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Assessment of Umbilical Cord Mesenchymal Stem Cells Cultivation using Fetal Bovine Serum or Human Platelets Lysate

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Abstract

Background: Mesenchymal stem cells (MSCs) are non-hematopoietic multipotent cells able to self-renewal and to differentiate into several mesenchymal lineages. MSCs are an attractive source for stem cell therapy due to their exciting characteristics including multilineage potentiality and immunomodulatory properties.

Objective: To assess the umbilical cord mesenchymal stem cells cultivation using fetal bovine serum or human platelets lysate.

Materials and methods: 10 newborns umbilical cord samples were collected from healthy maternal donors. Platelets concentrate was obtained from the blood bank to produce platelets lysate. MSCs were isolated from Wharton's jelly (WJ) tissue using the explant culture protocol. Stem cells cultivation was performed using Dulbecco's Modified Eagle's Medium. MSC morphology, growth expansion, and immunophenotype identification were performed at 80% confluence of cells.

Results: The study showed that the expansion potentiality of MSCs in HPL medium was significantly higher than FBS Medium. Moreover, the cultivation time for the 80% confluence was decreased in the human platelets lysate (HPL) medium than the fetal bovine serum (FBS) medium. Furthermore, the immunophenotyping assay showed that the HPL cultured MSCs expressed higher concentration of CD44 and CD73 compared to those cultured in FBS medium.

Conclusion: Wharton jelly is a rich source of mesenchymal stem cells and the human platelets lysate could be a good and a safe replacement to the fetal bovine serum in mesenchymal stem cells expansion.

Key words: Mesenchymal stem cells, Umbilical cord, Fetal bovine serum, Human platelets lysate, Wharton Jelly.

Introduction

MSCs obtained from the Wharton's Jelly (WJ) have gained much attention over the last years since they can be easily isolated without any ethical concerns. Furthermore WJ-MSCs represent a more primitive population than their adult counterparts. To yield clinically relevant cell doses, ex vivo expansion of MSCs is required. Most protocols still use fetal bovine serum (FBS) to

expand MSCs which has high cost, limited availability, and risk of contamination. HPL is an excellent choice as alternative to FBS due to its availability, low cost, safe and it is a human source. Mesenchymal stem cells (MSCs) are multipotent stromal non-hematopoietic cells with the ability to self renewal and to differentiate into several mesenchymal lineages. These cells were first described by Friedensteinet as fibroblastic-like cells from bone marrow[1].

MSCs possess unique biological characteristics such as self-renewal capacity, multi-lineages differentiation, homing and migration ability, immunomodulatory properties, and paracrine secretion activity that make them one of the valuable candidates for cell-based therapy. The International Society of Cellular Therapy defined MSCs by the following three criteria: MSCs must be adherent to plastic under standard tissue culture conditions, and must express certain cell surface markers such as CD44, CD73, CD90, CD105, and lack of expression to other markers including CD45, CD34, CD14, or CD11b, CD79 alpha or CD19, and HLA-DR surface molecules, and have the capacity to differentiate into osteoblasts, adipocytes, and chondroblasts under *in vitro* conditions[2].

In comparison with other stem cells sources, umbilical cord has advantages of easy harvesting (painless), no ethical concerns and abundance of MSCs. It is considered as source of cells that have more primitive properties than cells isolated from adult tissues and that make it a promising source of MSCs for clinical application and cells therapy. MSCs have been isolated from four different compartments of the umbilical cord: from Wharton's jelly, from tissue surrounding the umbilical vessels, from umbilical cord blood, and from the sub endothelium of umbilical vein. Researchers reported that Wharton's jelly is a rich source for MSCs and the efficacy of isolation of primary cell culture from Wharton's jelly amounts to 100%[3].

Umbilical cord derived mesenchymal stromal cells (UC-MSCs) are a focus for clinical translation but standardized methods for isolation and expansion are lacking. Creation of a protocol considering good manufacturing practices in isolation and expansion of UC-MSCs is required to obtain sufficient quantities for therapeutic uses. The culture medium is the most important component of the culture environment, because it provides the necessary nutrients, growth factors and hormones for cell growth, as well as regulating the pH and osmotic pressure of the culture. Fetal bovine serum (FBS) has been considered most frequently used to supply growth factors to MSC culture and stimulating cell proliferation and to provide transport proteins, minerals, trace elements, lipids, attachment factors[4].

To avoid the risk of using animal materials, the use of human platelets lysate has recently been an excellent choice as alternative to FBS due to its availability, low cost, safe and it is also a human origin. It can be autologous or allogenic obtained from single donor or pooled from multiple donors to provide sufficient volume for therapeutic scale and MSCs expansion. Platelets contain bioactive molecules and growth factors that are released from α-granules after platelet destruction by physical or physiological methods. All of these molecules could promote MSCs proliferation in comparison with FBS. Doucet and his colleagues demonstrated that factors in platelets granules could promote MSCs expansion according to its dose[5].

In this study we are adopting a xenogenic-free expansion protocol and using human platelets lysate (HPL) as alternative to FBS since platelet-derived growth factors can promote MSC *ex-vivo* expansion. The purpose of this study was to evaluate the efficacy of different concentration

of HPL on expansion of MSCs derived from Wharton's jelly.

Materials and methods

This was a descriptive cross-sectional study. A signed informed consent and a structured questionnaire were obtained from each mother prior to collection. The study was approved by Al Neelain University and the Ministry of Health (Sudan).The laboratory work was performed at Al Neelain Stem Cell Center of Al Neelain University (Khartoum, Sudan).
10 fetal umbilical cord tissues (Wharton jelly) were collected from mothers after healthy pregnancies either by cesarean section or vaginal deliveries at Dream Hospital (Khartoum). About 12 cm of umbilical cord tissue were harvested in sterile conditions for isolation, purification, and identification of MSCs.
Platelets lysate preparation: Platelets concentrate units (< 2 days store) were collected from the blood bank of the National Public Health Laboratory (Khartoum) for preparation of the platelets lysate. The platelets lysate was prepared by activation of the platelets granules using calcium chloride according to the protocol reported by Phuc Van Phan and his colleagues (2014)[6].
Culture media preparation: Low glucose Dulbecco's modified Eagle's medium (Gibco, Life Technologies, REF31600-083 / Lot 1850559) was supplemented with 1% Glutamine, and 1% antibiotic + antimycotic, and then human platelets lysate (HPL) medium or fetal bovine serum (FBS) were added to the media in different concentrations (5 %, 7 %, and 10%).
Isolation and culture of MSCs cells from umbilical cord: This was performed from Wharton jelly by the explant technique and enzymatic digestion protocol using trypsin enzyme and the WJ-MSCs were cultured (Protocol of Beeravolu, and his co-workers, 2017)[7]. Tissue pieces were seeded in 75 cm^2 tissue culture flasks and then 10 ml of culture media supplemented by different concentrations (5%, 7%, 10%) of HPL or FBS at 37ºC in a 5% CO_2 incubator. After 3 days of incubation, the culture media was removed and replaced by a fresh one. The appearance of cells from explants were observed daily under inverted microscope and the culture media were exchanged twice a week. After 10 days the tissue pieces were removed to release the cells from the explants and become expanded until they reach 80-90% confluence. The adherent cells were dissociated by using trypsin solution. Cell growth was subjected to viability counts using the Trypan Blue protocol with a dilution factor 10:1.
Immunophenotyping: The surface markers expression of MSCs expanded in FBS or HPL were analyzed with flow cytometry (Epics XL-MCL). The cells were then stained with anti-human monoclonal antibodies conjugated, against: CD34 and CD44 and FITC, conjugated against CD45 and CD73 (Immunostep.SL). Controls were used in all analysis steps.

Results

MSCs were successfully isolated from 10 W.J samples (100%) after expanding ed in DMEM medium supplemented with 5%, 7% and 10% of FBS or HPL. HPL was supper in term of propagation and colonogenic formation. MSCs reached 80-90% confluencein 10% HPL,7% HPL and 5% HPL, 10% FBS ,7% FBS and 5% FBS at within day 14-16 days,16-18 days, 17- 19 days >19 days and > 19 days respectively. Most of the cells expanded in HPL were spindle-shaped,

resembling fibroblasts it was appear most rapidly and in high amount in cells expanded in the presence of 10% HPL (within3 days), 7%HPL, 10% FBS and 5% HPL (within 4-5 days) and the same cell morphology but in few numbers were detected within 7-9 days of culturing in 7% and 5% FBS.

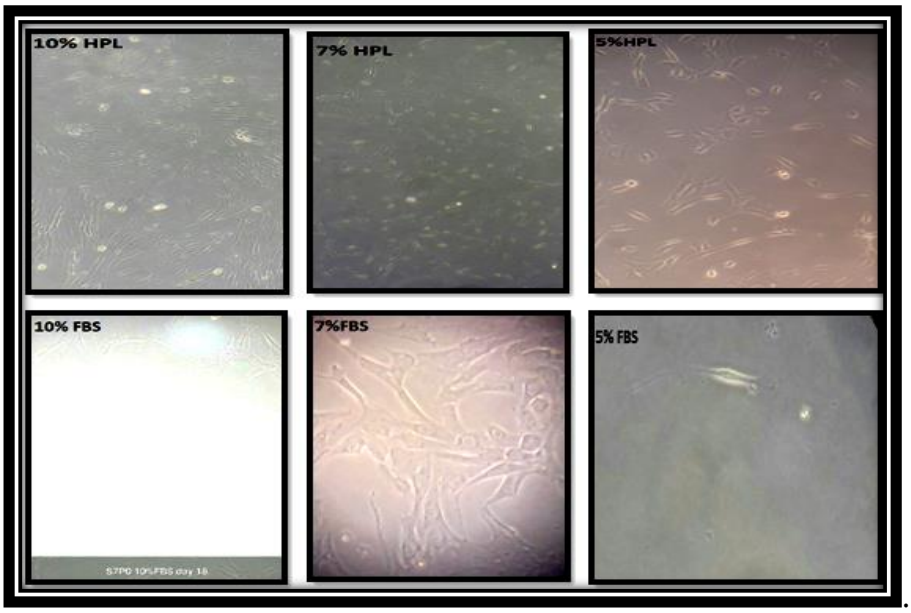

Fig. (1): Morphology of WJ-MSCs in different concentrations and FBS immunophenotypic identification

All adherent cells derived from umbilical cord did not express hematopoietic lineage markers (CD34, CD45) as assessed by flow cytometry. In addition, the majority of cells expressed high level spppp of positive markers (CD44, CD73).

Discussion

This study showed 100% efficiency of MSCs isolated from WJ, and our findings were supported by Irina and his co-authors (2015)[6], who found that the efficacy of isolation of primary cell culture from Wharton's jelly amounts is 100%.

This study evaluated different concentrations of HPL (5%, 7% and 10%) as alternative to FBS in expansion of UC-MSCs, we observed that HPL accelerate the proliferation rate of MSCs and the 10% HPL significantly improved the proliferation rate and colonogenic formation. Therefore the cells expanded in media supplemented with 10% HPL reached a 80-90% confluence, and early compared with media supplemented with other concentrations of HPL and FBS (even 10% FBS). Furthermore we observed strong expression (>90%) of CD44 and CD73 of MSCs cultures in media with 5% HPL, 7% HPL, 10% HPL and 10% FBS respectively. These findings were totally agreed with Doucet and his colleagues (2008)[5] who reported that, HPL supplemented media promoted MSCs expansion and thus decreased the time required to reach confluence. Besides, it

had increased the fibroblastoid colony-forming unit (CFU-F) size, as well as it had displayed distinct MSCs morphology when compared with media supplemented with FBS.
Conclusion: Wharton jelly is a rich source of mesenchymal stem cells and the human platelets lysate could be a good and a safe replacement to the fetal bovine serum in mesenchymal stem cells expansion.
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