CelCradle-500AP Technical Report III

Cultivation of VERO cells in high and low glucose culture medium

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1 Description

CelCradle-500AP provides an alternative choice from regular CelCradle-500 bottles specially designed to enhance cell immobilization, virus infection, transit transfection or cell harvest efficiency, or to harvest carriers directly. In this study, the culture of VERO in a high glucose (3 g/L) and a low glucose (1 g/L) culture medium in CelCradle-500AP bottles were illustrated. The result shows that the cell growth in culture medium with lower glucose slow down after 4 days. It indicates that the depletion of glucose does affect cell growth. However, cell remained still without discontinuing the growth. Therefore, Vero cell culture does not rely heavily on the supply of glucose. However, it is suggested to keep the initial glucose concentration to 3 g/L in order to provide a better energy source to support normal Vero cell growth.

2 Material

Device	Cell Line/Product	Medium	Seed
CelCradle-500AP	VERO ATCC CCL-81	2.7 L M199/10%FCS with 1 g/L glucose	1.0 x 10^8 cells/bottle
CelCradle-500AP	VERO ATCC CCL-81	2.7 L M199/10%FCS with 3 g/L glucose	1.0 x 10^8 cells/bottle

3 Protocol

3.1 Inoculum Preparation

Prepare 2 roller bottles with sub-confluence cells. Harvest cells by standard trypsinization protocol. Prepare 2.0 x 10^8 cells and separate cells into 4 50ml centrifuge tube containing 50ml culture medium each.

3.2 Inoculation

Prepare 2 L glass vessels for 2 and aseptically fill in 2.2 L culture medium containing 1 g/L and 3 g/L glucose. Assemble the reservoir vessels with tubing complete sets. Pre-warm 500 ml M199/5%FBS/1 g/L glucose and 500 ml M199/5%FBS/3 g/L glucose culture medium in a 37°C water bath. Bring two CelCradle-500AP bottles and place them in a biosafety cabinet. Connect the bottles with the 2 L reservoir vessels aseptically. Open the cap and add 400 ml culture medium in each bottle containing different concentration of glucose. Compress the bellows to wet the matrices with culture medium. Dispense 100 ml prepared inoculums on top of the matrix box in each CelCradle-500AP bottle and take them to CelCradle Stage immediately. Fix the bottles on CelCradle Stage controller in CO_2 incubator with 37°C and 5% CO_2 and start the compression immediately.

3.3 Immobilization

Set up immobilization parameters on the CelCradle Stage control box and start the controller by pressing “START” button. The inoculation parameters are set as below:

Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
2.0 mm/s	20 sec	2.0 mm/s	0 sec

3.4 Culture

After 3-4 hours, reset the parameters to culture parameters as below:

Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
1.5 mm/s	10 sec	1.5 mm/s	30 sec

Start recirculation by the second day (~24 hours) with pump rate 1 L/day, 24 cycle/day. Increase the pump rate to 1999 ml/day, 24 cycle/day by day 4.

The setup parameters are for reference only. These are not necessarily the optimum parameter conditions.

4 Result

Glucose Profile

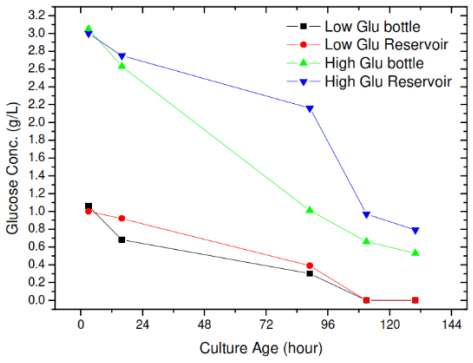

Figure. 1 The figure shows that the glucose was depleted by day 4 in the low glucose one both in the reservoir and the CelCradle bottle, while the high glucose one still got sufficient glucose by the end of culture.

pH profile

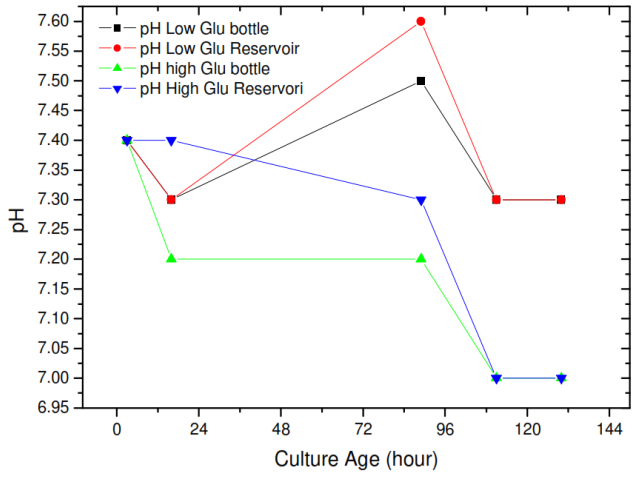

Fig. 2 The pH profile shows that the culture medium containing 3 g/L glucose will reduce pH to 7.0 within 6 days culture, while 1 g/L glucose one could keep pH at stable range between 7.3~7.5. Please note that the CO_2 is still kept at 5% in the incubator.

Cell Growth Profile

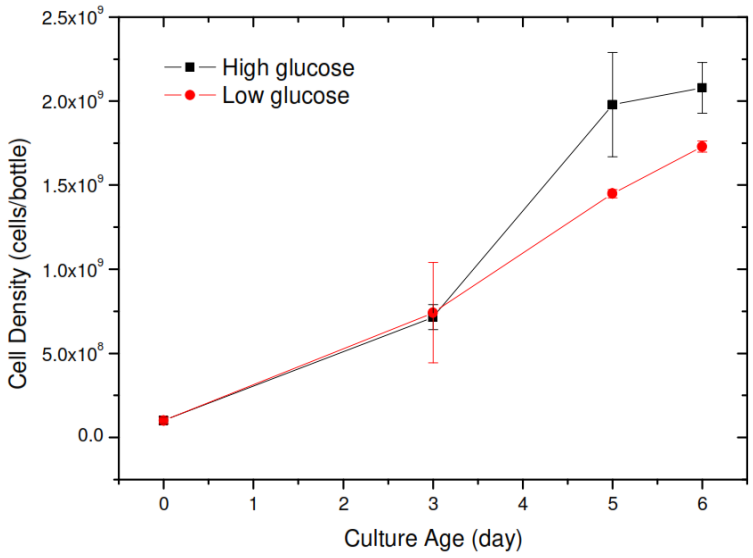

Fig.3 Both cell growth rates were the same in the first 3 days. However, the cell growth in culture medium with lower glucose slowed down after 4 days means the depletion of glucose does affect cell growth. Despite of this, cell remained still without discontinuing the growth. Therefore, Vero cell culture doesn't rely heavily on the supply of glucose.

The result shows that the low glucose one slow down after 4 days means the depletion of glucose does affect cell growth. pH in low glucose one (1 g/L) kept stable during whole culture period, while in the high glucose one (3 g/L) reduced to 7.0. The pH can be further controlled by reducing the CO2 concentration in the CO2 incubator. Therefore, we suggest to keep the initial glucose concentration to

3 g/L in order to provide a better energy source to support normal Vero cell growth and adjust down the CO2 concentration in order to keep the pH in the normal range.

5 VacciXcell Technical Support

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