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The Therapeutic Perspective of Amniotic Membrane, Villous Chorion and Wharton's Jelly-derived Mesenchymal Stem Cells in Myocardial Infarction

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ABSTRACT

This review was highlighted the novelistic therapeutic approaches of fetal-derived Mesenchymal Stem Cells isolated from Amniotic membrane, Villous chorion, and Wharton's jelly, which are an ideal source with similar characteristics such as other adult stem cells and potentially be used for regenerative medicine. However, the use of MSCs for therapeutic application is based on their subsequent large-scale *in vitro* expansion. In this context, we summarized the final cellular product to meet the various regulatory criteria, such as safety, integrity, purity, quality and quantity that have been ascribed to cell therapy products. We discussed the pro and cons about the potential capacity of fetal-derived MSCs as a suitable clinical therapy source for an ailment of myocardial complications with the assessment of *in vitro* differentiation potential into cardiac lineages and cells engraftment efficiency in a murine model, possible toxic effects of these cells when administered *in vivo* and other clinical evaluation been addressed.

Keywords: Amniotic membrane, Villous chorion, Wharton's jelly, Mesenchymal stem cells/multipotent stromal cells, Myocardial infarction

INTRODUCTION

In the recent years new insights were developed into Multipotent Stromal Cells/Mesenchymal Stem Cells (MSCs) isolation from different sources. The most significant source of MSCs studied was bone marrow (BM). However, the harvesting and expansion of BM-MSCs to higher passage level raise many complications. To overcome these disadvantages, alternative fetal sources are explored where MSCs could be isolated and expanded. Among the fetal sources, an ideal source is a human placenta, of which amniotic membrane, umbilical cord tissue, and the placental villi are the potential stromal cell sources [1-4]. Amniotic and chorionic mesenchymal cells are probably derived from the extra embryonic mesodermis. Chorionic mesenchymal cells are much less investigated than amniotic cells. Amniotic MSCs are preferably isolated from the amnion at term from the reflected portion of the membranes, in order to minimize the presence of maternal cells [5]. Chorionic cells are isolated from the membranes and from the fetal portion of the placenta. Both have a good adhesion and proliferation capacity on the plastic surface of culture containers for a limited number of passages [5,6]. In this state, this review brings into light that the placenta fetal tissue portions Amniotic Membrane (AM), Wharton's Jelly (WJ) and Villous Chorion (VC) are bringing to the solution, which could be a unique source of MSCs and functional capabilities of cells for myocardial regeneration and repair of the degenerated tissue.

Advantages

The placenta is the most important source of stem cells.

THE PLACENTA IS THE MOST IMPORTANT SOURCE OF STEM CELLS

Fetal-derived Mesenchymal Stem Cells (fMSCs) show remarkable stability when expanded *in vitro* and not evidently trigger any strong immune response. In allogeneic transplantations, MSCs rejection rate is minimal due to their immunomodulatory properties. The longevity, trans differentiability and expandability of these cells at a distant future, even after prolonged cryopreservation, are additional

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attraction [7]. fMSCs can able to differentiate into all three germ layers viz., mesodermal, ectodermal and endodermal lineages under *in vitro* conditions [2,8]. It isolated from biologic waste, ethically non-problematic, stem cells from the full-term placenta and may present an attractive completion to other classically established stem cells in different clinical approaches [9,10]. One of the most important benefits of using fMSCs for clinical use is availability. Moreover, it is possible to obtain placenta and Umbilical Cord Blood (UCB) from the same donor. Therefore, it is an attractive source of MSCs for co-transplantation in conjunction with UCB-derived hematopoietic stem cells [11].

CURRENT CLINICAL DEMANDS

Cell-based therapeutic approach with the help of stem or progenitor cells has so far held huge potential for the treatment of a vast array of degenerative and age-related diseases. Despite its efficiency the success of this medicinal approach is being challenged by many obstacles, which must be addressed for the exploitation of their clinical use [12]. Currently, many efforts are being performed for the development of different cell-based systems as test objects for determination of drug-related effects in pharmacological screening using MSCs. Still, it is necessary to determine clinical limitations and realistic clinical protocols. It is unknown to date whether MSCs engraft in the targeted area for a longer period of time and whether display self-renewal as well as multi-lineage potency *in vivo* [13]. Since the amount of MSCs needed in tissue regeneration is very high; scaling up is necessary for the successful clinical application.

Among all different sources, human fetal tissues are a hassle-free source of MSCs, known due to its contribution towards maternal tolerance. These cells believed to be having the capacity to yield between 1-4 × 10^7 cells when exploited for therapeutic application; do not elicit any allogenic or xenogeneic immune response. Besides, it is also understood that they are able to migrate to the targeted area upon administration, where they provide health microenvironment enabling engraftment of differentiated cells. In fact, when compared to fMSCs, BM-derived MSCs percentage is as low as 1 × 10^5 of mononucleated cells [14]. It has also been reported that the number of colonies forming units-fibroblast cells is 20-fold higher in umbilical cord tissue when compared with the MSCs from bone marrow [15]. MSCs from BM could be obtained from only 10% of the samples but umbilical cord tissue always gave sufficient numbers of MSCs [16]. MSCs can also be obtained from adipose tissue but this involves an invasive procedure such as liposuction. While stem cells are being used in a number of animal and human clinical trials of regenerative medicine and tissue engineering, scale-up of these stem cells is the current market demand. Even though BM-MSCs are well-established source for MSCs their need of numbers and grief surgical procedures makes fetal-derived MSCs a perfect choice due to their large quantity, ease of isolation, immune privilege, an absence of associated ethical issues, no requirement of invasive procedures for harvesting, and also have greater ability to culture in a clinical grade-large scale expansion to meet the current clinical demands [9,17-19].

THE PERSPECTIVE OF CLINICAL SCALED FMSCS

There are different protocols reported previously in terms of isolation, characterization and expansion of MSCs, but all MSCs exhibits the minimum criteria proposed by the International Society for Cellular Therapy (ISCT). However, the use of MSCs for therapeutic application is based on their subsequent large-scale *in vitro* expansion. A fast and efficient protocol for the generation of large quantities of MSCs is required to meet the clinical demand and biomedical research needs [20]. Furthermore, well-characterized cell lines, constantly growing while maintaining their typical properties, are highly valuable for research [21,22]. To address the requirement, we studied in detailed that clinical-scale expansion of fMSCs up to passage ten levels [2,18,19]. MSCs isolated can be expanded in the second passage itself to get as many as 5.57 × 10^9 cells within 21 days from amniotic membrane [19], 5.22 × 10^9 cells from Wharton's jelly [18] within 28 days and 2.16 × 10^9 cells within 14 days from villous chorion [2]. In briefly, the harvested cells from isolated villous chorion from one placenta yields 0.95-1.18 × 10^4 cells/cm^2 at passage 0 (P(0)), 2.63-4.97 × 10^4 cells/cm^2 at P(1), 3.49-4.77 × 10^4 cells/cm^2 at P(2) and 3.06-4.23 × 10^4 cells/cm^2 at P(10) accordingly. That is, it could expand these cells to a clinical quantity of 0.58 × 10^9-2.16 × 10^9 cells at second passage [2]. On the other side, the harvested cells from WJ of one cord yielded 1.93-2.46 × 10^4 cells/cm^2 at P(0), 4.46-5.37 × 10^4 cells/cm^2 at P(1), 4.42-5.93 × 10^4 cells/cm^2 at P(2) and 4.74-5.66 × 10^4 cells/cm^2 at P(10) accordingly, i.e., clinical quantity of 2.53 × 10^9-5.22 × 10^9 at second passages [18]. Similarly, the harvested cells from one amniotic membrane yielded 1.63-2.47 × 10^4 cells/cm^2 at P(0), 3.43-5.71 × 10^4 cells/cm^2 at P(1), 4.07-5.93 × 10^4 cells/cm^2 at P(2) and 3.31-5.66 × 10^4 cells/cm^2 at P(10) accordingly, i.e., clinical quantity of 1.52 × 10^9-5.57 × 10^9 cells at second passage level [19]. From this study results suggests that the growth of cells of AM, VC and WJ exhibit near limitless potential [1,2,18,19], very unique and lucrative candidates for cell-based therapies obviating the market need for their higher cell numbers and considering the application of these cells in clinical trials will meet the demands.

In our earlier study summarised that mean standard deviation (mSD) of villous chorion 1.34 × 10^9 ± 0.60 was inferior in total yield but harvested in fewer days compared to AM and WJ sources. The second passage amniotic membrane and Wharton's jelly total yield mSD was 3.34 × 10^9 ± 1.51, 3.99 × 10^9 ± 1.19 and duration of culture was average of 18, 26 days, respectively [2,18,19]. In fact, by

adding just one more round of expansion, it can be able to generate sufficient quantities of MSCs to treat ~150-185 patients from a single placenta, considering that a 70 kg patient needs approximately 2×10^6 MSCs/kg body weight for transplantation [23], though an optimal MSCs dose needs to be evaluated for the different indications.

In our acute toxicity study observed that WJ-MSCs isolated were expanded up to second passage to get as many as 1.3×10^9 cells from a single cord with no significant change in viability, stemness, karyotyping and sterility. These cells, when administered through intravenous and subcutaneous route, showed no signs of mortality, toxicity and pathological changes in Swiss albino mice of both the sexes. Minimum Tolerated Dose (MTD) and Minimum lethal dose (MLD) were estimated to be higher than 10×10^6 MSCs/kg body weights, which is ten times higher than the proposed human therapeutic dose. The study thus concluded in an assessment of possible toxic effects of these cells when administered *in vivo* and optimizing the route of administration with other clinical evaluation has been addressed [12]. It also studied up to tenth passage with no significant change in viability, phenotypic characterization, karyotyping and differentiate into all three germ layers which makes them the perspective of clinical scaled fMSCs to be used in therapeutic application [1,2]. These primitive cells have greater ability to expand in culture and have different physiology relatively because of their naive status [24-26].

THE PROSPECTIVE OF fMSCS INTO CARDIAC PROGENITOR CELLS

Myocardial infarction (MI) is one of the serious heart disorders causing millions of people to suffer and a substantial amount of mortality every year, 1 of 6 deaths all over the world are due to Myocardial infarction. Over the period of time during a search for alternative cellular medicine; an abrupt growth has been observed for fMSCs as a future cell-based therapeutic strategy for cardiac repair [27]. Various pathways of MSC mediated cardiac improvement have been suggested and are studied extensively including somatic reprogramming, transdifferentiation, paracrine signaling and direct electrophysiological coupling [28], Number of *in vivo* rodents and swine models have been studied as an experimental [29-31] and clinical [32] support to check the mechanism of engraftments of these cells to improve myocardial regeneration. Mesenchymal stem cells have been reported to acquire cardiomyocytes phenotype *in vitro* after treatment with 5' Azacytidine which proved to significantly enhance regeneration in infarcted area in a mouse model [29,33-35]. Azacytidine has been reported to cause DNA demethylation, gene activation, non-specific transcription and translation [1,36]. 5'Azacytidine is a DNA demethylating compound which induces uncontrolled myogenic specification in mesenchymal stem cells by random demethylation [37-39]. As our study conducted *in vitro*, the induced cardiomyogenic differentiation was observed to be successful with similar morphology such as rounded cell morphology, string bead like nuclei. In addition to this, differentiated cells have also shown to express some cardiac-specific markers such as CSM1, cTnT, GATA4, β-actin and NKx2.5 which confirmed AM, WJ, VC and pooled fMSCs successfully able to differentiate to cardiac progenitor cells with response to of 5' Azacytidine [1,29].

As our study conducted, *in vivo* manipulation of the murine hearts was carried out to investigate chronic biological and physiological processes involved and thereby influencing the outcome. So far it has not been determined whether there has been a therapeutic difference in cells obtained from different sources. Hence combined Umbilical Cord Blood and Wharton's jelly stem cells have been utilized as treatment post-MI to observe structural and functional changes. The analysis was done on the basis of comparison with non-transplanted as well as controlled (normal) mice. Post MI cardiac remodelling was observed in ventricular cross sections of all non-transplanted mice to be accompanied by structural changes in the Left Ventricular (LV) such as wall thinning and fibrosis (collagen deposition) 6 weeks post-MI. All sections treated with Wharton's jelly MSC showed a decrease in the ratio of infarction size to Left Ventricular confirming a significant reduction in the damage post-infarct on the contrary extent of myocardial regeneration in animals treated with UCB-MSCs was not as good as with the WJ-MSCs [29]. On the basis of these studies, fetal tissues Amniotic Membrane, Wharton's Jelly and Villous Chorion can contribute equally as a good stem cell source [1,2,18,19]. In addition to this, the pooled stem cells also should be selected considering their success in a clinical application [1] and considered for the therapeutic benefits such as homing to the damaged tissue, regulating immune and inflammatory responses at the targeted sites and thereby facilitating repair of the degenerated tissue.

CLINICAL APPLICATIONS PERSPECTIVE

Since it has been shown in various publications that MSCs have the ability to down-regulate immune response and support tissue repair mechanisms their use has been widespread for the treatment of many different diseases. Currently, many clinical studies are being initiated using MSCs from various sources viz., 39 studies from MSCs from bone marrow, 43 studies from the umbilical cord (UC) MSCs out of which 3 from WJ respectively and one study using MSCs isolated from amniotic membrane and no studies pertaining to villous chorion. UC-MSCs have for instance been applied in the treatment of 160 patients after myocardial infarction in a double-blind, placebo-controlled, multicentre second phase trial [40]. Several papers report on the differentiation of UC-MSCs into cardiomyocyte-like cells [41,42], but the functionality of these derived cells is also controversially discussed [43,44]. Successful and

promising pre-clinical and clinical studies on WJ-MSCs, AM-MSCs and VC-MSCs performed to date and their diverse properties offer these cells the possibility for future clinical use in the treatment of various therapeutics [40]. Till then some foremost technical hitches, such as translation from research to clinical grade scaling up, market authorization and clinical application will be get resolved.

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CONFLICT OF INTERESTS

There are no conflicts of interest to report.

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