Application Note

Cells: HeLa Cells
Tools: CardioExcyte 96, SOL

Inducing cell death using a photostatin on the CardioExcyte 96 and SOL

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Cells and compound PST-1 were kindly provided by LMU Munich/CytoSwitch

Summary

Microtubules are important for cell support, acting as a kind of internal scaffold providing both shape and an organized structure. Another important role of microtubules is the formation of the mitotic spindle which is critical in cell division[1]. For this reason, compounds which control microtubule dynamics such as Taxol and Vinca alkaloids are important as anti-cancer therapeutics and biological research tools[2,3]. Other compounds e.g. combretastin A-4 (CA4), have been shown to be powerful inhibitors of tubule polymerization[4]. Until now, the use of such compounds in both biological research and as anti-cancer agents has been limited because of their non-specificity; their bioactivity cannot be spatially or temporally directed, e.g. against particular tissues or cells. As an anti-cancer agent, CA4 was taken into Phase III clinical trials but was discontinued due to the cardio- or neurotoxic side-effects at high doses as healthy organs were affected. The use of light to control the activity of microtubule inhibitors may offer a solution to combat the specificity problem by controlling the activation of the compound using visible light in a spatial, temporal and reversible fashion[3]. The CytoSwitch startup project at the LMU Munich is developing photostatins as anti-cancer agents with reduced side-effects. Furthermore, CytoSwitch will commercialize photostatins as research tools for biological research.

In this study, HeLa cells were used on the CardioExcyte 96 equipped with the SOL and blue light was used to activate the photostatin PST-1, a photoswitchable analog of CA4[3]. When activated by light, PST-1 caused cytotoxicity indicated by a change in impedance.

Results

The impedance signal recorded on the CardioExcyte 96 changes as a result of alterations in confluency, cell contact (morphological shape) and conductivity of adherent cells and thereby provides a measure of toxicity.

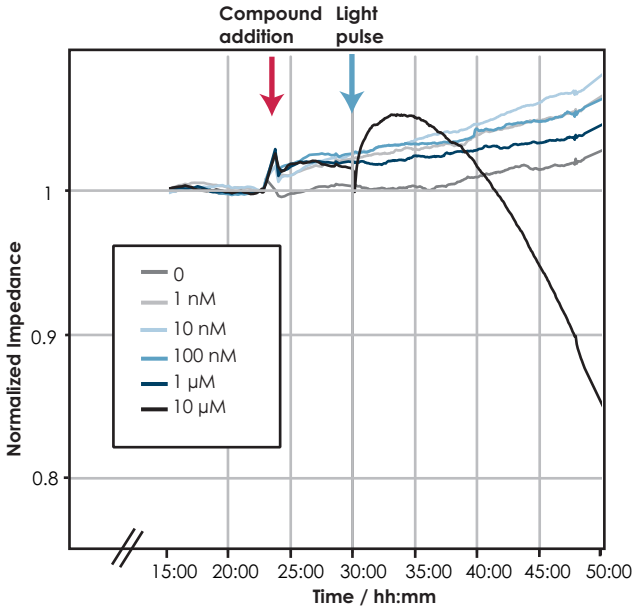

Figure 1: Effect of low concentrations of the photostatin PST-1 on HeLa cells before and after light pulses using the SOL. The compound was added at the timepoint shown (red arrow) at the concentrations indicated. The compound had no effect on the impedance signal before the light pulse was switched on for 75 ms repeated every 1 s for the remainder of the experiment, but the impedance signal reduced at the highest concentration (10 µM) after a delay of approximately 5 hours. No effect was seen at lower concentrations in either dark or light.

Application Note

The photostatin, PST-1, was applied to HeLa cells at low concentrations. No effect was seen before the SOL was switched on. Light pulses of 75 ms every 1 s were then provided by the SOL. After a delay of approximately 5 hours, a decrease in the normalized impedance signal at 10 µM was observed indicating a change in cell morphology (Figure 1).

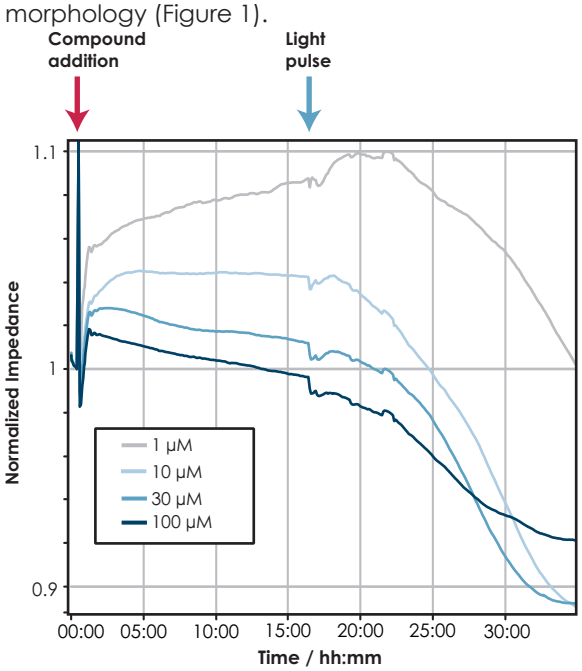

Figure 2: Effect of higher concentrations of PST-1 on the impedance signal of HeLa cells. At higher concentrations (30 - 100 µM), the impedance signal is reduced even before exposure to the light pulse (75 ms every 1 s). After exposure to light, the decline in the impedance signal is accelerated and is seen at all concentrations. This is in good agreement with the literature[3].

In subsequent experiments, the effect of higher concentrations of PST-1 was investigated (Figure 2). In these experiments, application of concentrations 30 µM or 100 µM to the HeLa cells caused a moderate decrease in the impedance signal even before exposure to light, indicating a change in cell shape as expected[3]. When the light was applied, the impedance signal decreased in all concentrations after a delay of approximately 4-5 hours as shown in the previous experiment.

In summary, the CardioExcyte 96 in combination with the SOL can be used to activate photoswitchable compounds, such as PST-1, which cause disruption of microtubules upon application of light even at low concentrations of the compound. The disruption of microtubules results in a morphological change which can be measured using impedance. Furthermore, cytotoxicity can be determined using this impedance measurement.

References

1. Jordan, A., *et al*. 1998. Med. Res. Rev., 18(4): 259–296
2. Peterson, J.R., & Mitchison, T.J. 2002. Chem. Biol. 1275–1285
3. Borowiak, M. *et al*. 2015. Cell. 162: 403-411
4. Pettit, G.R. *et al.* 1989. Experientia 45: 209–211

Methods

Cells

HeLa cells kindly provided by LMU Munich/Cytoswitch were used.

Impedance measurements

Impedance measurements were conducted according to Nanion's standard procedures for the CardioExcyte 96. HeLa cells were thawed, seeded on the Sensor Plate at an appropriate density to provide the desired confluency and cultured under propriety conditions for 24 hours prior to exposure to PST-1. The SOL was switched on for 75 ms every 1 s at a wavelength of 390 nm. All signals were normalized to a group of control measurements (n=5-11) on the same plate.

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