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Comparison of feto-maternal organ derived stem cells in facets of immunophenotype, proliferation and differentiation

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ABSTRACT

Scientific explorations on feto-maternal organ stem cells revealed its possible applicability in treatment of various diseases. However, establishment of an ideal placental tissue stem cell source in regenerative application is inconclusive and arduous. Hence, this study aims to resolve this tribulation by comparison of mesenchymal stem cells (MSC) from fetal placenta – amniotic membrane (AM-MSC), chorionic plate (CP-MSC) tissue and the maternal placenta-Decidua (D-MSC), thereby facilitating the researchers to determine their pertinent source. The cells were expanded and scrutinized for expression profiling, proliferation and differentiation ability. Remarkable expressions of certain markers in addition to its prospective mesodermal differentiation confirmed their mesenchyme origin. Despite the specified alikeness among these sources, reliable and non-invasive procurement of AM-MSC coupled with its higher growth potency makes it the most constructive stem cell source. However, exhibited similarities demands further investigations on extensive expandability and cytogenetic stability of these sources prior to its therapeutic applicability.

1. Introduction

The reproductive success in mammals especially primates depends on the proper development of feto-maternal interface tissue. A failure in its development leads to complications during pregnancy, such as recurrent miscarriages, preeclampsia as well as placenta accrete (Khong, 2008; Salker et al., 2010; Founds et al., 2009). Indeed the proper development of these tissues ensures establishment of fetal immunotolerance by maternal body in a semiallograft acceptance manner (Blanco et al., 2009). Thus details into the components of this tissue and its development, maintenance as well as physiologic functions such as immunotolerance and regenerative capacity had created great deal of excitement to contemporary researchers. The feto-maternal interface tissues form a conduit for nutrient access and waste elimination by the developing fetus (Orwig et al., 1997). The feto-maternal organ, also called as placenta has two major components, the maternal part – decidua and the fetal part – chorion and amnion (Abdulrazzak et al., 2010; Pipino et al., 2012).

The maternal component – decidua is a specialized tissue formed from the differentiation of uterine stem cells under the influence of pregnancy hormones. Decidualization during pregnancy occurs by the scrupulous control of the ectopic invasion of chorionic trophoblast. This is attained by coordinated phagocytosis and extracellular matrix remodeling of the anti-mesometrial and mesometrial decidua (Afonso et al., 1997). Prior research on the composition of decidua reveals its fibroblastic nature (Riddick and Kusmick, 1977), whereas later research pursuits conclude it to be composed predominantly of leukocytes, glandular cells and stromal cells (Bulmer, 1995). Thus, the composition and regenerative capacity of decidua has still not been accepted unequivocally.

A principal redundant source under the scan of cell therapists is the fetal placenta. There are multitude supportive evidences that make placenta a better source of stem cells; these include its high plasticity as evidenced by its early stage embryological development as well as its role in feto-maternal tolerance. In addition, the non-invasive nature of this pregnancy discard tissue makes it an attractive source for stem cells (Evangelista et al., 2008). The fetal component of term placenta amnion contains amniotic epithelium and amniotic mesenchyme where as the chorion composed of chorionic mesenchyme and chorionic trophoblast (Evangelista

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et al., 2008). The plasticity of amniotic membrane is evidenced by the presence of amniotic mesenchyme as well as the embryonic nature of amniotic epithelium (Ilancheran et al., 2007; Miki et al., 2005; Tamagawa et al., 2004).

Chorion is another postnatal placental tissue that had undergone meticulous scrutiny to identify mesenchymal stromal cells. Previous reports illuminate higher yield (Parolini et al., 2008) coupled with extensive proliferative capacities for chorion derived stromal cells (Sakuragawa et al., 2004; In't Anker et al., 2004; Portmann-Lanz et al., 2006; Soncini et al., 2007; Witkowska-Zimny Malgorzata and Wróbel Edyta, 2011). Besides, their suitability in cellular therapeutics is better illustrated by their fibroblastic morphology, plastic adherence, surface antigenic expression as well as multidifferentiation capacity (Ilancheran et al., 2009). Similarly, the redundant nature of this tissue source makes it a suitable candidate for cellular therapies and transplantation biologists.

In lieu of the aforesaid attributes, both fetal and maternal part of the placenta are gaining consensus in topical research. Hence, this study compared the feto-maternal organ derived mesenchymal stem cells isolated and cultured from term placenta sources such as amniotic membrane, chorionic plate tissue and decidua in facets of properties such as surface antigenic profiling, proliferative capacity and differentiation ability. Revealing the stemness property of array of feto-maternal organ tissues in this study facilitates the researchers to further investigate on their pertinent source of stem cells for its utilization in regenerative medicine. Furthermore, this study also demonstrates the ideal stem cell source of the feto-maternal organ tissues for cell based therapies with regards to specified aforesaid attributes.

2. Methods

2.1. Sampling

Human placental samples ($n = 5$) were obtained from healthy mothers, whose age group ranged between 23 and 32 years, with BMI $24.6 \pm 3.43\,kg/m^2$ during routine elective cesarean section births and Decidua tissues ($n = 5$) were macroscopically dissected from the central region of the maternal placental surface. The samples were collected in 0.9% saline. Written informed consent was obtained several weeks prior to delivery from each mother in accordance with the regulations and requirements of ethical committee of Lifeline Multispecialty Hospital, Chennai, India.

2.2. Cell culture

Harvested tissues were washed with Phosphate Buffered Saline (PBS) (Hi-Media, Mumbai, India) containing 1% antibiotic-antimycotic solution (Invitrogen, New York, USA) and mechanically minced into 2–3 mm in diameter pieces. The minced tissues were further digested using 0.075% collagenase type-1 (Hi-Media) solution. The collagenase activity was stopped by addition of 10% Fetal Bovine Serum (FBS) (Invitrogen). The cell digests were centrifuged and erythrocytes present in the cell fraction were lysed using 0.7% NH_4Cl solution. The cells were recovered by centrifugation; the pellet was resuspended in PBS and filtered to obtain a single cell suspension. Cell viability was evaluated and enumerated using Trypan blue exclusion technique.

Cells were seeded at a cell density of $3 \times 10^5/25\,cm^2$ flask (Nunc, Roskilde, Denmark) and cultured in filter sterilized DMEM-LG [Dulbecoo's Modified Eagle Medium-Low Glucose] (Invitrogen) solution with 10% FBS (Invitrogen) and 1% antibiotic-antimycotic solution. Standard culture conditions were maintained throughout, such as 37 °C, 5% CO_2 and 95% humidity for 2–4 days before the first medium change and a 70% cellular confluence was obtained. The primary culture was subcultured until passage3 (P3) with media change done twice every week.

Table 1
Details of antibody conjugated cell surface markers.

S.No	CD Marker	Fluorochrome	Cat No.	Company
1	CD34	PE	348057	BD-Biosciences
2	CD31	FITC	555445	BD-Biosciences
3	HLA-DR	PER CP	347364	BD-Biosciences
4	CD14	PE	560919	BD-Biosciences
5	CD73	PE	550257	BD-Biosciences
6	CD90	PER CP	15-0909-73	e-Biosciences
7	CD105	APC	17-1057-73	e-Biosciences
8	SSEA4	Alexaflour 488	53-8843-41	e-Biosciences
9	CD166	PE	559263	BD-Biosciences
10	CD106	FITC	551146	BD-Biosciences
11	ABCG2	PE	12-8888-73	e-Biosciences
12	CD117	APC	17-1179-73	e-Biosciences
13	CD54	PER CP	555512	BD-Biosciences
14	CD49d	PE	12-0499-73	e-Biosciences
15	CD140b	PE	558821	BD-Biosciences

2.3. Surface antigenic profiling

The surface antigenic profiling was performed using BD (Becton and Dickinson, NJ, USA) FACS Aria. Approximately 1×10^5 cells were stained with saturating concentrations of fluorochrome conjugated antibodies (Table 1) and were incubated in dark. The incubated cells were washed with wash flow buffer (phosphate buffer supplemented with 2% (v/v) FBS (Sigma–Aldrich, MO, USA) and 0.1% (w/v) sodium azide, NaN_3 (Sigma–Aldrich)). The cell pellet obtained was resuspended in 500 μl of BD FACS flow, vortexed and analyzed. Sample data acquisition and analysis were performed using BD FACS-DIVA Software.

2.4. Growth curve and PDT

The growth characteristics of cells were evaluated by plotting growth curves at passage 3 (P3). The rate of growth of each cell population was calculated by counting the total number of cells in duplicates, every day for 10 days. The results were depicted on a log-linear scale. The population doubling time of amniotic membrane, chorionic plate and deciduas derived stem cells were carried out at P3 in a 12 well plate in duplicates with a seeding density of 3×10^5 cells per plate at day 0, the cells were harvested and counted every day. The growth of the cells was calculated until day 10 on a daily basis

$$\text{PDT} = \frac{\text{Days of exponential phase}}{(\log N2 - \log N1)/\log 2}$$

2.5. Mesoderm differentiation

Osteogenic Differentiation: Osteogenic differentiation was carried out using osteogenic induction medium DMEM-LG supplemented with 10% FBS, 1% Antibiotic (Invitrogen), 0.1 μM dexamethasone (Sigma–Aldrich), 10 mM β-glycerophasphate (Sigma–Aldrich) and 2 mM Ascorbic acid (Sigma–Aldrich). MSC at passage 3 were recovered and approximately 3×10^4 cells were seeded onto culture plate in growth medium. Cells were incubated at 37 °C under 5% CO_2 and 95% humidity with changes of medium until confluency. Upon reaching 80–90% confluency, medium was replaced with osteogenic induction medium with timely replacements. Osteogenic differentiation was further confirmed by Von Kossa as well as Alizarin Red staining.