1122

1. INTRODUCTION

Mesenchymal stem cells (MSCs) are particularly attractive in cell-based therapy [1] because of their distinct immunosuppressive properties and multi-differentiation capacity [2, 3]. MSCs are traditionally found in the bone marrow. However, they can also be isolated from other tissue such as human umbilical cord [4–7]. Human umbilical-cord-derived MSCs (hUC-MSCs) are shown to display similar or preferable properties compared to bone marrow-derived MSCs (BM-MSCs) including plastic adherence, specific surface markers, self-renew ability, convenient collection, safety, and potential to differentiate into certain lineages in vitro [8, 9]. These advantages make hUC-MSCs gain more attention in recent years.

Currently, hUC-MSCs are being explored as a promising candidate for therapeutic applications. Wu and Zhou [10, 11] have discussed the therapeutic potential of hUC-MSCs in cardiomyocyte regeneration, and their findings demonstrate that transplanted hUC-MSCs provide benefit in cardiac function recovery, suggesting hUC-MSCs represent a source of stem cells for cellular therapy and myocardial tissue engineering. Liao et al report that hUC-MSCs treatment may become an ideal strategy of cell-based therapy for central nervous system injury and disease [12]. Moreover, the effects of hUC-MSCs on liver fibrosis have also been observed by Professor Han’s group [13]. Recently, a series of experimental results indicate that hUC-MSCs are exceptional candidates for muscle repair [8], bone tissue engineering [14] and the treatment of Rheumatoid Arthritis (RA) [15].

The above findings suggest that the hUC-MSCs hold great promise as a tool for understanding regeneration medical development and as therapeutic agents. As well known, a large population of cells sustained in high viability is fundamental to a successful cell-based therapy. Therefore, the detection of cell viability changes is a key step for the population expansion and quality assurance of hUC-MSCs. Several optical methods may be usable in this respect,
including laser scanning confocal fluorescence microscopy by means of exogenous fluorophores and multi-wavelength fluorescence lifetime spectroscopy based on cellular endogenous fluorophores [16]. However, whether for exogenous fluorescent probes or endogenous fluorophores, photobleaching is always a concern. In contrast, Raman spectroscopy is a promising alternative that is label-free and not limited by the issue of photobleaching. It can provide information on the intrinsic molecular structures and composition of biological samples, and thus shows a tremendous application potential in biomedical fields, such as detecting the carotenoids’ concentration [17, 18], elucidating the excited state vibrational dynamics of β-carotene [19], identifying the biofilm formation by Staphylococcus epidermidis [20], and estimating the damage of low density lipoprotein in blood serum [21], etc. In addition, the emergence of new technologies like tunable narrow band light source [22, 23] will greatly expand the application of Raman spectroscopy.

In our recently published work [24], this technology has been employed on the hUC-MSCs for detecting their viability transitions. It is encouraged to find out that the most significant Raman spectral variations associated with the cell viability decrease are presented at 1342, 877, and 744 cm\(^{-1}\), which show a great potential for characterizing the cell viability changes. Considering (the biochemical effects involved in the reactions of Reactive Oxygen Species (ROS) [25], we have inferred that these Raman spectral changes are highly likely due to the interactions between the intracellular ROS and biological macromolecules. However, given the diversity of cellular components and the complexity of molecular interactions, the reasons and the mechanisms of the Raman spectral variations remain not clear. In this regard, several studies in our group are being carried out for insight into the role of ROS in the cell viability transitions.

As an important aspect, the dependence of the Raman spectral variations on the yield of ROS in the cells are explored in this paper. Here, we employ a ROS probe (DCFH-DA) to monitor the change of intracellular ROS level in hUC-MSCs with their viability decline. In addition, the Raman spectra of the cells with different viability are also recorded using Raman micro-spectroscopy. The results we obtained show that the relative intensity variations of Raman peaks at 1342, 877, 744 cm\(^{-1}\) and the relative yield variation of ROS in the cells with time (cell viability decline) can be described by a same model function (with different fitting parameter values). And the correlations of these relative changes were evaluated by correlation coefficient method. It is found that they have perfect linear correlation coefficients respectively. This study demonstrates further that the relative yield variation of the ROS in hUC-MSCs with different viability can linearly lead to the relative intensity variations of Raman spectra in the cells.

2. MATERIALS AND METHODS

2.1. Isolation and Culturing of hUC-MSCs

The hUC-MSCs were isolated from umbilical cords collected from healthy parturient women with well-developed fetus [24]. Briefly, the cords were minced into 1 mm\(^3\) small fragments and washed thoroughly with PBS to remove contamination. The fragments were then treated with 0.075% collagenase II (Sigma) and 0.25% trypsin (Sigma) at 37°C for 30 min. The digested mixture was passed through a 200 μm filter to obtain cell suspensions. The dissociated cells were washed twice with PBS, planted on uncoated culture flasks, and then cultured in Dulbecco’s modified Eagle’s medium with low glucose (DMEM-LG/F-12, DF12; Gibco) and 10% Fetal Bovine Serum (FBS, USA). The culture medium was replaced 2 days first and then after every 3 or 4 days. The cells were serially passaged and expanded in a humified incubator at 37°C with 5% CO\(_2\).

hUC-MSCs with different viability were used for experiments. Decrease of the cell viability was induced by cell starvation that making the cells suspended in PBS at a low temperature of 4°C for several days. The cell viability was measured using the Trypan Blue method everyday, and the viability is reflected by the ratio of the number of viable cells to the total number of all cells.

2.2. Raman Spectroscopy

A Raman micro-spectrometer (LabRAM HR800, HORIBA Jobin Yvon, France) was used to record the Raman scattering from the center of single hUC-MSCs. In brief, excitation light at 632.8 nm was provided by a helium-neon laser with the output power of 17 mW. A 50× objective was used to focus the excitation light and collect the Raman signal. For each spectral scan, Raman spectrum was recorded in the range of 600–1800 cm\(^{-1}\), and the signal was integrated for 100 s. All the spectra were preprocessed and analyzed using the methods as described previously [24].

2.3. Intracellular ROS Measurement

The relative levels of ROS in hUC-MSCs with different viability were accessed using 2’,7’-dichlorofluorescin diacetate (DCFH-DA, Sigma), a fluorescent probe for intracellular ROS measurement. The DCFH-DA itself is non-fluorescent. It can diffuse through the cell membrane readily and be hydrolyzed by intracellular esterases to produce non-fluorescent 2’,7’-dichlorofluorescin (DCFH), which can no longer pass through the membrane so that the probe can easily be loaded into the cells. DCFH is then ox-