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Analysis of Cytotoxic Effect using the Live Cell Imaging System

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Abstract

Objective: To explore the possibility of using the live cell image system to detect cytotoxicity and oxidative stress. Methods: 5x104 293T cells or 1x104 cardiac microvascular endothelial cells (CMECs) were seeded on a 24-well plate, then the 24-well plate were placed on the live cell imaging system in an incubator. 25 μM and 250 μM H2O2 were added to 293T cells, while 100 nM, 250 nM, 500 nM and 1000 nM doxorubicin (Dox) were added to CMECs respectively, and then the cells density and proliferation were observed and analyzed with a live cell image system. The images of each well were captured every 30 minute for H2O2-treated 293T cells or an hour for Dox treated CMECs respectively. The cell density was analyzed automatically by the set program included in live cell image system. Results: The death of 293T cells induced by H2O2 was able to be observed in real time by the live cell imaging system via analysis of cell density. 25 μM H2O2 had no significant effect on the death of 293T cells, while 250 μM of H2O2 was able to induce 293T cell death. In six hours after the Dox treatment, no dividing cell is identified in all concentration treated groups compared to control group. In 15 hours after Dox treatment, morphological changes and cells debris were found in all concentration treated groups. In additon, it was found that as the concentration of Dox increased, the density of CMECs was decreased. When the concentration of Dox reached 250 nM, the density of CMECs was significantly decreased. Conclusion: Live cell imaging system is able to quantitative analysis of cytotoxic effect via comparison of difference of cell proliferation and cell density in real time monitor strategy. The analysis of cytotoxic effect using the live cell imaging system is feasible and reliable.

Keywords

Live Cell Imaging System; Cytotoxicity; Proliferation.

1. Introduction

In the field of life science research, the establishment of cytotoxicity models [1] and oxidative stress experiments [2] were widely used in various studies. The proliferation and toxicity to cells could be detected by MTT assay or CCK8 assay. However, these methods could only be used to detect the results after the termination of reaction, and could not monitor the process of cell reaction in real time, while, sometimes false positive results were included in these assay [3]. In addition, to search for optimal reaction conditions, these methods require a lot of reagents and time for a large number of screenings. The live cell imaging system is the 24 channels microscope for the incubator with

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automated remote monitoring of cell cultures. The live cell imaging system is able to monitor and measure directly the growth state and the amount of cells in the incubator by the image strategy, which is a marker-free and real time analysis method. This method has the advantages of fast speed, low cost and dynamic observation. To verify the feasibility of this technology for analysis of cytotoxicity, in this study, H2O2 inducing oxidative stress cytotoxic effect in 293T cells and doxorubicin (Dox) inducing cytotoxic effect in cardiac microvascular endothelial cells (CMECs) were observed by the live cell imaging system in this study. The results of this study documented that analysis of cytotoxic effect using the live cell imaging system is feasible and reliable.

2. Methods and Materials

2.1 Laboratory Animal

Three-month-old male Sprague-Dawley (SD) rats (250-300 g) were used in this study. Rats were housed for 2 weeks to allow them to acclimate before the experiment, provided food and water regularly. Animal care, surgery and handling procedures were performed in accordance with the regulations established by the Ministry of Science and Technology of the People's Republic of China ([2006] 398) and approved by the Animal Care Committee of Jinan University.

2.2 Isolation and Culture of Cardiac Microvascular Endothelial Cells(Cmecs) from Young Rats

The rats were sacrificed by cervical dislocation, and sterilized by soaking in 75% ethanol for 3-5 minutes. The rat heart was cutted out on a clean bench and washed with ADS buffer. The hearts were minced into small pieces approximately 1-2 mm3 in size, then vortexed in a water bath at 37 °C with 0.2 mg/ml type II collagenase and 0.1 mg/ml DNase for 40 minutes. The suspension was filtered through a 100 μm filter and the filtrate was centrifuged at 200×g for 10 minutes. The supernatant was then removed and the pellet was resuspended in 2 mL of PBS buffer (pH=7.4). The cell suspension was added slowly and gently to a gradient buffer consisting of 40% percoll and 20% percoll, followed by centrifugation at 400 x g for 30 minutes. After percoll gradient centrifugation, the intermediate cell layer was collected and PBS buffer was added to 10 mL, followed by centrifugation at 1000 × rpm for 3 minutes. The cell pellet was resuspended in CMEC culture medium (M199 basal medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 500 μL endothelial cell growth factor) and cultured in an incubator containing 5% CO2 and 95% air at 37 °C. The cells were passaged after reaching 90% confluence, and the CMEC culture medium was changed every three days.

2.3 H2O2 Treatment Inducing Oxidative Stress Cytotoxic Effect in 293T Cells

293T cells were seeded in a 24-well plate containing 10% FBS in DMEM medium at a density of 5×104 cells/well, and cultured in an incubator containing 5% CO2 and 95% air at 37 °C. The H2O2 solution was diluted with new medium to the concentration of 25 μM and 250 μM. After 24 hours, the new medium was replaced and placed in a live cell dynamic imaging and analysis system (zenCELL owl device, InnoME GmbH, Germany) for 24 hours, and each picture was taken at 30-minute intervals.

2.4 Dox Inducing Cytotoxic Effect in CMECs

CMECs were seeded in 24-well plates of EC-M199 containing 10% FBS at a density of 104 cells/well, and cultured in an incubator containing 5% CO2 and 95% air at 37 °C. The Dox solution was diluted with new medium to the concentration of 100 nM, 250 nM, 500 nM, or 1000 nM, and the new medium was replaced after 24 hours, and placed it on the zenCELL owl, a live cell dynamic imaging and analysis system (zenCELL owl device, InnoME GmbH, Germany), to observe for 5 days, and each picture was taken at per-hour intervals.

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2.5 Statistical Analysis

All experimental data were analyzed and graphed by GraphPad Prism 8 statistical analysis software. The one-way ANOVA was used for the analysis between groups, and $P<0.05$ indicating that the difference between groups was statistically significant.

3. Results

3.1 Live Cell Imaging System is Able to Measure the Cytotoxic Effect of H2O2 Inducing 293T Cells Death

The 293T cells were treated with H2O2 (0 μM, 25 μM and 250 μM) for 24 hours. Each photo was taken per half hour at the time interval and the image of cells every 6 hours was shown (Figure 1B).

Untreated and 25 μM showed the same proliferation activity (Figure 1B) leading to a confluent monolayer over a time period of 24 hours. The cells gradually increased with time through the real-time observation, which proved that low concentration of H2O2 was not enough to induce oxidative stress inducing cell death in 293T cells. However, the higher concentration (250 μM) H2O2 apparently caused irreversible damage to cells, which became round-shaped and had detached from the surface after 6 hours. While the control cells and 25 μM groups had adhered at 6 hours. As it was showed in Figure 1A, the cell coverage was decreased in the 6th hour after 250 μM H2O2 treatment which was consistent with the image in Figure 1B. The results of live cell imaging system analysis revealed that 250 μM of H2O2 is able to induce the cell death of 293T cells, while 25 μM of H2O2 had no significant cytotoxic effect in cells.

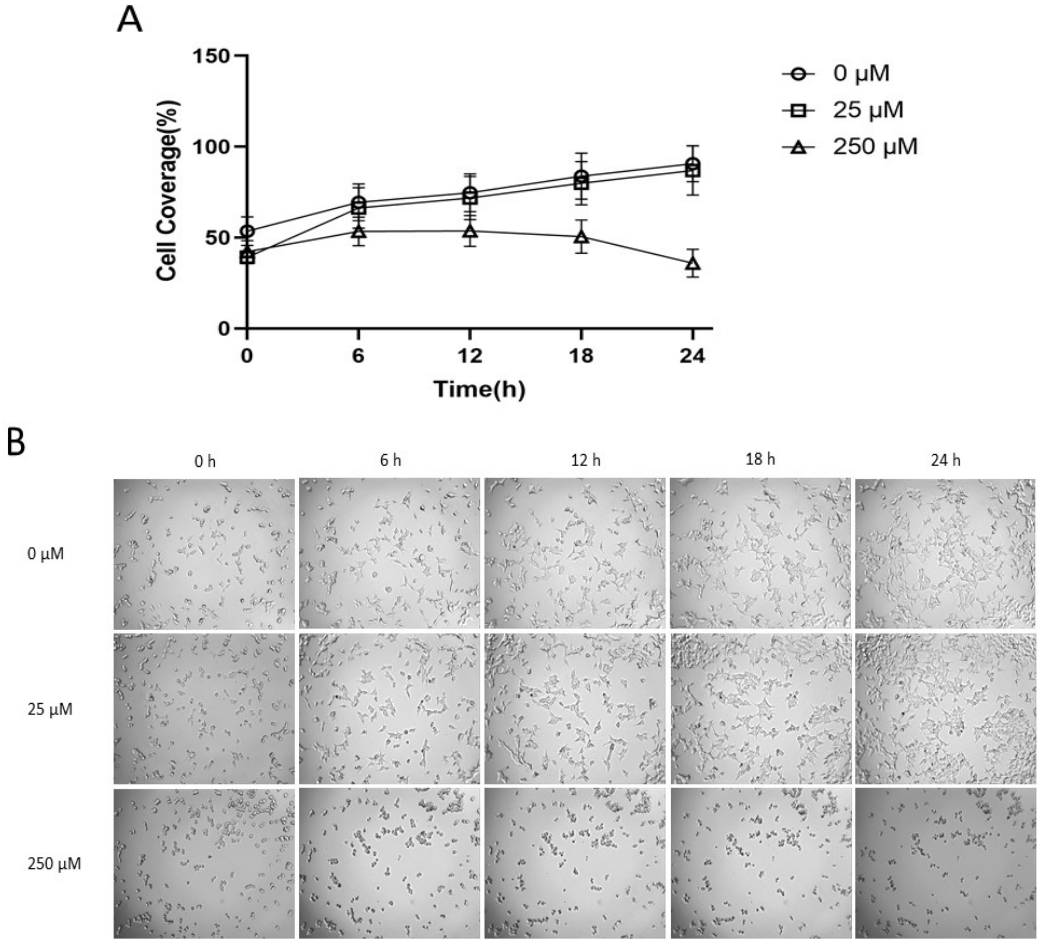

Figure 1. A. The cell coverage of 293T cells treating with 0 μM, 25 μM and 250 μM H2O2. B. The images of 293T cells of the three groups every 6 hours over a 24-hour period captured by live cell imaging system.

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3.2 Live Cell Imaging System is Able to Measure the Cytotoxic Effect of Dox Inducing Cell Death of CMECs

The CMECs was treated with different concentrtion of Dox (100 nM, 250 nM, 500 nM and 1000 nM). In six hours after the Dox treatment, no dividing cell is identified in all concentration treated groups compared to control group which show proliferative activity (Figure 2A). In 15 hours after Dox treatment, morphological changes and cells debris were found in all concentration treated groups (Figure 2B). In additon, it was found that as the concentration of Dox increased, the density of CMECs was decreased (Figure 2B). When the concentration of Dox reached 250 nM, the density of CMECs was significantly decreased (Figure 2A). The results of live cell imaging system analysis revealed that 250 nM and more than this dosage of Dox is able to induce the cyototoxic effect for CMECs.

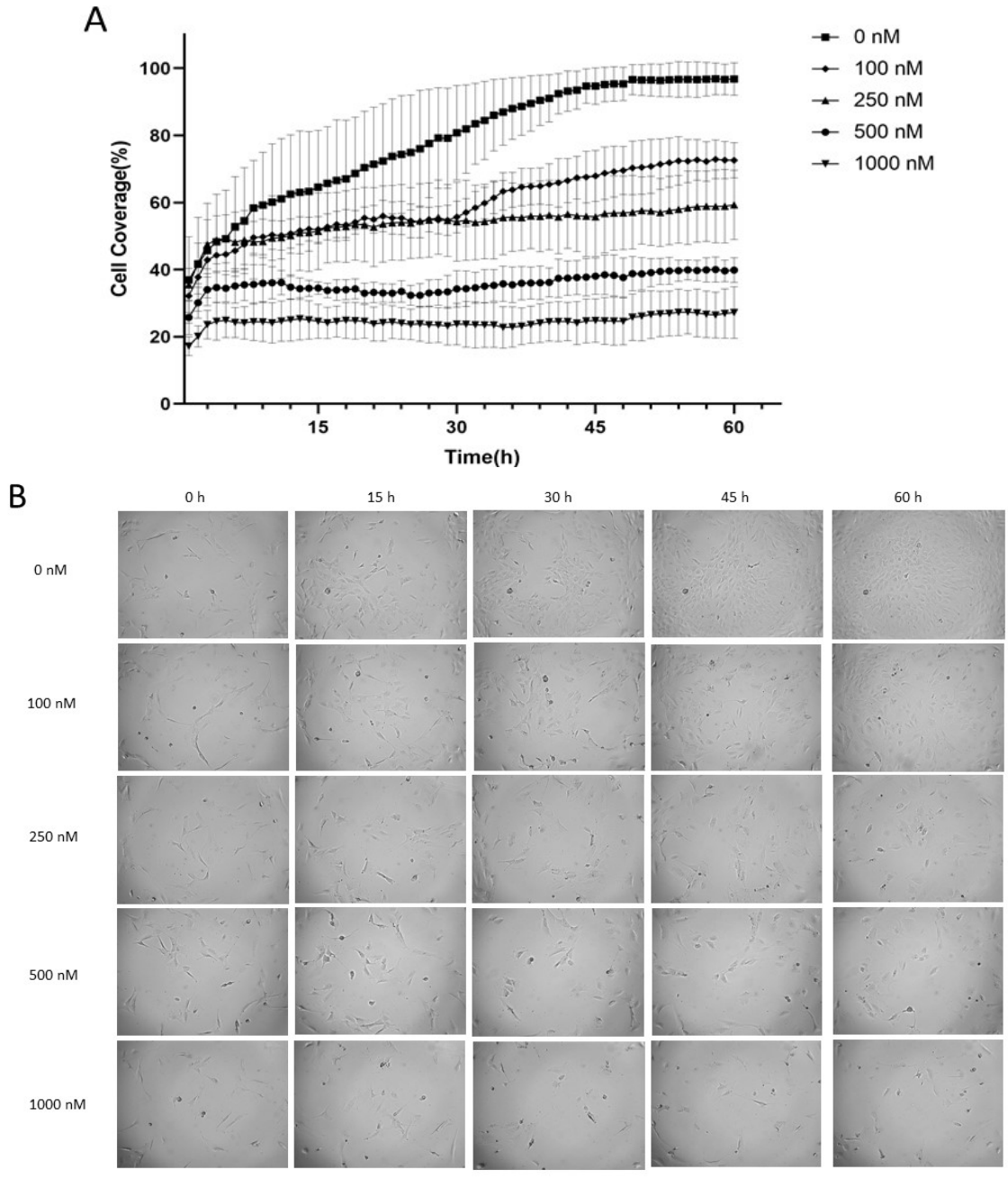

Figure 2. A. The cell coverage of endothelial cells treating with 0 nM, 100 nM, 250 nM, 500 nM or 1000 nM doxorubicin. B. The images of CMECs of the five groups every 15 hours over the first 60-hour captured by live cell imaging system.

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4. Discussion

In this study, H2O2 inducing oxidative stress cytotoxic effect in 293T cells and doxorubicin inducing cytotoxic effect in CMECs were observed and analyzed by the live cell imaging system. The results of this study demonstrated that live cell imaging system was perfectly suitable for analysis cell growth and proliferation in real time when it was set and applied in incubators which is able to observe 24 fields simultaneously. The images which are captured by live cell imaging system were well matched with cell density in each field. Indeed, Li et al. reported that the results of live cell imaging system for analysis of the proliferation of Ophiopogonin D in cardiomyocyte cell line H9C2 was well consistent with the results of CCK-8 methods. The results of this study also demonstrated that live cell imaging system is able to quantitative analysis of cytotoxic effect via comparison of difference of cell proliferation and cell density in real time monitor strategy. In addition, the results of present study documented that analysis of cytotoxic effect using the live cell imaging system is feasible and reliable.

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