



Study the Isolation of mesenchymal stem cells from newly born infants of the fetal-chorionic plate

Mustafa A.A. Altwell* and Mohammed Abdalmalek Ali Al-Bedhawi

Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad,
Iraq

*Email: mostafa.abdullah1300a@ige.uobaghdad.edu.iq

Abstract

Stem cells are exclusive population of cells located in all stages of lifetime that holds capability to self-renewal plus specialize to group of cell lineages of multiple organs and tissues and considered a major source of differentiating cell types in the body, stem cells has been considered as part of new cell therapy of numerous damaged organs for instant embryonic stem cells in term of neurological cases like stroke and parkinson's as these cells are transplanted in the body for focal organ damage repair, differentiation and immunoregulatory response, chemoattractant and stimulate tissue regeneration Current study detected the presence of mesenchymal stem cells isolated from Iraqi newborn infants placenta using enzymatic method of collagenase I enzyme and cultured with DMEM-low glucose, through observation of their genetic markers expression such as CD105, CD90 and CD73 using RT-PCR showed positively folding as well as the onset of their expression levels reduction as an initiation process for osteoblastic gene expression using epidermal growth factor plus osteogenic differentiation factors including β -glycerophosphate disodium salt pentahydrate, Dexamethasone and L-ascorbic acid.

Keywords: mesenchymal stem cells, CD105, CD90, CD73, Epidermal growth factor, osteogenic differentiation factors, placenta, collagenase I

1.Introduction

Stem cells are unspecialized self-renewal undifferentiated with ability to convert to any cell type under various external signaling stimuli responses (1) they present within numerous tissues and organs during each developmental stages of life from embryonic to adult and has asymmetric division capability to ensure it is survival by dividing into one specialized cell and one unspecialized stem cell (2) at the embryonic level, stem cells are important for generating all organs and growth of the fetus while at the adult level they are responsible for maintenance and homeostasis of all tissues (3) mesenchymal stem cells (MSC) indeed are adult stem cells with general features of self-renewal ability as well as multipotent power plus of that it shows immunomodulation efficacy, hormones and exosomes (4) due to their variety differentiation capability of ectodermal, mesodermal and endodermal layers, MSC has enlightened to be one of the most essential type of stem cells to be



used as regenerative medicine approaches for wide range of organs repair (5) Mesenchymal cells can be found among various organs and within several tissues comprised as a part of the cellular matrix and are located inside dental pulp, liver, even skin (6) however the most tissues they are found in with abundant settlement are umbilical cord and placental tissue, bone marrow and adipose tissue (7) MSC project numerous markers such as CD90, CD105 and CD73 and these cells presented inside placenta as well as umbilical cord (8) placental tissue generally contains mesenchymal stem cells which are isolated from different sites of placenta includes amniotic fluid and membrane, chorionic plate, chorionic villi or intervillous space and decidua basalis (9) MSC also found not only in placenta but in bone marrow but not abundant and might be more matured (10) Under specific triggering conditions, MSC could differentiate into one of the trilineage cell line which is either chondrogenic, lipogenic and osteogenic cells (11) Because of multilineage differentiation capabilities of MSC potency and their self-renewal feature and immunomodulatory responses they have been under focus (12) MSC considered as adult stem cells with multiple differentiation features for regeneration (13) MSC also may present in adipose tissue although could be less naïve (14) MSC showed to have promising and viable sources for modern clinical treatments for tissue repair and regeneration (15)

2. Material and method

2.1 Sample Collection

The tissue of human placenta was obtained from newly born Iraqi baby from Al-yarmouk hospital in Baghdad governorate, instantly washed with distilled water packed into ice bag and moved directly to Iraqi center for cancer research to continue the procedure, after the delivery into the facility, the tissue placed in a tray and prepared for dissection of 1gm for the mincing process Without forgetting to take small piece handled within trizol for later molecular test.

2.2 Sample Preparation

After 1gm of placenta tissue were dissected from the fetal part, it was washed with phosphate buffer saline about three times then placed in glass petri dish



submerged also with PBS for mincing it into several small entities gently using needle holder to hold the small pieces and scissors cutting into tiny particles, Then collecting these tiny tissue particles from the submerged PBS within petri dish into centrifuge tube containing 5ml of free-serum DMEM mixed with 1mg of lyophilized collagenase type I and then placed about 45 minute inside water-bath of above 39°C for the enzyme to activate notifying that each 10 minute the tube were held out of the water-bath for shaking gently to mix the components then replaced into the water-bath and so on for the separation process of the mesenchymal stem cells from the placental tiny tissue particles

2.3 Cell Culturing and Differentiation Factors Additive

After the process of making MSC free from the placental tissue, free osteogenic differentiation factors-serum media first incubated to reach optimum 37°C then poured into two cell culture flask about 7ml each and then 4ml from the centrifuge tube containing collagenase-free serum DMEM with mesenchymal stem cells were collected from the supernatant and inoculated into each flask and kept inside non Co2 incubator overnight for two days and under inverted microscope was showed as spherical cells for the first 24hours.

On the day three of culture, media were changed from free osteogenic differentiation factors-serum DMEM media into osteogenic differentiation factors serum-DMEM media to proceed the differentiation process within three days until day five

2.4 Gene Expression

The Quantitative Real Time PCR (qRT-PCR) was carried out using the QIAGEN Rotor gene Q Real-time PCR System, by evaluating levels of CD73, CD105 and CD90 with GAPDH housekeeping gene as a control one by preparing of primers using Harvard primer-bank site

3. Results and Discussion

The results of the current study revealed good MSC gene expression located both inward the chorionic plate of the placental tissue as well as after isolation



from the tissue of an Iraqi baby sample, the biologic activity of MSC among different tissue parts of the placenta may indicate their strength and stemness, According to the results recorded from the experiment done MSC within chorionic plate of surface markers such as CD105, CD73 and CD90 folding expression which are marked as indicator of MSC showed positive in the tissue itself plus the isolated cells from the same tissue, using RT-PCR to catch higher folding value of those markers

Location of MSC	Name of the gene	Expressing Fold
Cp Tissue	CD90	1.000
	CD105	1.000
	CD73	1.000
Isolated MSC	CD90	6.634
	CD105	4.891
	CD73	1.625

The current study plan was a five-days old of the cells starting by day one isolation and culturing of MSC from chorionic-plate of the placenta until day three after adding osteogenic differentiation factors until day five including β -glycerophosphate, L-ascorbic acid and Dexamethasone as a major source for directing MSC to be committed for becoming osteocytes later in addition to epidermal growth factor mixed with the DMEM-media added to the culture from day one of culture for accelerating MSC growth and regarded as extra factor to induce cellular behavior turning MSC into osteocyte. We targeted two osteogenic genes such as osteopontin (sparc) and osteonectin to determine at day five of adding osteogenic differentiation factors plus to epidermal growth factor to see if there is any accelerated response and if at day five does MSC turn to shut down their stemness genes and prepare itself cellularly to orient their molecular

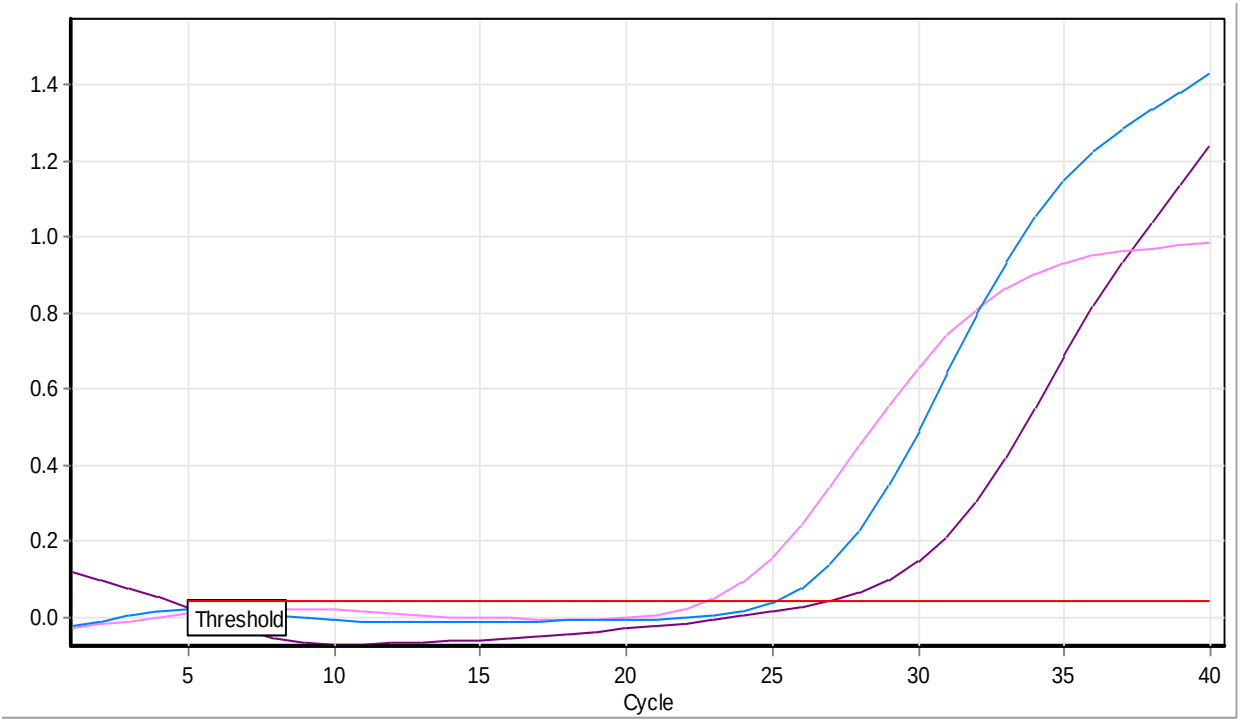


interaction signals to inactivate MSC gene expression and gradually increase osteogenic gene expression,

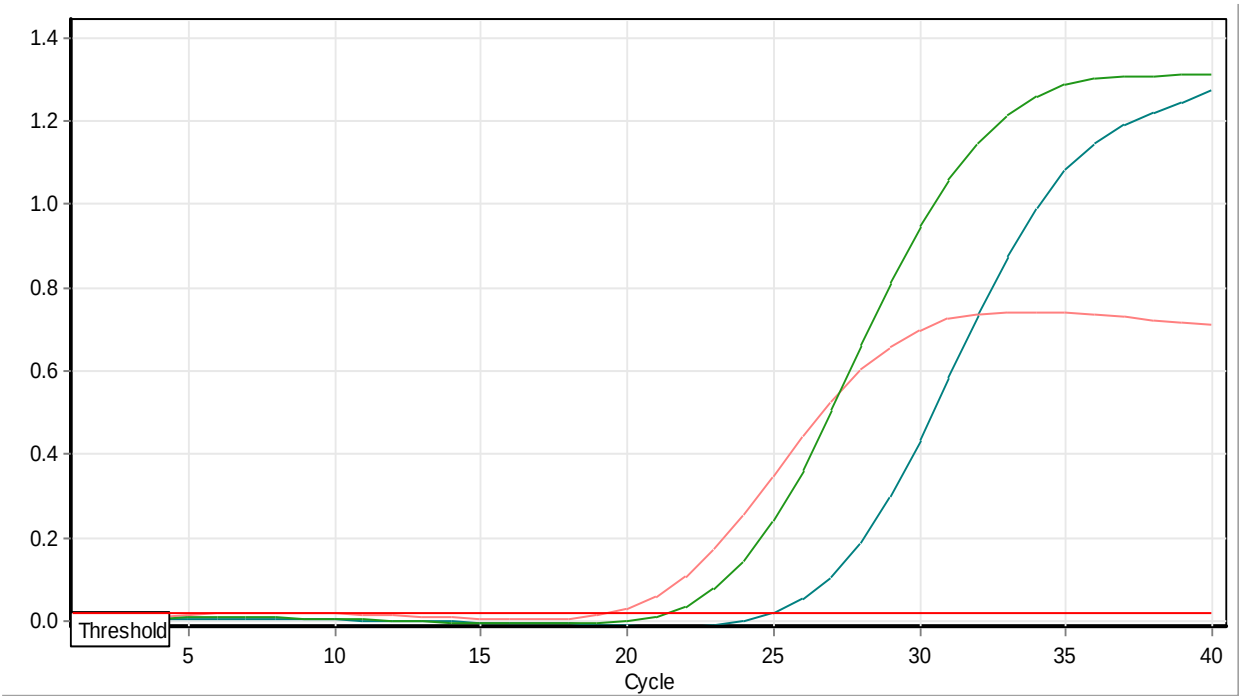
Name of the gene	Expression fold
Osteonectin	0.050
Osteopontin	0.005
CD90	0.633
CD105	0.173
CD73	0.933

While the threshold curve cycles of charts of mesenchymal stem cells isolated from placental chorionic plate (CD73, CD90 and CD105) and osteogenic markers (OSTEONECTIN and OSTEOPONTIN) are shown below:

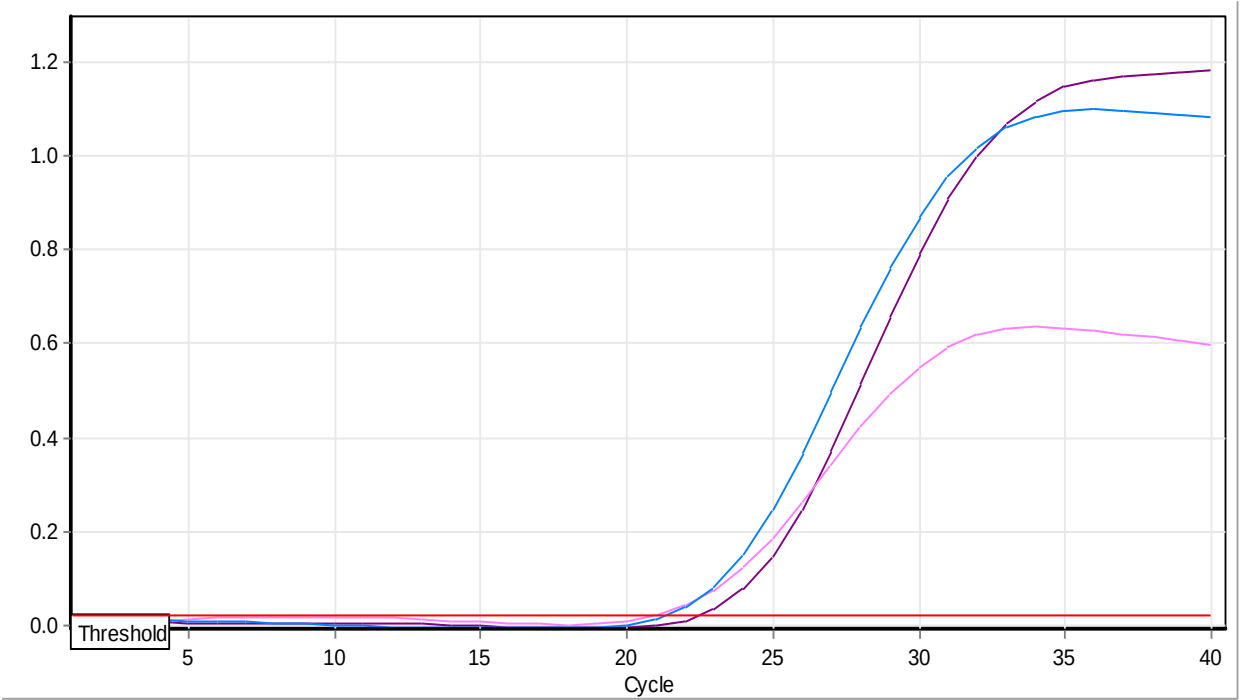
CD90:



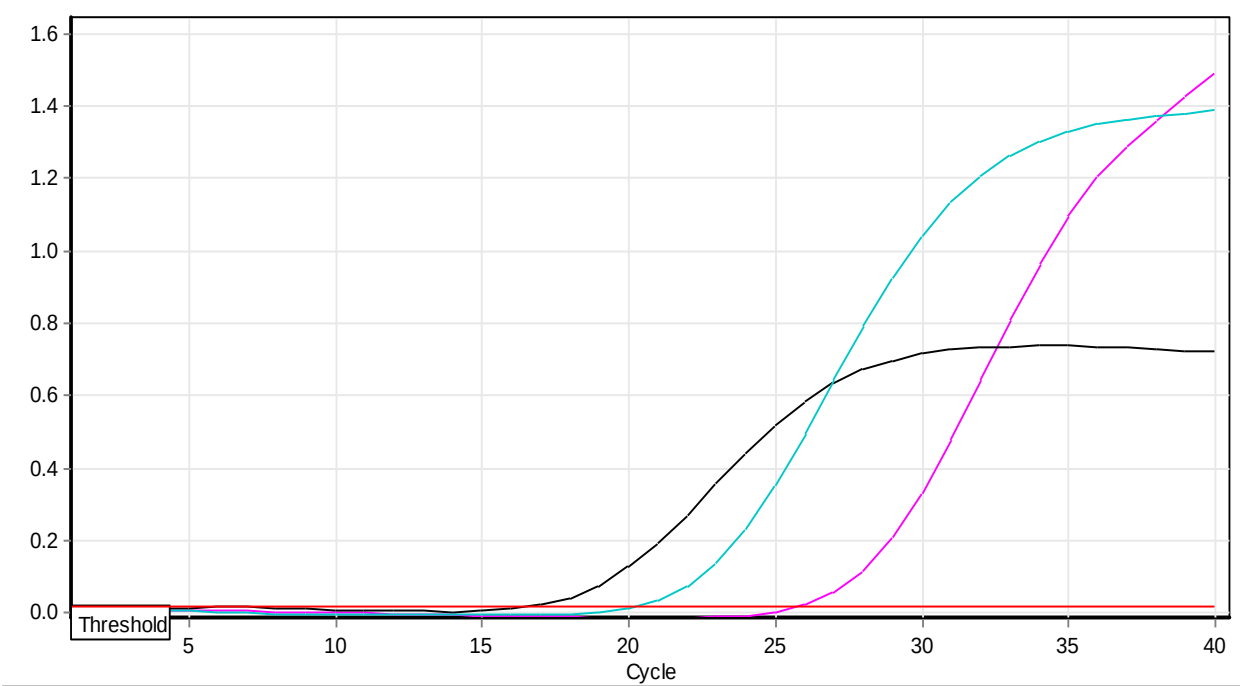
CD105:



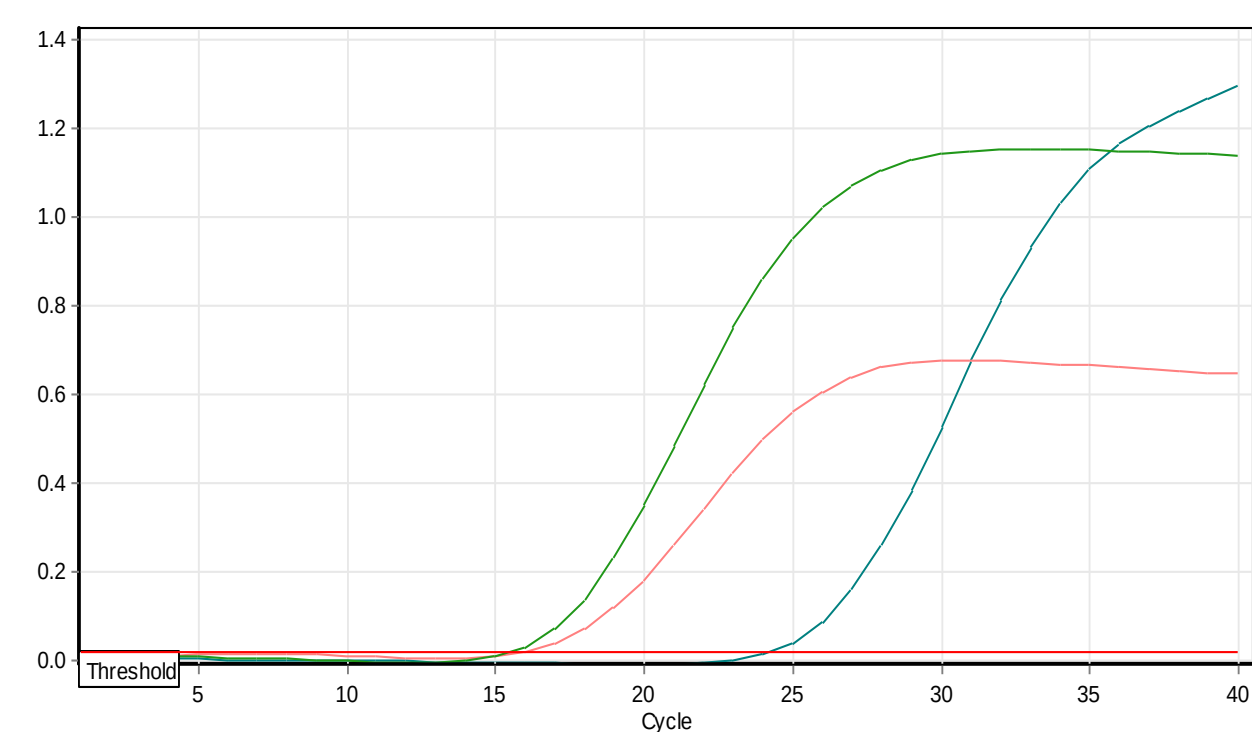
CD73:



OSTEONECTIN:



OSTEOPONTIN:



Although there were no significant change or upregulation of any of the two osteogenic genes and remaining downregulated and appears no response regardless of adding osteogenic differentiation factors at day five of cellular collection from the culture, it was noticed that MSC genes expression were also downregulated of the same cells that was collected which may indicates the starting point of stem cells to alter their stemness genes downregulated and prepare the cell molecularly directing stem cells toward specializing but since the experiment done for short period of time it might be not enough for the cell to begin convert but at least we succeed in detecting the turning point itself of the MSC where their genes gradually initiate to decrease.

The timing onset of mesenchymal stem cells to convert into any cell type generally and osteocyte specifically is dependent on many factors such cell culture conditions like media specifications, oxygen and carbon dioxide level, addition of



differentiation factors and the dose of the materials according to which cell type required to be committed for and as well if there are scaffolding structure considered as a media bowl for MSC

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