Journal of Thai Interdisciplinary Research
Volume 12, Number 3, Pages 50 - 53

Viability and telomere length analysis of Mesenchymal Stromal Cells

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Abstract

Mesenchymal stromal cells (MSCs) have provided various potential clinical applications, particularly due to their immunomodulation and tissue repair abilities. To develop cellular therapy products based on human MSCs, some basic requirements should be implemented. These include the choice of MSCs source, cultivation media and methods, and sterility tests. In this study, cell viability and telomere length analysis have been applied to ensure optimal and consistent therapeutic results of MSCs therapy.

Keywords: HT Q-FISH, Mesenchymal Stromal Cells, telomere length, senescence

Article history: Received 28 September 2016, Accepted 5 June 2017

1. Introduction

Mesenchymal stromal cells (MSCs) are non-hematopoietic stem cells initially found in bone marrow. These multi−potent cells actually present in varieties of tissues and can be isolated and expanded *in vitro* from bone marrow, adipose, and trophoblastic tissues such as placenta and umbilical cords. Using specific media, MSCs can be induced to differentiate into different types of tissue such as cartilage, fat, and bone [1].

The use of MSCs derived from different tissue sources has been proposed for treatments of a variety of conditions and diseases [2]. Administration of MSCs can promote tissue repair without the need of engraftment due to their abilities to produce a large numbers of growth factors and cytokines, some of which exhibit anti-inflammatory and anti-fibrotic activities [3]. In addition, MSCs can prevent hypoxic cells from undergoing apoptosis by the mechanism of mitochondria transfer [4]. Since cultivated MSCs have been manipulated to exert the biological functions intended for clinical use, they should be classified as advanced therapy medicinal products (ATMPs). In order to commercialization of clinical grade MSCs for cell therapies, some important regulation requirements should be considered. The characteristics of MSCs should be defined based on the criteria established by the International Society for Cellular Therapy including 1. adherence to plastic, 2. expression of CD73, CD90, and CD105 antigens, while being CD14, CD34, CD45, and HLA-DR negative, and 3.ability to differentiate into osteogenic, chondrogenic and adipogenic lineage [5].

In addition, the cultivation media including serum and supplementation have to be freed from hazardous or infectious substances. The standard operating operation (SOP) of culturing process and materials should be documented. Culturing process need to be performed in a clean room or a clean incubator designed in compliance with the International Standard ISO 14644.

Source of MSCs

Bone marrow is the first source of MSCs commonly used for experimental and clinical studies. Adipose tissue is another source that is even richer in MSCs than bone marrow. MSCs in adipose tissue are found in the stromal vascular fraction and can be isolated after collagenase digestion [6, 7] or by automated devices [8]. Both MSCs derived from bone marrow (BM-MSCs) and adipose tissue (AD-MSCs) have been clinically tested for autologous treatment for various diseases. Some believe that the use of MSCs from autologous sources for clinical application is preferable since it should reduce risks of potential disease transmission and incompatibility. However, recent studies have shown that MSCs derived from elderly adult tissues may contain somatic mutations and have undergone aging process leading to unpredictable or poor therapeutic results [9, 10].

Trophoblastic tissues such as Wharton's jelly tissue from umbilical cord (UC) and placenta membrane offer abundant sources of MSCs [11]. To eliminate the risk of transmissible agents, donor screening,

DOI 10.14456/jtir.2017.20

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sterility check, and also the tests for mycoplasma, hepatitis B, C, HIV, and syphilis are mandatory. UC-MSCs are considered to be more primitive and do not carry somatic mutations. Their immuno-modulative properties have been shown to be superior to those of BM-MSC and AD-MSCs [12]. These qualities have made UC-MSCs the best MSCs of choice for allogeneic cell therapy. They can be cryopreserved for later use as "off the shelf" products. However, the study has shown that few hours post thawing, the immune-modulatory functions of MSCs could be impaired [13], thus cryopreserved MSCs should be re-cultured into log phase of cell growth (Fresh MSCs) before clinical application. In addition, the use of fresh MSCs will eliminate any problem that might have come from trace amount of DMSO in the cryopreservation media.

Since MSCs are telomerase negative and have a limited capability for self-renewal, extensive sub-culturing and expansion of MSCs can result in telomere shortening leading to cellular senescence and growth arrest [14, 15]. Thus, it is important that optimal passage numbers of MSCs culture should be determined. Telomere length is a marker of cellular senescence and measurement of telomere length of would provide critical information on the replicative capacity of the cells and may be applied as one of the criteria in the selection of MSCs for therapeutic use.

The purpose of this study is to determine the viabilities of UC-MSCs and the telomere length of MSCs from different sources and different passage numbers. Since the telomere length distribution of the cells is not symmetrical, PCR based assay that provide measures of the average telomere length may not be the best method to analyze the telomere lengths [16]. In this study, we have used automated high-throughput quantitative fluorescence in situ hybridization assays (HT Q-FISH) since the method allows the quantification of both mean telomere length and the percentage of short telomeres per cells [17].

2. Materials and methods

MSCs derived from umbilical cord, adipose tissue, and bone marrow tissue were cultivated in phenol red free Dulbecco's Modified Eagle Medium (DMEM) low glucose media (Gibco) supplemented with 1% penicillin/streptomycin, 5% human platelet lysate and cultured at 37°C in a 5% CO2 incubator. The growth medium was replaced every third day. Viability assays of UC-MSCs were performed by flow cytometry analysis using Guava ViaCount Assay according to the manufacturer protocols (Millipore). Telomere length analysis was studied by using automated high-throughput quantitative fluorescence in-situ hybridization assays to assess metaphase chromosomes after hybridization with a fluorescent CCCTAA probe (Lifelength.com, Madrid, Spain).

3. Results and discussion

3.1 Viability testing

The viability assays of UC-MSCs showed that immediate post-thawed viability of UC-MSCs was only 75% (Figure 1). Moreover, when the cells were left in the freezing media after thawing, they continued to die quickly within 48 hours (Figure 1). Therefore, our results support the idea that for a better therapeutic result, fresh MSCs instead of frozen MSCs should be applied for clinical application.

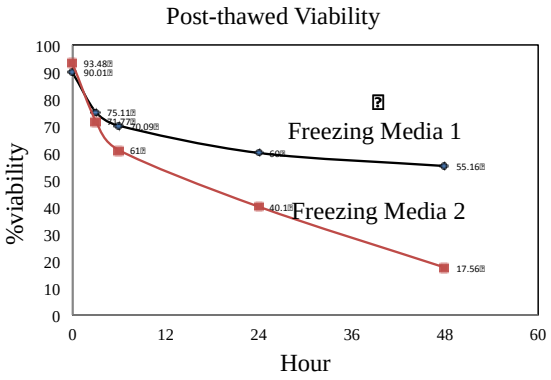

Figure 1 Viability of UC-MSCs immediately post thawing and after being left in the freezing media at room temperature for up to 48 h.

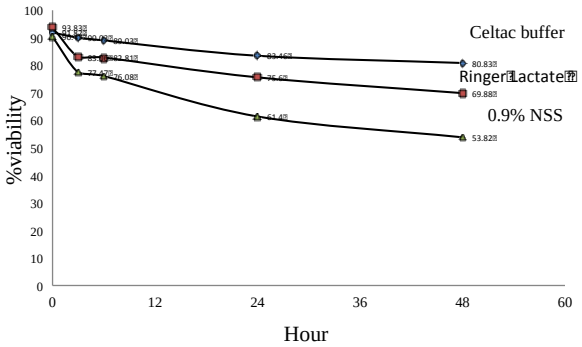

Figure 2 UC-MSCs in culture were trypsinized collected, and kept in Celtac® buffer, ringer late buffer, or normal saline at room temperature. The percent of viability was tested at 3 h. 6 h, 24 h., and 48 h.

To determine the perfect media for MSCs storage, the viability of fresh MSCs kept in 0.9% normal saline (NSS), ringer lactate, and Celtac media for 48 hours were compared. MSCs kept in normal saline

will die faster than those kept in ringer lactate or special buffer media (Figure 2). Our result showed that normal saline or any other non-buffer media should not be used for MSC storage since they would have deteriorating effects on the viability.

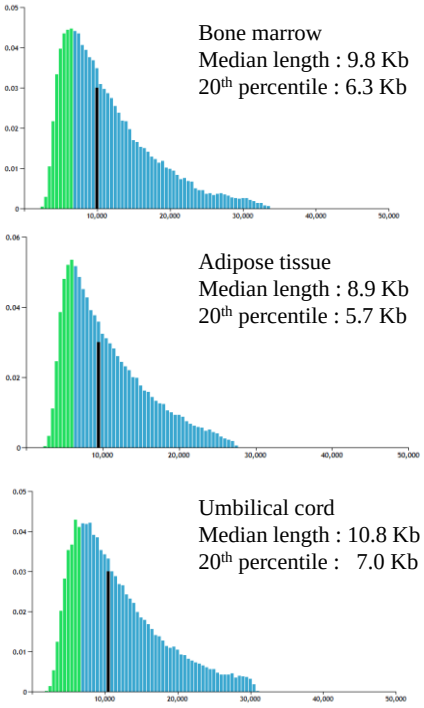

Figure 3 Telomere length analysis of MSCs (passage 5) derived from various tissue using High-Throughput (HT) Q-FISH technique

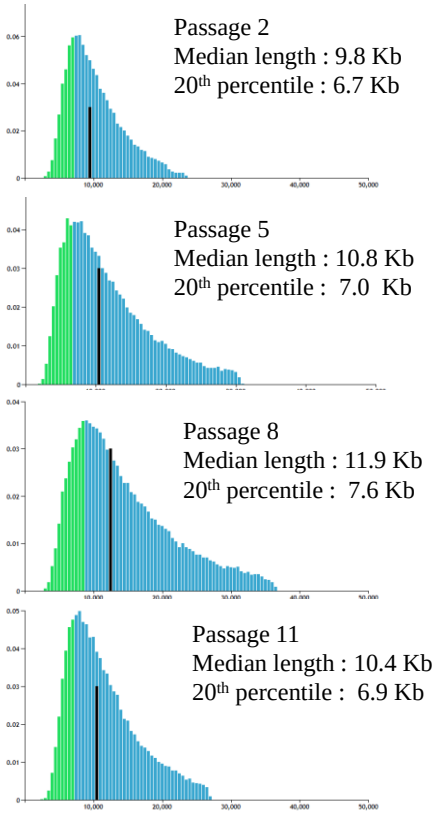

Figure 4 Telomere length analysis of UC-MSCs from different passages using High-Throughput (HT) Q-FISH technique

3.2 Telomere length

We compared the telomere length of MSCs derived from bone marrow, adipose tissue, and umbilical cord. The results showed that the median telomere length of UC-MSC (10.8Kb) was higher than those of AD-MSCs (8.9 Kb) and BM-MSCs (9.8 Kb) (Figure 3).

In addition, the median telomere length of UC-MSCs from passage 2, 5, 8, and 11 was 9.8 Kb, 10.8 Kb, 11.9, and 10.4 Kb, respectively (Figure 4). The results suggest that UC-MSCs from culture passage 6-9 should be more suitable for clinical application.

4. Conclusions

The umbilical cord provides a good source of MSCs due to abundant availability, lower risk of carrying somatic mutations, and cost-effectiveness of the expansion process. Our data indicate that while UC-MSCs can be cryopreserved in a cell bank; however, frozen cells need to be re-culturing before application due to high percentage of dead cells after thawing. Our data also indicated that to maintain their viability during the process of transportation, these cells should be kept in buffer and not in normal saline.

Since each lab will have its own protocols for cultivating and manufacturing MSCs, we recommend that measuring the viability and telomere length of MSCs could provide useful information and a new standard for evaluation of cell qualities before clinical application.

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