Resazurin Dye as a Reliable Tool for Determination of Cell Number and Viability in Mesenchymal Stem Cell Culture

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Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 3, pp. 173-175, July, 2010
Original article submitted July 27, 2009

Human mesenchymal stem cells are a valuable cell source for tissue engineering. Determination of cell number and viability is crucial. However, this can be tested only at the end of cell culture. This study shows that Resazurin dye staining is a reliable tool for evaluation of cell number and viability in culture without cell perturbation.

Key Words: *Resazurin dye; mesenchymal stem cells; cell count*

Cells from the mesenchymal differentiation lineage have been shown to be excellent cell sources for tissue engineering. However, these cells are not ubiquitously available. In numerous experimental studies, cell lines or repeatedly passaged cells were used accepting the risk of reduced differentiation potential. The use of the first passage cells seems to be important for minimizing dedifferentiation, especially for cartilage tissue engineering. Monitoring of adherent cells by light-optic microscopy (trypan blue exclusion test) requires trypsinization of the culture. Therefore, the development of reliable and non-toxic methods for quantification of cell number and viability during culturing is an important problem.

Resazurin dye in a non-fluorescent form is readily reduced to a fluorescent form due to metabolic activity of living cells [1]. Intracellular oxidoreductases, mitochondrial and cytosolic, are involved in the process [2]. Fluorescence- or absorbance-based instruments are suitable for detection of the reduced compound [3]. For viability studies, the method was correlated to the established method of trypan blue exclusion [4] or ^{3}H-thymidine incorporation assay [5] and showed accurate determinations for nonadherent cells [6,7]. To our knowledge, no studies have been performed with adherent cell lines like human mesenchymal stem cells. Therefore it remains unclear whether Resazurin dye can be used for these adherent cells without harm and if the accuracy of this assay meets the standard criteria.

MATERIAL AND METHODS

Mesenchymal stem cells were obtained via Jamshidi punction from the iliac crest of male patients undergoing spine fusion. After separation by Ficoll gradient fractionation, the cells were resuspended in Dulbecco's modified Eagle's low glucose medium containing 10% fetal bovine serum. According to our standard culture conditions 2×10^{6} nucleated cells per 100-mm dish were seeded in monolayer culture in atmosphere of 5% CO_2 and 37°C. The cells were counted after attaining 80% confluence.

For comparison of trypan blue exclusion and Resazurin dye method, the supernatant was collected after 1-h incubation with 0.44 mM Resazurin (Roth, Germany), three 100-µl aliquots were transferred to a 96-well plate, and absorbance was measured at 485 nm in a microplate reader and analyzed using Tecan software. Trypan blue exclusion was performed as described elsewhere [8]. The correlation coefficient

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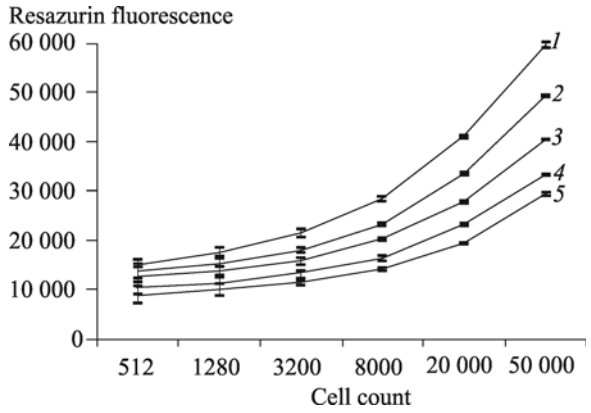

Fig. 1. Intensity of fluorescence of reduced Resazurin in culture medium as a function of cell count in the culture after adding Resazurin in concentration of 0.66 (*1*), 0.50 (*2*), 0.37 (*3*), 0.28 (*4*), and 0.21 mM (*5*).

between cell number and relative fluorescence units (RFU) was determined.

In a parallel experiment, the cells were seeded at different densities (from 512 cells/ml up to 50,000 cells/ml) and Resazurin was added to the culture medium to a final concentration of 0.21-0.66 mM as described elsewhere [9]. The intensity of fluorescence (RFU) was measured after 1-h incubation.

The effect of Resazurin on cell viability was studied. To this end, the cells were seeded to 96-well plates at a density of 2000 cells per 300 μl per well and calcein AM (acetoxymethyl ester) was added. The cells were treated with Resazurin in different concentrations (0.1-1.0 mM). Cells not exposed to Resazurin served as the control. Cells in some wells were treated with 70% ethanol for 30 minutes to obtain dead cells. Fluorescence was measured on a microplate reader at excitation and emission wavelengths of 485 and 530 nm, respectively.

Quantitative data were statistically analyzed using Student's *t* test ($p<0.05$).

RESULTS

Quantification of the cell number in a wide cell concentration range was feasible by the Resazurin dye method. Resazurin dye was used in a wide concentration range without losing correlation with the cell number (Fig. 1). The concentrations 0.21 mM and 0.66 mM yielded similar results, but our preferred concentration was 0.44 mM according to multiple studies on non-adherent cell lines. The use of Resazurin dye allows determination of cell numbers in a wide range, at least from 512 to 50,000 cells/ml. The correlation coefficient for this range of cell concentration was over 0.98. Consistency of this procedure leads to a conclusion that even lower cell concentration could be used without losing accuracy.

Resazurin dye had no adverse effects on cultured adherent cells, *e.g.* mesenchymal stem. Cell viability measured using vital dye calcein AM showed that Resazurin in none of the applied concentrations induced cell death during short (1 h) and long-term (12 h) exposure (Fig. 2).

Resazurin added to the culture medium did not appreciably affect cell proliferation and differentiation compared to untreated control cell cultures. Use of Resazurin dye revealed high correlation with standard methods of cell counting, *e.g.* trypan blue exclusion test (Fig. 3).

Resazurin dye as a tool for determination of cell proliferation of different non-adherent cell lines was used in multiple studies. However, we are not aware of a report in the literature, which determined the cell number of adherent cells like mesenchymal stem cells in this way.

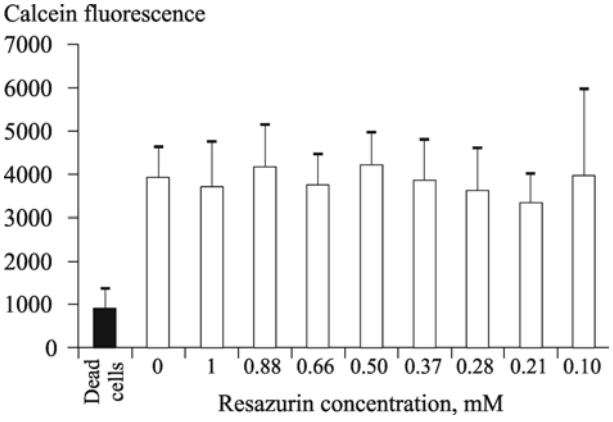

Fig. 2. Effect of 1-h incubation with Resazurin in different concentrations on cell viability (evaluated by calcein labeling) in culture. Fluorescence excitation and emission wavelengths are 485 and 530 nm, respectively. Dark bar: accumulation of calcein by dead cells (30 min treatment with 70% ethanol). All differences from the control are reliable at $p<0.01$.

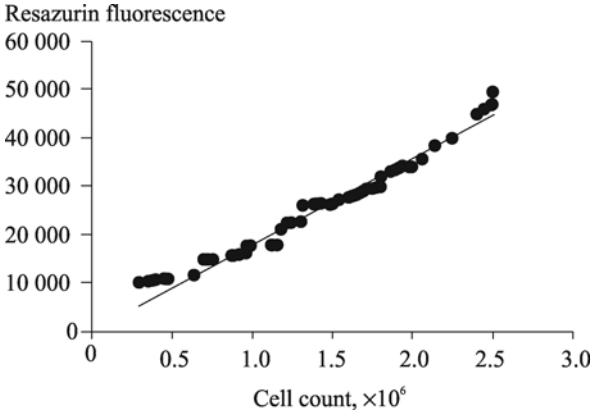

Fig. 3. Correlation of the cell counts obtained by Resazurin labeling method and trypan blue exclusion test. Abscissa: cell count determined by trypan blue exclusion test.

The most important advantage of Resazurin dye compared to standard cell count techniques is the possibility of determining the cell number during culturing without reduction of cell proliferation and differentiation potential. This is in line with published reports, where no adverse effects of Resazurin on non-adherent cell lines were detected [10,11]. Thus, Resazurin dye assay may provide a powerful tool to check cell viability and provide a correlation with cell number in adherent mesenchymal stem cells during culturing.

This work was supported by the DFG (German Research Foundation) and an intramural grant. We would like to thank Richard Kujat for his support and Daniela Drenkard for her excellent technical assistance.

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