Distinguish on the viability of human umbilical cord mesenchymal stem cells using delayed luminescence*

CHEN Ping (陈平)[1], **LI Xing** (李星)[1], **WANG Yan** (王岩)[1], **BAI Hua** (白华)[2], **and LIN Lie** (林列)[1]**

1. Key Laboratory of Optical Information Science Technology, Ministry of Education of China, Institute of Modern Optics, Nankai University, Tianjin 300071, China

2. School of Electronics and Information Engineering, Tianjin Polytechnic University, Tianjin 300387, China

(Received 3 June 2014)

In this paper, we report the discrimination of the viability of human umbilical cord mesenchymal stem cells (hUC-MSCs) with photo-induced delayed luminescence (DL). We measure the DL decay kinetics of hUC-MSCs using an ultraweak luminescence detection system, and find the significant difference in the weight distributions of the decay rate for hUC-MSCs with high and low viabilities. Spectral discrimination of hUC-MSCs with high and low viabilities is thus carried out by comparing the DL kinetics parameters, including the initial intensity, the peak decay rate and the peak weight value. Our results show that the novel optical method for the viability diagnosis of hUC-MSCs has a promising prospect.

Document code: A **Article ID:** 1673-1905(2014)05-0391-4

DOI 10.1007/s11801-014-4099-2

Mesenchymal stem cells (MSCs) are a kind of particularly attractive candidate for clinical applications due to their intrinsic properties, including multi-differentiation capacity, hematopoietic-supporting activity, immunomodulatory potency and self-renewal[1-6]. Previous studies have shown that MSCs can be isolated from not only the bone marrow but also other organs, such as human umbilical cord[7,8]. Compared with the bone marrow-derived MSCs (BM-MSCs), human umbilical cord MSCs (hUC-MSCs) are safe, abundant and without ethical problems. Accordingly, hUC-MSCs have attracted increasing attention in recent years. Research findings suggest that hUC-MSCs have great potential applications in understanding regeneration medical development and cell-based therapy[9-12]. It is well known that a large population of cells sustained in high viability is fundamental to a successful cell-based therapy. Therefore, the viability diagnosis is a key step for the population expansion and quality assurance of hUC-MSCs.

Label-free cell-based assays are desirable for cytological analysis. A new method based on photo-induced delayed luminescence (DL) is worthy of attention and exploration. The phenomenon of DL is known as the long-lived afterglow of biological systems after being illuminated with white light or monochromatic light[13]. Many researchers have already carried out some attempt of using DL in biology and medicine as a quantitative diagnostic method for different physiological and pathological changes[14-21]. Owing to the striking correlation and advancement of ultraweak irradiation detection technology, it is possible to obtain information on change of viability of cells by using DL as a fast and sensitive optical indicator. For developing a novel cytological method, we report the use of DL to spectrally discriminate hUC-MSCs with different viabilities in this paper.

The hUC-MSCs were isolated from umbilical cords which are collected from healthy parturient women with well-developed fetus[22]. Briefly, the cords were minced into 1 mm^3 small fragments and washed thoroughly with phosphate buffered saline (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 passed through a 200 μm filter to obtain cell suspension. 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 every 2 days at first and then every 3 or 4 days. The cells were serially passaged and expanded in a humidified incubator at 37 °C with 5% CO_2.

The hUC-MSCs with different viabilities were used for experiments. The decrease of the cell viability is in-

* This work has been supported by the National Natural Science Foundation of China (Nos.60508004 and 60778043), the National High Technology Research and Development Program of China (No.2011AA030205), the Doctoral Fund of Ministry of Education of China (No.20110031110035), and the Tianjin Municipal Science and Technology Commission (No.08ZCDFGX09400).

** E-mail: linlie@nankai.edu.cn

duced via cell starvation by 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 dye exclusion method every day, and the viability is defined as the ratio of the number of viable cells to the total number of all cells.

To perform DL decay kinetics measurements, an ultraweak luminescence detection system is employed. The experimental setup of the system for monitoring DL decay kinetics is shown in Fig.1. A xenon flash lamp (L7685, Hamamatsu Photonics, Japan) with pulse width of 5 μs, adjustable pulse energy and flash frequency was chosen for illumination. The detection system consists of a highly sensitive photomultiplier tube (PMT, R943-02, Hamamatsu Photonics, Japan) cooled down to −30 °C, a preamplifier (SR445, SRS, USA), a photon counter (Multichannel Scaler/Averager, SR430, SRS, USA), two shutters and a sequential control unit. The two shutters are located in the excitation and signal light paths respectively, and are able to complete open and close actions one after the other within 1 ms interval, which serve to eliminate the disturbance by the afterglow of light source and ensure the safe measurement of the luminescence signal by the PMT. DL decay kinetics measurements were carried out under the same excitation conditions with mean power of 20 μW and illumination time of 335.54 ms at constant temperature of 22 °C and relative humidity (RH) of 40%. A bin width of 327.68 μs was used for all measurements. A 290–370 nm band pass filter, a 410 nm dichroic mirror and a 400 nm long pass filter were combined for the detection of DL. For each sample, three repeated measurements were performed.

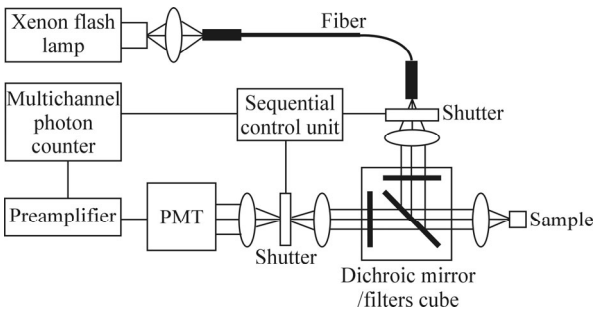

Fig.1 Schematic diagram of the experimental setup

The hUC-MSCs with different viabilities are used for DL experiments. We define that if the ratio of the number of viable cells to the total number of all cells is greater than 80%, the viability is identified as high viability, while if the ratio is less than 20%, the viability is identified as low viability. Fig.2 shows the average DL decay curves of hUC-MSCs with both high and low viabilities in the duration of 3.5–120 ms after illumination, and each curve represents the mean value of three individual measurements. The system noise is evaluated, and the background count is about 12 c/s in the magnitude, which can be neglected compared with the DL intensities of the cells within 120 ms after illumination. The outstanding signal-to-noise ratio (SNR) of this detection method provides the basis for reliable measurement of ultraweak luminescence signal.

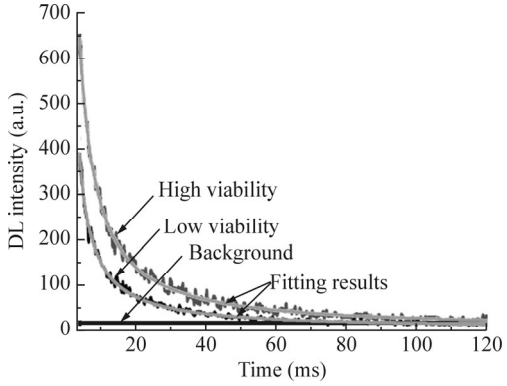

Fig.2 Experimental and fitted DL decay curves of hUC-MSCs with high and low viabilities

A significant difference in DL decay features of hUC-MSCs with high and low viabilities is observed from Fig.2. Compared with the hUC-MSCs with high viability, the initial photon counts for hUC-MSCs with low viability decrease apparently. Ideally, we should find an appropriate mathematical model to quantitatively characterize the DL decay. It is found that the DL decay kinetics of different kinds of biological systems can be fitted by the hyperbolic function. In this paper, the DL decay curves of hUC-MSCs with both high viability and low viability are characterized by a hyperbolic function as

$$I(t)=\frac{I_0}{(1+t/t_0)^m}, \tag{1}$$

where I_0 and $I(t)$ denote the DL intensities at the initial time t=0 and time t after illumination, t_0 is the characteristic time of decay rate, and m is the index factor. DL characteristics are described by I_0, t_0 and m, which can be obtained by fitting the experimental data. Fig.2 shows the fitted curves for DL decay of hUC-MSCs with high and low viabilities, for hUC-MSCs with high viability, I_0=655 c/327.68 μs, t_0=1.917 24 ms, m=1.144 11 and R^2=0.997, and for low viability hUC-MSCs, I_0=391 c/327.68 μs, t_0=0.732 27 ms, m=1.113 32 and R^2=0.986.

Previous investigations have shown that for different biological systems, the hyperbolic relaxation is a characteristic active response of an ergodic coherent state[13]. However, some other research results showed that this decay can also be generated by the non-coherent superposition of various exponential relaxations with different decay rate constants, which means that the DL is a comprehensive embodiment of various decay processes of luminescence in the biological systems[23]. In the case of cells, the previous results demonstrated that the DL contains a variety of light-emitting decay processes[22]. Therefore, it is reasonable to describe the DL decay ki-

netics as the superposition of exponential functions with different decay rate constants and weight coefficients, which can be expressed as

$$I(t)=\int_0^{\infty} Af(\nu)\mathrm{e}^{-\nu t}\mathrm{d}\nu\,, \tag{2}$$

where A is the normalized constant and is set to be I_0 to account for the initial intensity of DL, v is the decay rate, and $f(v)$ stands for the weight distribution for the factor v. From the point of view of mathematical analysis, the distribution function $f(v)$ is the Laplace transform of $I(t)/I_0$. Therefore, the mathematical expression of $f(v)$ can be obtained by performing an anti-Laplace transform processing, which is expressed as

$$f(\nu)=t_0^m\nu^{m-1}\mathrm{e}^{-\nu t_0}/\Gamma(m)\,, \tag{3}$$

where the gamma function is $\Gamma(m)=(m-1)!$. Fig.3(a) shows the weight distribution of the decay rate v for the DL of each sample based on Eq.(3).

As shown in Fig.3(a), there is a significant difference in weight distributions of the decay rate v for DL of hUC-MSCs with high and low viabilities. To quantitatively analyze the difference, the peak parameters of the weight distribution $f(v)$, including the peak decay rate v_{max} and its corresponding peak weight value F_{max}, are employed to describe the DL decay kinetics.

In the weight distribution curve, $\mathrm{d}f(v)/\mathrm{d}v=0$, we can calculate v_{max} and F_{max} as

$$\nu_{\max}=\frac{m-1}{t_0}\,, \tag{4}$$

$$F_{\max}=f(\nu_{\max})=t_0(m-1)^{m-1}\mathrm{e}^{1-m}/\Gamma(m)\,. \tag{5}$$

From Eqs.(1), (4) and (5), we obtain three characteristic parameters of I_0, v_{max} and F_{max} of the DL. Fig.3(b)–(d) show the comparisons of the characteristic parameters for hUC-MSCs with high and low viabilities. It can be seen that the initial intensity I_0 of (682±51) c/327.68 μs and the peak weight value F_{max} of $(1.235\pm0.097)\times10^{-3}$ for DL of hUC-MSCs with high viability are respectively 105% and 134% higher than those of hUC-MSCs with low viability, which are I_0=(332±63) c/327.68 μs and $F_{max}=(0.527\pm0.063)\times10^{-3}$. On the other hand, the peak decay rate v_{max} of 92.82±20.29 s^{-1} for hUC-MSCs with high viability is less than that of the hUC-MSCs with low viability, which is 193.74±36.81 s^{-1}. Statistically, a bilateral t-test is employed for comparing the differences in characteristic parameters. The p-values for I_0, v_{max} and F_{max} are 0.031, 0.035 and 0.009 for hUC-MSCs with high and low viabilities, respectively. The low p-value ($p<0.05$) indicates that the differences in the three parameters are statistically significant for hUC-MSCs with high and low viabilities. Therefore, a comparison of these parameters shows the solution of distinguishing the hUC-MSCs with high and low viabilities from healthy controls.

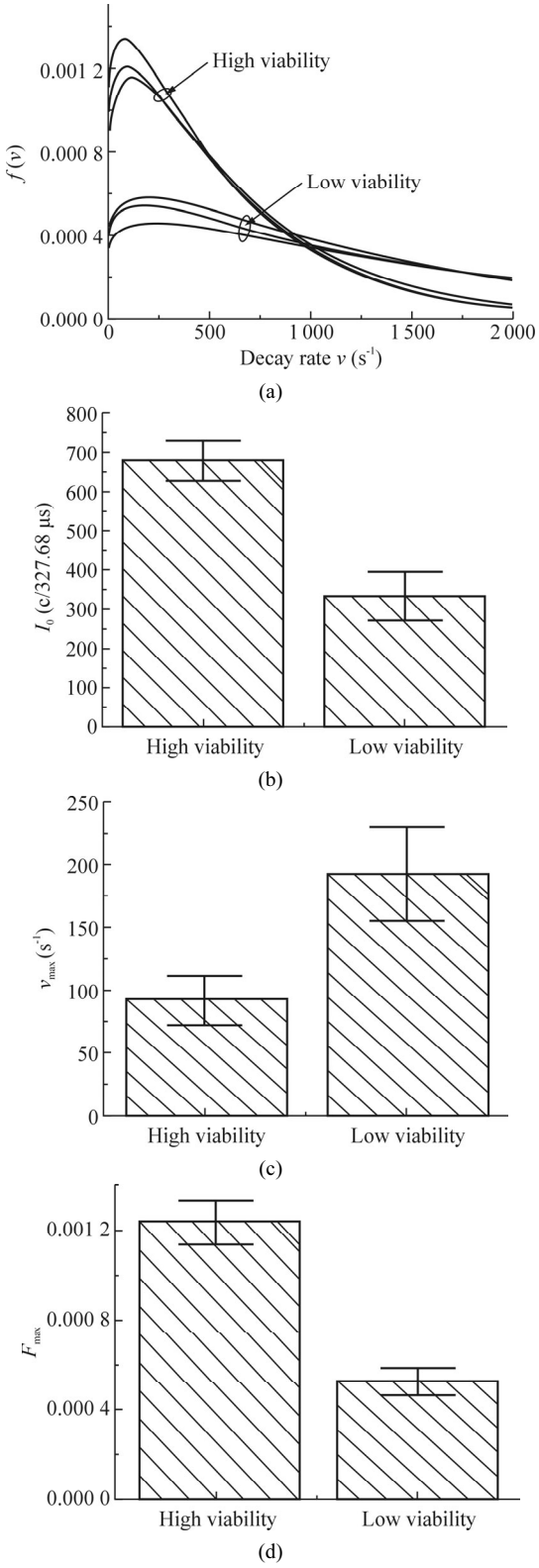

Fig.3 (a) Weight distribution of the decay rate v for the DL of hUC-MSCs with high and low viabilities; Comparisons of (b) I_0, (c) v_{max} and (d) F_{max} for hUC-MSCs with high and low viabilities

In this paper, using an ultraweak luminescence detection system, we measure DL from hUC-MSCs with both high and low viabilities and obtain the outstanding SNR.

We identify a significant difference in the weight distribution of the decay rate for the hUC-MSCs with high and low viabilities. With the initial intensity (I_0), the peak decay rate (v_{max}) and the peak weight value (F_{max}) as the characteristic parameters, explicit discrimination of hUC-MSCs with high and low viabilities can be realized. In future work, we expect to identify the cell viability responsible for causing the variation in kinetics parameters of the DL of stem cells.

References

[1] P. Bianco, X. Cao, P. S. Frenette, J. J. Mao, P. G. Robey, P. J. Simmons and C.-Y. Wang, Nature Medicine **19**, 35 (2013).

[2] S. H. Ranganath, O. Levy, M. S. Inamdar and J. M. Karp, Cell Stem Cell **10**, 244 (2012).

[3] S. Wang, X. Qu and R. C. Zhao, Journal of Hematology and Oncology **5**, 19 (2012).

[4] J. W. Lee, X. Fang, A. Krasnodembskaya, J. P. Howard and M. A. Matthay, Stem Cells **29**, 913 (2011).

[5] A. R. Williams and J. M. Hare, Circulation Research **109**, 923 (2011).

[6] H. Motaln, C. Schichor and T. T. Lah, Cancer **116**, 2519 (2010).

[7] L. L. Lu, Y. J. Liu, S. G. Yang, Q. J. Zhao, X. Wang, W. Gong, Z. B. Han, Z. S. Xu, Y. X. Lu, D. Liu, Z. Z. Chen and Z. C. Han, Haematologica **91**, 1017 (2006).

[8] D. Baksh, R. Yao and R. S. Tuan, Stem Cells **25**, 1384 (2007).

[9] Y. Liu, R. Mu, S. Wang, L. Long, X. Liu, R. Li, J. Sun, J. Guo, X. Zhang, J. Guo, P. Yu, C. Liu, X. Liu, Z. Huang, D. Wang, H. Li, Z. Gu, B. Liu and Z. Li, Arthritis Research & Therapy **12**, R210 (2010).

[10] C.-G. Fan, Q.-J. Zhang and J.-R. Zhou, Stem Cell Reviews and Reports **7**, 195 (2011).

[11] T.-C. Fang, C.-Y. Pang, S.-C. Chiu, D.-C. Ding and R.-K. Tsai, PLoS ONE **7**, e46504 (2012).

[12] A. Bongso and C.-Y. Fong, Stem Cell Reviews and Reports **9**, 226 (2013).

[13] F.-A. Popp and Y. Yan, Physics Letters A **293**, 93 (2002).

[14] F. Musumeci, L. A. Applegate, G. Privitera, A. Scordino, S. Tudisco and H. J. Niggli, Journal of Photochemistry and Photobiology B: Biology **79**, 93 (2005).

[15] F. Musumeci, G. Privitera, A. Scordino, S. Tudisco, C. L. Presti, L. A. Applegate and H. J. Niggli, Applied Physics Letters **86**, 153902 (2005).

[16] L. Lanzanò, A. Scordino, S. Privitera, S. Tudisco and F. Musumeci, European Biophysics Journal **36**, 823 (2007).

[17] H.-W. Kim, S.-B. Sim, C.-K. Kim, J. Kim, C. Choi, H. You and K.-S. Soh, Cancer Letters **229**, 283 (2005).

[18] J. Kim, C. Choi, J. Lim, H. You, S.-B. Sim, Y.-K. Yom, E.-H. Kim and K.-S. Soh, Journal of Alternative & Complementary Medicine **11**, 879 (2005).

[19] P. Chen, L. Zhang, F. Zhang, J.-T. Liu, H. Bai, G.-Q. Tang and L. Lin, Biomedical Optics Express **3**, 1787 (2012).

[20] I. Bókkon, R. Vimal, C. Wang, J. Dai, V. Salari, F. Grass and I. Antal, Journal of Photochemistry and Photobiology B: Biology **103**, 192 (2011).

[21] K. Lenger, R. P. Bajpai and M. Spielmann, Identification of Unknown Homeopathic Remedies by Delayed Luminescence, Cell Biochemistry and Biophysics, 1 (2013).

[22] H. Bai, P. Chen, H. Fang, L. Lin, G. Q. Tang, G. G. Mu, W. Gong, Z. P. Liu, H. Wu, H. Zhao and Z. C. Han, Laser Physics Letters **8**, 78 (2011).

[23] A. Scordino, A. Triglia and F. Musumeci, Journal of Photochemistry and Photobiology B: Biology **56**, 181 (2000).