

APPLICATION NOTE

Enhancing drug response evaluation through real-time monitoring of spheroid cytotoxicity

Using Celloger® Pro

■ Introduction

Cell culture systems are indispensable in the field of biomedical research, where 2D cultures are widely used because of their cost-efficiency and convenience. Nevertheless, the limitations of 2D culture systems, such as the loss of cell-to-cell or cell-to-matrix interactions and tissue-specific structures, hinder their capacity to mimic *in vivo* conditions, especially in disease models like cancer.¹ Due to these limitations, there is a growing interest in 3D culture systems that provide more realistic model resembling a complex *in vivo* environment. These 3D systems hold immense promise for monitoring crucial factors such as cytotoxicity, drug resistance, and cellular responses within the cancer microenvironment.^{2,3} Moreover, they have significantly advanced drug safety and efficacy evaluations, facilitating early-stage drug discovery and development.⁴

The study presented herein highlights the power of Celloger® Pro, a cutting-edge live cell imaging system, in investigating the effects of an anticancer drug, Staurosporine, on 3D spheroids made from HEK293-GFP stable cells. This application note details the comprehensive results obtained using Celloger® Pro, demonstrating its capacity to dynamically capture and quantify cellular responses to drug treatment in a three-dimensional context.

■ Method

HEK293-GFP cells were seeded at 10,000 cells/well in a 96-well cell floater plate (SPL, 34896) which facilitates the formation of spheroid structure. Following overnight incubation, various concentrations of staurosporine (SSP) were treated: 0 μM (control), 0.1 μM , and 1 μM . Additionally, 4 μM of EthD-1 (Sigma, 46043), a fluorescent marker that selectively stains dead cells, was treated. Subsequently, real-time imaging was conducted using a Celloger® Pro. Images were captured at 30-minute intervals over 24 hours, with a 2X lens.

■ Result

To investigate the response of HEK293-GFP spheroids to the anti-cancer drug SSP, we monitored the real-time reduction in spheroid size using brightfield and green fluorescence imaging. Simultaneously, cell death was evaluated at different time points by measuring the intensity of red fluorescence. As the concentration of SSP increased, there was a corresponding rise in red fluorescence intensity, indicating signs of cell death. This trend became more pronounced over time (Fig. 1A). The relative red fluorescence intensity was quantified and graphically presented using Celloger® Pro's analysis software, which provided an insightful illustration of the spheroids' response to drug-induced cell death (Fig. 1D).

Additionally, the progression of cell death corresponded with a reduction in the spheroid's size, evidenced by the green fluorescence channel images. This clearly demonstrated the correlation between decreased spheroid size and intensified red fluorescence in the merged green and red fluorescence images, providing a direct visualization of the drug's impact on cellular viability (Fig. 1A). To further validate the decrease in spheroid size induced by SSP treatment, we precisely measured the spheroid's diameter using Celloger® Pro analysis software (Fig. 1B). The spheroid's size was quantified and effectively visualized through graphical representation, offering a clear and comprehensive depiction of the observed changes (Fig. 1C). These results underscore Celloger® Pro's capability to provide real-time insights into the dynamic changes occurring within 3D cellular structures.

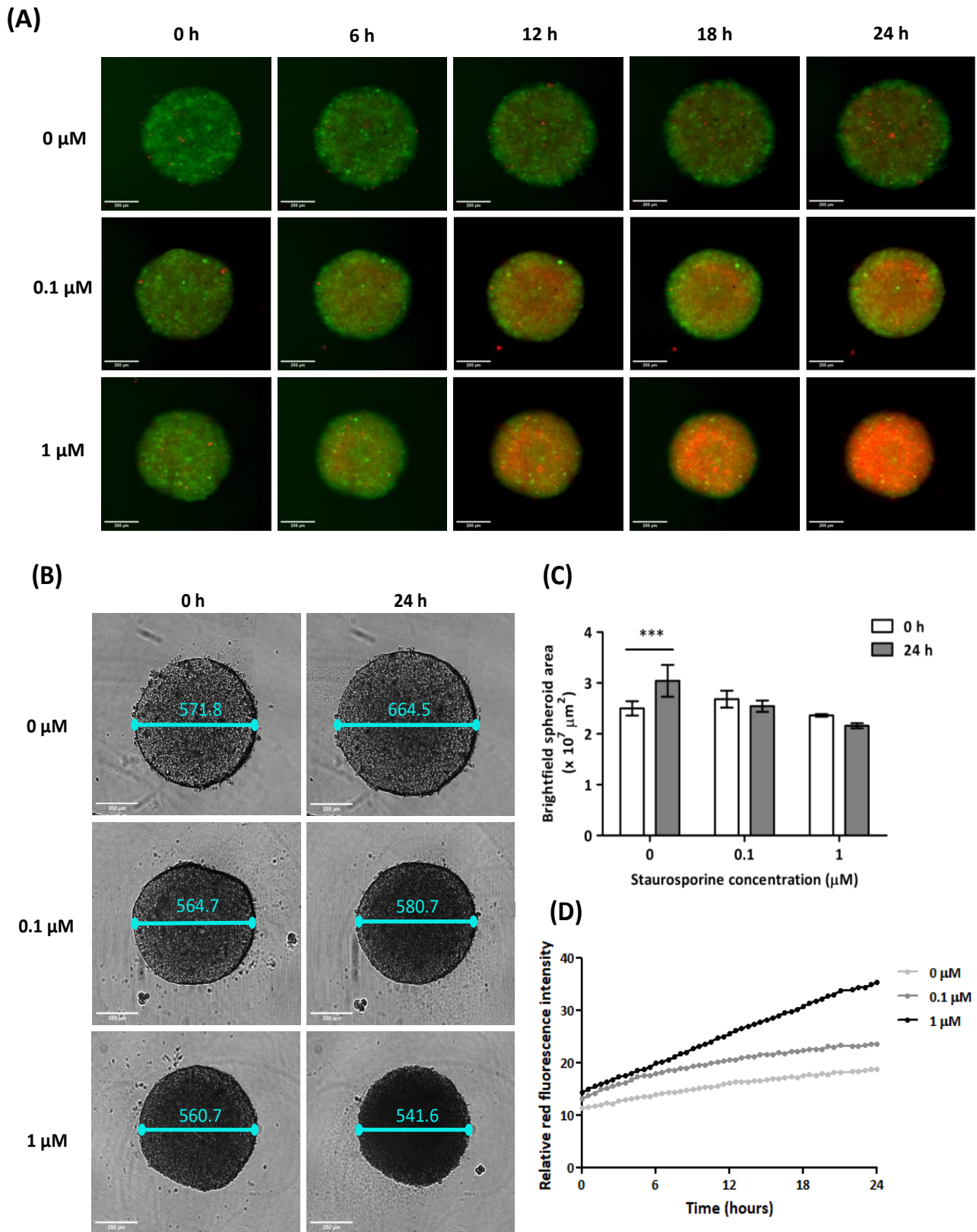


Figure 1. The results of spheroid cells with 0, 0.1, and 1 μM of SSP.

(A) Merged of green and red fluorescence images for each concentration of SSP (scale bar: 200 μm). (B) Brightfield images with spheroid's diameter (scale bar: 200 μm). (C) Comparative graph of spheroid area at 0 and 24 hours for each concentration of SSP. $n=3$ for each group. *** $P < 0.0001$ (D) Relative red fluorescence intensity graph over time.

■ Conclusion

The results presented in this application note exemplify the invaluable capabilities of Celloger® Pro in probing complex cellular phenomena. By dynamically tracking the size reduction of 3D spheroids under SSP treatment and quantifying fluorescence intensity changes in response to cell death markers, Celloger® Pro proves to be an indispensable tool for both qualitative and quantitative live cell imaging. By combining the power of 3D spheroid culture with the real-time imaging capabilities of the Celloger® Pro, we have finally demonstrated its potential to revolutionize drug response evaluation and contribute to the advancement of early-stage drug discovery. Researchers can utilize its features to unravel the subtleties of cellular responses to drug treatments in a three-dimensional context, leading to deeper insights and accelerated advancements in anticancer research and beyond.

Reference

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2. Ravi, Maddaly, et al. "3D cell culture systems: advantages and applications." *Journal of cellular physiology* 230.1 (2015): 16-26.
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4. Huang, Zhaoming, Panpan Yu, and Jianhui Tang. "Characterization of triple-negative breast cancer MDA-MB-231 cell spheroid model." *OncoTargets and therapy* (2020): 5395-5405.