DETERMINE THE OPTIMAL AMOUNT OF CELL-TAK TO IMMOBILIZE SUSPENSION CELLS FOR FURTHER ANALYSIS ON THE CYRIS® PLATFORM

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ABSTRACT

The following protocol describes the preparation, run and results of a preliminary dose-response experiment in the CYRIS® analysis platform to determine the minimum effective density of Cell-Tak to immobilize YAC-1 suspension cells on our metabolic sensor plate for further analysis in the CYRIS® analysis platform.

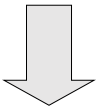
Different blood cells for example lymphocytes or suspension cell lines need to be immobilized for analysis with our automated cell analysis platform CYRIS®. For this application we developed a protocol using *Cell-Tak Cell and Tissue Adhesive* from Corning® to immobilize suspension cells on the surface of our sensor plates. This special adhesive is based on proteins isolated from the marine mussel *Mytilus edulis* and is widely used to immobilize suspension cells on different surfaces such as glass or plastic. For optimal immobilization of suspension cells to our sensor plate it is recommended to determine the minimum effective density of Cell-Tak by a preliminary dose-response experiment as described here.

INTRODUCTION

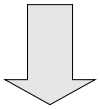
In order to analyze non-adherent cells like blood cells such as lymphocytes or suspension cell lines with our automated cell analysis platform CYRIS®, we developed a protocol to immobilize suspension cells on the surface of our sensor plates. This is necessary because the periodic flow of liquid through the cells generated by the device would otherwise flush them out of the sensor well and thus make them no longer measurable. For this application we use *Cell-Tak Cell and Tissue Adhesive from* Corning®. This special adhesive is a formulation of so-called polyphenolic proteins isolated from the marine mussel *Mytilus edulis*. The marine mussels naturally secrete these proteins which allow the mussels to anchor strongly to solid surfaces under water, such as ocean rocks (Waite J.H. & Tanzer M.L. 1981). These polyphenolic proteins are composed of tandemly repeated oligopeptides with a similar amino acid sequence rich in hydroxyproline (hyp), 3,4-dihydroxyphenylalanine (dopa) and lysine (lys) (Waite J.H. 1983). The Corning® Cell-Tak adhesive can be used to immobilize suspension cells on different surfaces such as glass or plastic. There are different coating methods possible. Here we used the adsorption method to immobilize suspension cells of the YAC-1 cell line on the foil surface of our metabolic sensor plate to further analyze the cells with our multi-parametric cell analysis platform CYRIS®. This coating method is simple and budget-friendly. The resulting coating is uniform, thin and transparent for microscopic imaging. The adsorption method is based on a pH change resulting in precipitation and adsorption of the Cell-Tak adhesive to the surface. The Cell-Tak adhesive is in solution at a lower pH value and comes out of solution when the pH is increased. To guarantee optimal immobilization of suspension cells to our sensor plate it is recommended to determine the minimum effective density of Cell-Tak by a preliminary dose-response experiment as described here. It is recommended to read the entire protocol first to avoid problems with time management.

ASSAY WORKFLOW

Coating of the Sensor Plate with Corning® Cell-Tak Adhesive by Adsorption



Seeding of Suspension Cells onto the Coated Sensor Plate



Performing the CYRIS® Experiment

MATERIAL AND METHODS

Materials Required

Reagents

- Cell-Tak Adhesive (Corning®)
- DMEM high glucose medium
- Fetal calf serum (FCS)
- INCYTON measuring medium
- Phosphate buffered saline (PBS) (pH 7.4)
- Sodium bicarbonate buffer ($NaHCO_3$) (0.1 M, pH 8)
- Sodium hydroxide solution (NaOH) (1 M)

Devices and disposable materials

- Centrifuge
- INCYTON Cyris® flox
- INCYTON metabolic sensor plate
- Pipettes and pipette tips (2-200µl, 50-1000µl)
- Standard cell incubator
- Vacuum pump, flexible tube and glass Pasteur pipettes
- 15 ml sterile conical tube
- 0.5 ml micro reaction tube

Method

Regents for measuring of OCR of YAC-1 cells in CYRIS®

Prepare sterile measuring medium supplemented with 10 % FCS and prewarm to 37 °C in a water bath. Preparation of DWP (5) for the 24-hour baseline measurement: Fill each well of one sterile DWP with 8 ml warm measuring medium with 10 % FCS and place it directly into the CYRIS® incubator at position 5 for acclimatization. Cover it for transport from the sterile workbench to the incubator. Prepare waste DWP: put an empty DWP in the CYRIS incubator on position 3 for wasted media.

Deep-well filling in list form:

Measured function	Substance	DWP position	Total volume of measuring medium	Wells to be filled with measuring medium	Volume of measuring medium per well
Baseline	None	5	192 ml	1-24	8 ml
Waste	/	3	/	/	/

Fluidic

Equip the robot head with 24 sterile pipette tips and attach it to the robot arm in the CYRIS climate chamber.

Adsorption coating procedure

As a high density of Cell-Tak will not necessarily improve the performance of your experiment but will increase the costs of an experiment, it is recommended to determine the minimum effective density of Cell-Tak by a preliminary dose-response experiment as described here.

Prepare the following Cell-Tak solutions as indicated below in list form. Use a 0.5 ml micro reaction tube to dilute the indicated amount of Cell-Tak into the $NaHCO_3$ buffer. Then add the indicated volume of NaOH, mix thoroughly and dispense immediately into the sensor plate as indicated below in list form. Incubate the sensor plate at 37 °C for 1 hr. In the meantime prepare the cell suspension as describe in the cell culture section.

Preparation of Cell-Tak solutions with different Cell-Tak concentrations in list form:

$NaHCO_3$ (0.1 M, pH 8)	342 µl	324 µl	306 µl	288 µl	270 µl	252 µl
Cell-Tak stock solution (1.85 µg/µl)	Add 12 µl	Add 24 µl	Add 36 µl	Add 48 µl	Add 60 µl	Add 72 µl
NaOH (1 M)	Add 6 µl	Add 12 µl	Add 18 µl	Add 24 µl	Add 30 µl	Add 26 µl
Total volume	360 µl	360 µl	360 µl	360 µl	360 µl	360 µl
Final Cell-Tak concentration	0.062 µg/µl	0.123 µg/µl	0.185 µg/µl	0.247µg	0.308 µg/µl	0.372 µg/µl
Final Cell-Tak solutions	**1**	**2**	**3**	**4**	**5**	**6**

Coating of a sensor plate with different Cell-Tak solutions in list form:

Final Cell-Tak solutions	Wells to be filled with Cell-Tak solution	Volume of Cell-Tak solution per well
1	1, 7, 13, 19	88 µl
2	2, 8, 14, 20	88 µl
3	3, 9, 15, 21	88 µl
4	4, 10, 16, 22	88 µl
5	5, 11, 17, 23	88 µl
6	6, 12, 18, 24	88 µl

After incubation aspirate the Cell-Tak solutions via the side channels of the sensor plate using a glass pipette connected to a vacuum pump and wash the wells once with 100 µl PBS each. Aspirate the PBS via the side channels of the sensor plate and immediately add 150 µl of prepared cell suspension into each well of the sensor plate as described in the cell culture section.

Cell culture

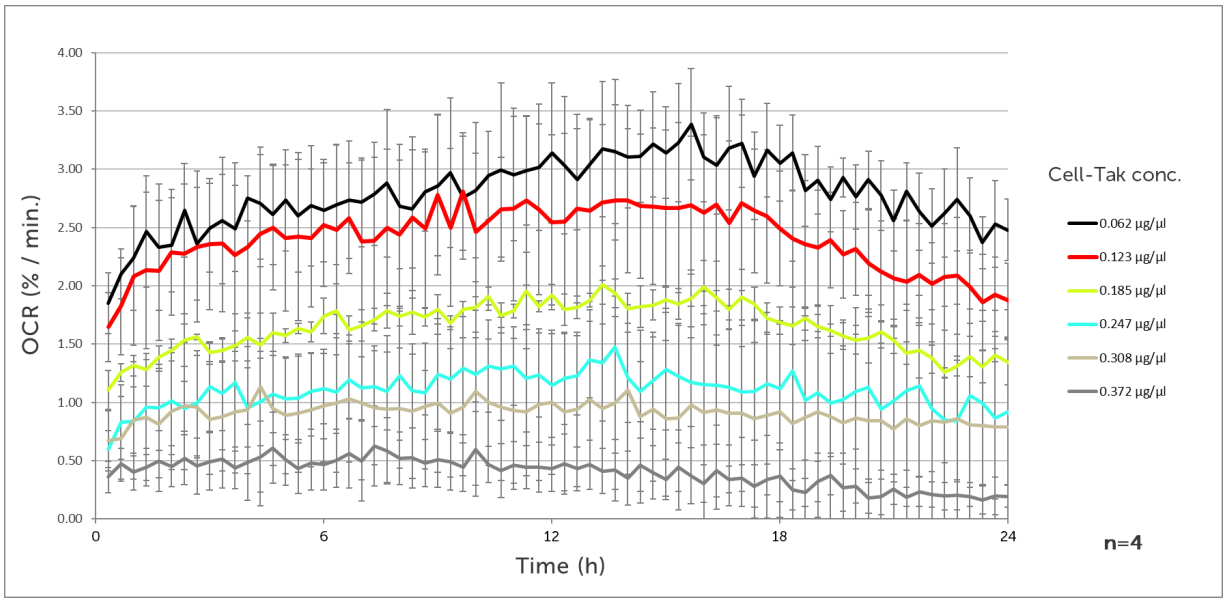
YAC-1 cells (a mouse lymphoma cell line) are maintained in DMEM high glucose supplemented with 10 % FCS (culture medium). On the day of the experiment, count the cells and dilute the cell suspension in a 15 ml sterile conical tube in culture medium to a concentration of 4 x 10^6 cells/ml. Incubate the cell suspension in a standard cell incubator (37 °C, 5 % CO_2, 95 % humidity) until further use.

Immediately before dispensing the cell suspension into the precoated sensor plate, centrifuge the cell suspension at 300 x g for 5 min, discard the medium supernatant and resuspend the cell pallet in the necessary amount of warm measuring medium supplemented with 10 % FCS to recreate a concentration of 4 x 10^6 cells/ml. Carefully add 150 µl cell suspension containing 600,000 cells into each well of the precoated sensor plate. Cover the sensor plate with a standard multiwell plate lid and leave it in the sterile workbench for 20 minutes to allow the cells to distribute evenly in the wells. Transfer the sensor plate into a standard cell incubator (37 °C, 5 % CO_2, 95 % humidity) and incubate for 60 min to allow the cells to attach properly. After incubation aspirate the medium via the side channels of the sensor plate using a glass pipette connected to a vacuum pump and distribute 500 µl fresh measuring medium supplemented with 10 % FCS between the two side chambers of the culture wells 1-24. Make sure that the medium levels in each of the three chambers are the same. Cover the plate with the special fluidic lid and insert the sensor plate into the platform. Start a standard measuring cycle over 24 hours with 100 µl medium exchange and 1 image per well every 20 minutes. The experiment is performed and monitored automatically, and the acquired data is displayed in real time.

RESULTS AND DISCUSSION

Oxygen consumption rates

Raw measuring of OCR data can be exported directly to spreadsheet applications. This makes it possible to analyze the data according to one's own preferences. In this example, we evaluated the oxygen consumption rate of YAC-1 cells immobilized to our sensor plate by precoating of the plate with different Cell-Tak concentrations. We formed groups according to the different precoatings and calculated the mean value and standard deviation of the oxygen consumption rate for each group plotted against time. The course of the OCR gives a good impression of the consistent adhesion and good constitution of the cells during the experiment.



OCR of YAC-1 cells immobilized to our sensor plate by precoating with six different Cell-Tak concentrations to determine the optimal amount of Cell-Tak to achieve the best immobilization result.

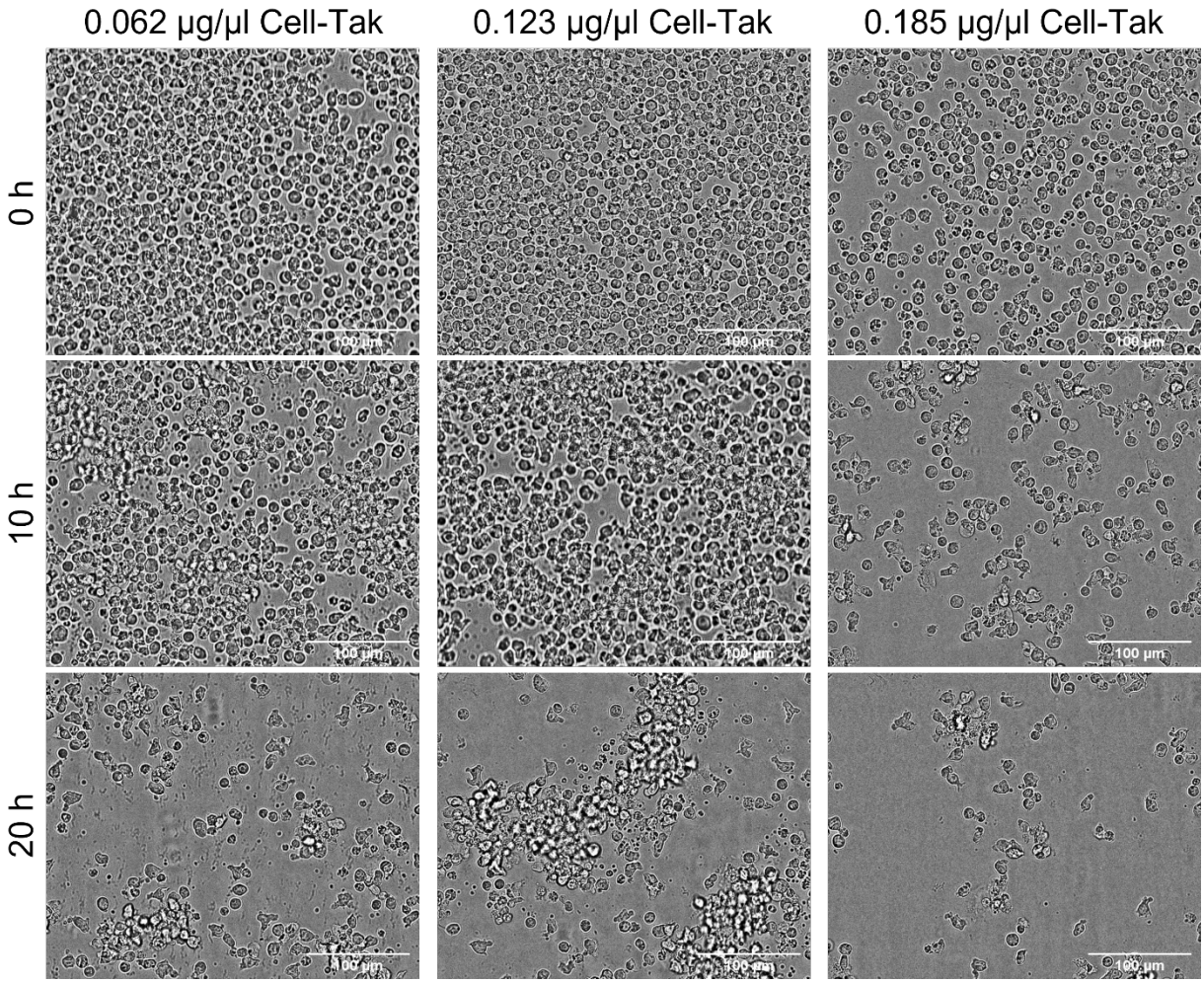
Based on OCR rates of YAC-1 cells immobilized to our sensor plate by precoating of the plate with different Cell-Tak concentrations, it can be observed that YAC-1 cells are effectively immobilized to the surface of the sensor plate by a precoating of the plate with 0.062 µg/µl or 0.123 µg/µl Cell-Tak. Already at the beginning of the experiment, the OCR rates of these two groups are higher compared to the other four groups, indicating that in these two groups the cells were effectively immobilized to the surface of the sensor plate. Moreover, the OCR rates of YAC-1 cells immobilized to the sensor plate by precoating with 0.062 µg/µl or 0.123 µg/µl Cell-Tak, increase until about 14 hours, implying that the cells adapt and proliferate during this time and consume more oxygen. After about 14 hours, the OCR rates decrease slowly until the end of the experiment at 24 hours. This suggests that the cells are detaching from the surface of the sensor plate and washed out. This is in line with the evaluation of the imaging data.

A precoating with higher Cell-Tak concentrations (> 0.123 µg/µl) seem to be less effective in this setting, as already at the beginning of the experiment the OCR rates of YAC-1 cells immobilized by a

precoating with Cell-Tak concentrations ranking from 0.185-0.372 µg/µl are lower compared to the groups of precoating with 0.062 µg/µl or 0.123 µg/µl Cell-Tak. This suggests that the adhesion capacity of precoatings with higher Cell-Tak concentrations (> 0.123 µg/µl) is reduced, resulting in a lower immobilization of the YAC-1 cells to the surface of the sensor plate. It might be possible that the adhesion capacity of the precoating with higher Cell-Tak concentrations is reduced by cross-linking between amino acids of polyphenolic proteins.

Imaging

Microscope images are taken periodically of all wells. This parallel imaging makes it possible to create time series and complements the metabolic data. In this example, the microscopic images of YAC-1 cells after immobilization with different Cell-Tak concentrations at different time points are shown. In line with the OCR data, it can be seen in the imaging that the cells are immobilized more efficiently on the surface of the sensor plate by a precoating with lower Cell-Tak concentrations.



Microscopic images of YAC-1 cells immobilized by a precoating with different Cell-Tak concentrations (0.062 µg/µl, 0.123 µg/µl, 0.183 µg/µl) at the beginning of the measurement (0h) as well as 10 and 20 hours after the beginning (10 h, 20 h).

CONCLUSION

This application note shows a preliminary dose-response experiment to determine the minimum effective density of Cell-Tak for optimal immobilization of suspension cells like YAC-1 cells to our sensor plate. It could be shown that a precoating with lower Cell-Tak concentrations (0.062 µg/µl and 0.123 µg/µl) was more effective and that with this setting the time slot for effective measuring of YAC-1 is up to 18 hours. Depending on the cell line and cell density, we recommend defining the optimal Cell-Tak concentration for your application.

REFERENCES

Waite J.H. and Tanzer M.L. (1981). Polyphenolic substance of Mytilus edulis: novel adhesive containing L-Dopa and hydroxyproline. Science, 212(4498), 1038-1040.

Waite J.H. (1983). Evidence for a repeating 3,4-dihydroxyphenylalanine- and hydroxyproline-containing decapeptide in the adhesive protein of the mussel, Mytilus edulis L. The Journal of biological chemistry, 258(5), 2911–2915.

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