in 2001 show that bone marrow`s pre-constructs can transform into heart cells after injection to rat`s cardiac ischemic muscle and replace the dead tissue. Baharvand *et al*. [10] in 2005 showed that parts of cellular external matrix derived from cardiac fibroblast (Cardiogell) supports primary maturity of cardiac cells derived

from mESCs in lab conditions. Menasche *et al* [11] report in 2002 for the first time explain the use of myoblast cells in enhancing heart`s performance in human. For the first time in 2006 Ko et. Al., succeeded in producing cardiac tissue from rat cells (ES) in vitro environment. Potassium canals performance in cardiomyocytes derived from embryonic stem cells was performed using Baharvand *et al*., [10] in 2012 which RT-PCR test results indicate the expression of differentiation between cardiac specific genes such as alpha and beta myosin of heavy and light chain and ventricular auricular factors in cardiomyocytes. In this project considering previous researches and knowing stating cardiac cells discharge cardiac growth factors and cardiac essences contains them, decided to use heart essence as an induction factor on the ground of omentum stem cells and succeed in creating cardiac cells and investigate differentiation effect which was successful. Hence, through cellular culturing and consecutive passaging we found that countless number of cells that have covered the flask`s floor in a monolayer fashion are stem cells and are morphologically spindly. As a result to prove stemness we have employed identification tests such as flowcytometry which determines antigens which are expressed on the cell surface and RT-PCR test which determined expression on the surface of MRNA which are used in synthesis of cellular genes, like other researchers. We found that omentum cells like other mesenchymal cells contains mature and embryonic markers which will possess a set of embryonic pluripotent stem cells. In the performed flowcytometry test it was determined that cultured cells are stem and expressed mesenchymal surface antigens including CD90 and CD44 with the highest amount. However, CD45 which is specific to fat tissues is not expressed and can be concluded that cultured cells are mesenchymal and are omentum fat tissue free. The result of the performed RT-PCR indicates that WT_1 and OCT_4 markers which are expressed in mature and embryonic stem cells are also expressed in omentum stem cells with amounts of 396bp and 352bp respectively. In this study we showed that MSCs obtained NMRI rat`s omentum induced by the cardiac essence as a sole inducer can take the morphology of semi-cardiac cells [12-15]. Cells take a highlighted and lengthy appearance with an ovate core which is the characteristics of cardiac cells. This project was conducted in the absence induction factors such as cardiac growth factors, Bfgf, sphingosine-1-phosphate and TGF-B. Considering the applied method in this study differentiation between MSCs of NMRI rat`s omentum in the period of 30-35 days and cardiac cells was performed. But it was determined that differentiation process between MSCs and semi-cardiac cells of mesodermal lineage using only cardiac essence is a long and time consuming process and final differentiation of cardiac cells in the presence of a suitable inducer of heart tissue is performed better and in a shorter time and cardiac essence alone does not contain all the growth and induction factors needed to form a pulsating mature cardiac cell.

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