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RESEARCH ARTICLE

CULTURING METHODOLOGY OF MESENCHYMAL STEM CELLS ISOLATED FROM HUMAN WHARTON'S JELLY

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ABSTRACT

Adult mesenchymal stem cells are mostly multipotent stem cells and are capable of self-renewal throughout the entire organism's life. The adult stem cells differentiate into other mature cell types. The adult stem cells are already committed and other cells, which are normally intermediate cells with increased commitment, are called primordial cells. It has many sources from human and the major source of mesenchymal stem cells by umbilical cord blood and wharton's jelly. This study has been completely used by wharton's jelly of human.

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INTRODUCTION

The Wharton's jelly of the umbilical cord contains mucoid connective tissue and fibroblast-like cells. Using flow cytometric analysis, we found that mesenchymal cells isolated from the umbilical cord express matrix receptors (CD44, CD105) and integrin markers (CD29, CD51) but not hematopoietic lineage markers (CD34, CD45). Interestingly, these cells also express significant amounts of mesenchymal stem cell markers (SH2, SH3). Adult mesenchymal stem cells are mostly multipotent stem cells and are capable of self-renewal throughout the entire organism's life (human). The adult stem cells differentiate into other mature cell types. The adult stem cells are already committed and other cells, which are normally intermediate cellswith increased commitment, are called primordial cells. These types of stem cells reside within mature tissues or wharton'sjelly,umbilical cord blood and serve as limitless sources for new cells, enabling maintenance and repair of the tissue by continuously regenerating mature tissues either as part of normal physiology or as part of repair after injury. The wharton's jelly or Umbilical cord blood and bone marrow in higher organisms harbor cells, termed as adult stem cells, and these cells are reminiscent of un-programmed stem cells.

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Cells have many sources and the bone marrow, wharton's jelly, Umbilical cord blood are the most accessible source. Mesenchymal stem cells (MSCs), also known as wharton's jelly, marrow stromal cells, are defined as self-renewable, multipotent progenitor cells with the capacity to differentiate into tissues of the mesodermal lineage, including bone, cartilage, adipose, tendon and muscle (Poutos and Giannoudis, 2005). MSCs have also shown to possess the capacity to differentiate into hepatic stem cells (Peterson *et al*., 1999).

Isolation of mononuclear cells from Wharton's Jelly

The Adult bone marrow and umbilical contains at least two types of stem cells: hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), sometimes also referred to as marrow stromal cells (Majumdar *et al*., 1998). The isolation of mononuclear cells (MNCs), although do not belong to the set of molecular procedures, is the first step of cell sample processing used in many molecular diagnostic test in stem cell culture. Erythrocyte, plasma is often performed prior to nucleic acid extraction or before the separation of specific cell subsets to increase the yield and purity of these procedures

MATERIALS AND METHODS

Piration of Sample from bone Marrow: The samples were taken from different types of donars and the samples were collected after birth baby and noted sex of the baby and their

mother's age becaues measure the cell viability. Tissue was taken out after one or two hours process has been started for grow good quality cells.

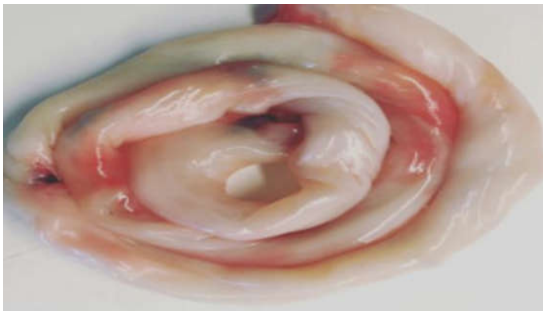

Figure 1. Umbilical cord

Cell Culture Laboratory

Institutional review board approval and health and family welfare (H2) Department, government Arignaranna Memorial Cancer Hospital, karrapetai Kanchipuram were obtained for all procedures. With the consent of the parents, fresh human umbilical cords were obtained after birth and stored in sterile normal saline an hours before tissue processing (wharton's jelly) to obtain mesenchymal cells. After removal of blood vessels, the mesenchymal tissue was scraped off from the Wharton's jelly with a scalpel and centrifuged at 250 g for 5 minutes at room temperature and the pellet was washed with serum-free Dulbecco's modified Eagle's medium (DMEM). Next, the cells were centrifuged at 250 g for 5 minutes at room temperature and then treated with collagenase (2 mg/ml) for 16 hours at 37°C, washed, and treated with 2.5% trypsin for 30 minutes at 37°C with agitation. Finally, the cells were washed and cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and glucose (4.5 g/l) in 5% CO_2 in a 37°C incubator. Cell culture equipment was accessed from Frontier Lifeline Research Institute, Gumidipoondi, Chennai, and the method of culturing MSCs was clearly established. Stem cell culture laboratory, maintained in a sterile condition, was accessed and included a laminar flow, biological safety cabinet, 5% CO_2 incubator maintained at 37°C, inverted microscope with interference phase optics for observing cultured cells and a haemocytometer (glass) for counting cells with the microscope.

Growth Media for MSCs

The human umbilical cord samples were harvested from many different types donors and safely blood vessels were removed for avoid contamination at the same time separately removed source of wharton's jelly for derived cell pellets. These cells precipitated at bottom of the centrifuge tube in the form of pellets. At the beginning of the isolation, 20ml or 30ml of bone marrow was collected from the selected pregnancy and the pellets were resuspended in 2ml of PBS. After 20 – 21 days the cells were measured by haemocytometer and cells were estimated to be 2.2×10^6 ml.

Identification of mesenchymal stem cell isolated from wharton's jelly of human

The primary cell was cultured and MSCs were isolated from the wharton's jelly of human. The mesenchymal stem cell was identified after confluent at the same time image 5.1B shows 20% to 40% and 60% to 70% confluency. The mesenchymal stem cell morphological structure was identified used confocal microscope (Fig. 2A &3B).

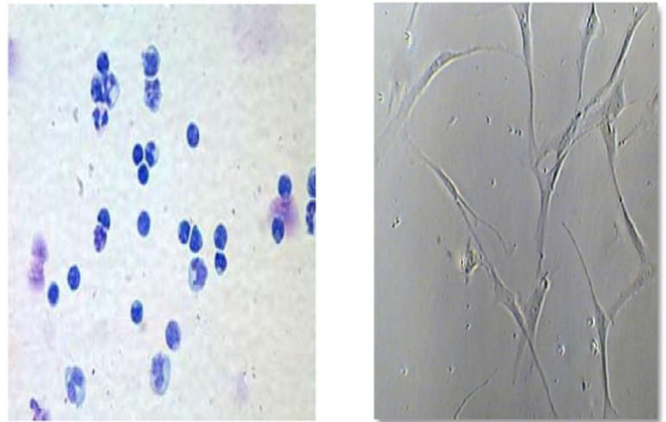

Figure 4A MSCs observed under the 4B MSCs Cultured on day 22 with Inverted phase contrast microscope (100×) 20%-40% Confluency (inv.Microscope 100×)

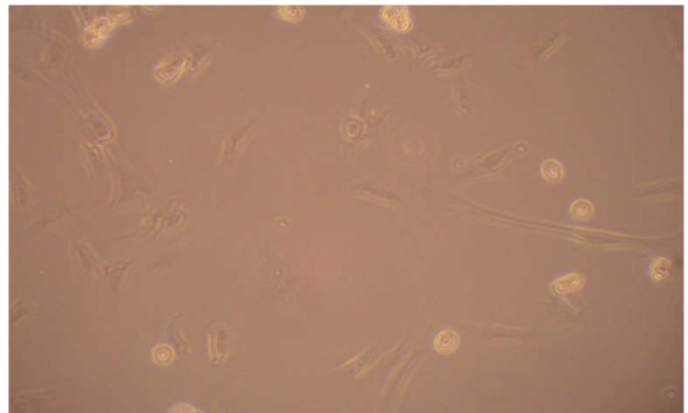

Figure 5. MSCs Cultured on day 32 with 60%-70% Confluency (inverted phase con.microscope)

Subculturing of Wharton's jelly mesenchymal stem cells

At the beginning of proliferations the cells were spindle-shaped and bipolar, and within 15-21 days, an adherent single layer was achieved with a confluency of 60% to 70% (Figure 5.6B). When the cells were at 70% confluency, it was considered as the ideal time for the first passage. Ocular evaluation of the cultures of MSCs by phase contrast microscopy was made to demonstrate changes in the morphology (tripolar/triradiate) at different time duration of the culture and passage of the cells. With the expansion or subculture to develop the cells, these cells were confluent at 50-70% within 21-30 days after due passages. The mesenchymal stem cells were fully attached toflask when observed under electron microscope and looked spindle-shaped. The confluent cells were identified and the MSCs compared to the morphophenotype. The cells of 70% confluencywere accepted for experimental analysis. The first or primary isolation of WJ derived MSCs was resorted to by the critical steps of isolation of cells from a WJ and focused by density gradient centrifugation, followed by recovery and expansion of cells that adhered to tissue culture flasks in standard serum-containing medium (passage zero cells/Po). Passage zero (Po) cells were subsequently expanded by plating at a low density, which enhanced the percentage of rapidly

proliferating spindle-shaped cells. Those cells turned to belarge and flat mature hMSCs, following the processing of zero passage cells to four to six times. First passage (P1) was performed after 21 days and confirmed 70 to 80% confluencywith cells showing polygonal morphology (tripolar/ triradiate) and reticular cytoplasm. In the current pursuit, subculturing was carried until third passage (P3).

DISCUSSION

In the present study, attention was focused on primary culture of mesenchymalstem cells obtained from Wharton's jelly of healthy pregnancies. The umbilical cord was collected by sterile equipment and cultured to passage 3. The sample 1 cells grew out into spindle shapes and sample 2 also like same shapes. The mesenchymal stem cells were growing without any contamination up to 3rd passages. Variations were observed in two different types of human umbilical cord.Mostly adult mesenchymal stem cells are multipotent stem cells certain stage cell are controlling its growth condition. In this study, multipotent stem cells were chosen having derived fromwharton's jelly of healthy pregnancy after birth the baby. It was very specific mesenchymal multipotent stem cells. This allowed for higher cell yields through *in vitro* expansion with a concomitant increase in culture homogeneity, which favored flexibility and reproducibility in the donors. However, before collecting the WJ from Specific donors, the undifferentiated stem cells were cultured to second passage cells, which grew quickly and morphological differentiations were also identified (Castro *et al*., 2002; Nygren *et al*., 2004). Therefore, it has been proposed that a key criterion for demonstration of differentiation of bone marrow cells was mesenchymal multipotent stem cells being continued to long passages such as 25 passages (Wagers *et al*., 2004; Chien 2004). Fortunately, several recent studies done in the field of electromagnetic radiation (Lechner *et al*., 2004) on the animal stem cells and human stem cells, some researchers initially found impacts in bone marrow-derived mesenchymal stem cells (Choi *et al*., 2003).Thus, the data clearly demonstrated the a significant impact of this radiation on bone marrow-derived cells using by RT-PCR analysis in exposed guinea pigs and hematopoietic stem cells also showed non-adherent cells. The mesenchymal stem cells not only respond to multiple stimuli, but also have an impact on the organism. It is, therefore, important to consider each stem cell interaction in every direction.For over a few decades, the adherent MSCs culture assay has been used as an effective approach to identify and select BMMSCs.

Conclusion

In conclusion, Wharton's jelly is an advantageous MSC source, because the harvest of this type of stem cells is not painful or invasive and because, in addition to their effect on wound healing, they seem to have a significant impact on the treatment of keloids. Furthermore, the cells led to better nerve regeneration (Gärtner *et al*., 2012), better neuroprotection and less inflammation. Also, the isolation of WJ-MSCs seems to be more efficient and the cells have a higher capacity of proliferation and are less senescent than ADSCs. Even if proper studies comparing the wound-healing abilities of different MSC sources are still lacking in the literature, at least a new preclinical and clinical research field investigating the potential of WJ-MSCs in wounds and wound healing has arrived.

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