CelCradle-500 Technical Report XVI

Cultivation of hybridoma OKT 3 cells for MAb production

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1 Description

CelCradle-500 provides a powerful cell culture tool to achieve high cell density and high productivity of target bioproducts because of its unique feature of offering high oxygen transfer and low shear stress culture environment. Users can easily collect highly concentrated cells, virus or secreted products from CelCradle systems. In this study, the application of CelCradle-500 and CelCradle-500P for the growth of suspended hybridoma OKT 3 cells (ATCC® Number: CRL-8001) and production of human IgG with different operation setting is elucidated. OKT 3 cells (1×108) were seeded in each CelCradle-500 unit. For CelCradle-500 and CelCradle-500P, a final cell number of 3.64×10 and 8.96×109 in one CelCradle unit were obtained after 12 and 10 days cultivation respectively. In addition, total yield is higher in CelCradle-500P but similar antibody concentrations were obtained in both CelCradle-500 and CelCradle-500P system. Thus, the total antibody quantity is depended on the volume of the culture medium that cell utilized. This study shows CelCradle-500 and CelCradle-500P can be applied to hybridoma OKT 3 cultivation for antibody production. However, the optimum condition for cultivation of each hybridoma cell line may require users to setup.

2 Material

Device	Cell Line/Product	Medium	Seed
CelCradle-500 CelCradle-500P Spinner Flask (500ml working volume)	OKT 3/mIgG	IMDM/10%FCS IMDM: (Gibco, Cat.: 12200-036) FCS (Biological industries, Cat.: 01-001-1A)	1 x 10^8 cells/bottle

3 Protocol

3.1 Inoculum Preparation

Inoculate OKT-3 cells in 9 x T150 flasks with 3.0 x 10^6 cells in 30 ml IMDM/10%FCS pre-warmed culture medium each. Incubate in 37°C, 5% CO_2 incubator for 3 days. Collect 3.0 x 10^8 OKT 3 cells by centrifugation and re-suspend cells with 3x30 ml fresh medium in 3x50 ml tubes. These cells were ready for CelCradle-500, CelCradle-500P and spinner flask inoculation. (Note: Cell viability should be above 90%)

3.2 Preparation before cell seeding

Place two CelCradle Stage controllers in two 37℃ incubators. Set up the inoculation parameters (See below). Warm up culture media in 37℃ water bath. Take out two CelCradle-500 bottles aseptically and place it in a biosafety cabinet. Open the cap and add each bottle with 470 ml fresh culture medium in the bottle. Place CelCradle Stage system in 37°C incubators, and set the parameter as described in

Table 1. Fill CelCradle-500 and CelCradle-500P bottles and spinner flask with 470 ml pre-warmed fresh medium.

3.3 Inoculation

Open the cap and distribute 30 ml media containing 1.0 x 10^8 suspended cells above the matrix box of CelCradle. Bring the bottle and lock up on the CelCradle Stage controller in incubator at 37°C immediately. Press "START" button to start the controller. The inoculation parameters are set as below:

Rising rate	Top Holding Time	Down Rate	Bottom Holding Time
2.0 mm/s	1 min	2.0 mm/s	0 sec

Inoculate with the same cell density in CelCradle bottles to spinner flask. Stir with 60 rpm.

3.4 Culture

Keep the same parameters as inoculation for culture for the first 5 days. During cultivation, the pH, residual glucose concentration was examined for modulating the culture condition and determining the following operation strategy. Below are the tips:

(A) For CelCradle-500

NaHCO3 (ICN) in culture medium is 3.7 g/L with initial pH=7.4. Medium exchange by vacuum aspiration only, and avoid vigorously disturbing the bottle during operation. Gradually adjust CO2 supply from 5% to 0% whenever pH reduces below 7.0.

(B) For CelCradle-500P

The NaHCO3 in culture medium in CelCradle-500P bottle is 3.7 g/L with initial pH=7.4. The NaHCO3 in culture medium in reservoir vessel is 4.0 g/L with initial pH=7.6. Connect the perfusion tubing set with CelCradle-500P before seeding cells. Gradually adjust CO2 supply from 5% to 0% during culture. Turn on perfusion pump on day 3. The initial pumping rate is 1 L/day. Monitor the pH and glucose concentration during cultivation. If the pH value or the glucose concentration in CelCradle-500P drops below 6.9 or 1.5 g/L, increase the pumping rate to 2 L/day.

4 Experimental Design

The parameter setting for spinner flask, CelCradle-500 (semi-batch), CelCradle-500P (re-circulation) is described as in Table 1.

Table 1. Parameter setting

Exp. Group	Strategy	Rising Rate (mm/s)	Down Rate (mm/s)	Top Holding Time (min)	Bottom Holding Time (min)
Spinner Flask (SP)	500 ml working volume, 60 rpm, exchange 1/4 culture medium daily from day 3				
CelCradle 500 (C)	Seeding	2.0	2.0	1.0 min	0 min
	Culture 1 (first 5 days)	2.0	2.0	1.0 min	0 min
	Culture 1 (after 5 days)	2.0	2.0	1.0 min	30 mins
CelCradle 500P (P)	Seeding	2.0	2.0	1.0 min	0 min
	Culture 1 (first 5 days)	2.0	2.0	1.0 min	0 min
	Culture 1 (after 5 days)	2.0	2.0	1.0 min	30 mins

Table 2 illustrates the experimental design of CelCradle culture system including different medium exchange (ME) strategy, cultivation duration, and total utilized medium volume during cultivation.

Table 2. Medium exchange strategy and culture days

System	ME Strategy	Day	Total Medium Volume (L)
Spinner Flask (SP)	ME from day (1/4 V) / day	12	1.625
CelCradle 500 (C)	3, 2, 2, 2, 2	11	2.5
CelCradle 500P (P)	5.5L	10	6

Medium exchange strategy of semi-batch system is different from perfusion system. For instance, the medium exchange of C-7 (3,2,2,2,2) means preceding medium exchange on day 3,5,7,9, and 11 respectively. Perfusion culture does not need medium exchange but continuous re-circulate culture medium between CelCradle-500P bottles and reservoir vessel but the recirculation rate should increase as cell density increased.

5 Result

Cell Growth

As shown in figure 1, total cell number in CelCradle-500P system was the highest among the other groups (approximately 8.96x109/ CelCradle-500P) after 10 days cultivation. CelCradle-500 system (about 3.64x109/ CelCradle-500), and spinner flask got similar cell density (3.09x109) after 12 days cultivation. However, if we look into figure 1(A), it was found that that CelCradle-500 and -500P shows similar average cell density. It indicates that sufficient supply of cultural medium in CelCradle-

500P can reach higher cell mass within similar culture days, but it does not alter the final cell density under medium volume basis.

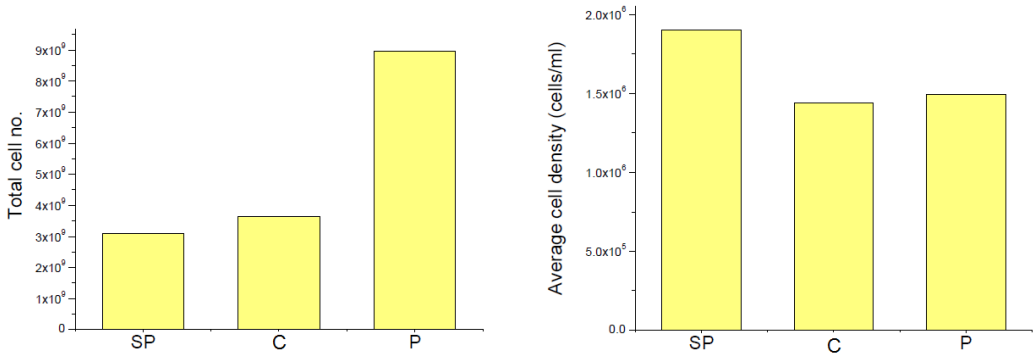

Figure 1. Cell Growth: (A) Total cells, (B) Cell density

MAb Production

Although different culture systems and running conditions were preceded, the result shows similar monoclonal antibody concentration in both CelCradle systems (figure 2, Table 3), while the MAb concentration in spinner flask is lower.

Table 3. MAb concentration in different culture systems

System	ME Strategy	Day	Ab (mg/L)
Spinner Flask (SP)	day	12	0.734
CelCradle 500 (C)	3, 2, 2, 2, 2	11	1.258
CelCradle 500P (P)	5.5L	10	1.053

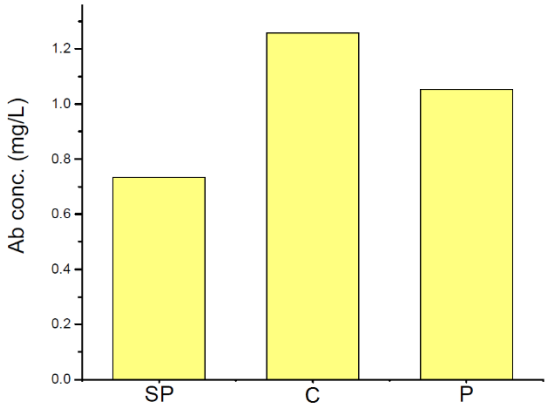

Figure 2. Antibody concentration in different culture systems

Figure 3 (A) and (B) shows total antibody yield and productivity per day of spinner flask (SP), CelCradle-500 (B), and CelCradle-500P (P) system respectively. The total yield of monoclonal antibody is P > C > SP (figure 3(A)). This suggested that the antibody production depends on the type of culture systems

and consumed culture medium (table 2). Although the cultivation time of these 3 systems is different from each other, the antibody productivity per day still keeps the same trend (P > C > SP).

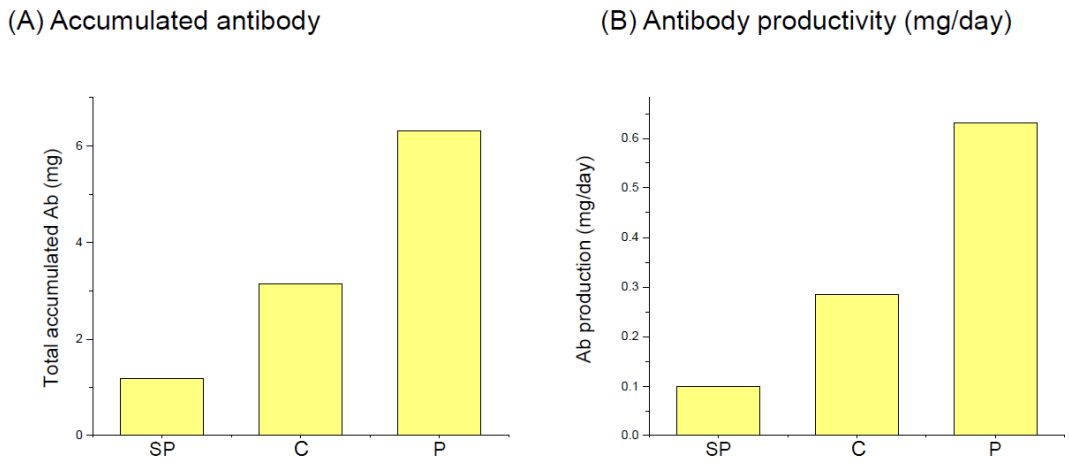

Figure 3. MAb production and productivity in different culture systems

Figure 4 shows antibody productivity per 10^6 cells. Compare with B and P system, although total accumulated antibody of P is higher than B (figure 3(A)), the antibody productivity/10^6 cells of P is slightly lower than B. Compare figure 4 with figure 1 (A): although total cell number in P is approximately 2.5 folds higher than B, the antibody productivity/10^6 cells of P is slightly lower than B.

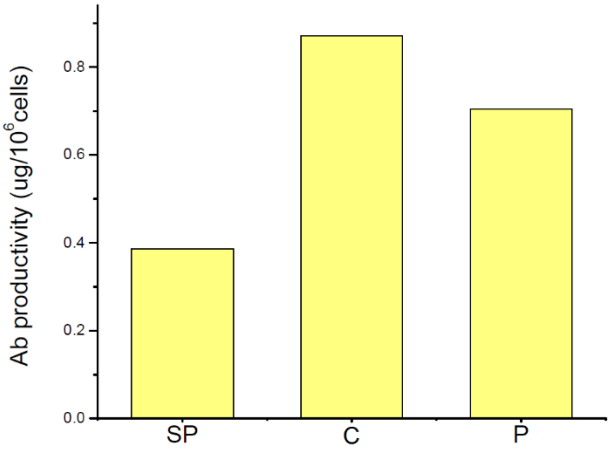

Figure 4. Antibody productivity per million cells

Figure 5 shows medium consumption rate (L/day) of OKT 3 cells. The medium consumption rate of these 3 systems also shows similar pattern (P > C > SP) to total accumulated antibody (figure 3(A)) and antibody productivity/day (figure 3(B)). Combine figure 3 and figure 5 for comparison, it revealed that more culture medium consumed, more cells and antibody was produced. It indicates that antibody production of OKT3 needs good nutrient supply.

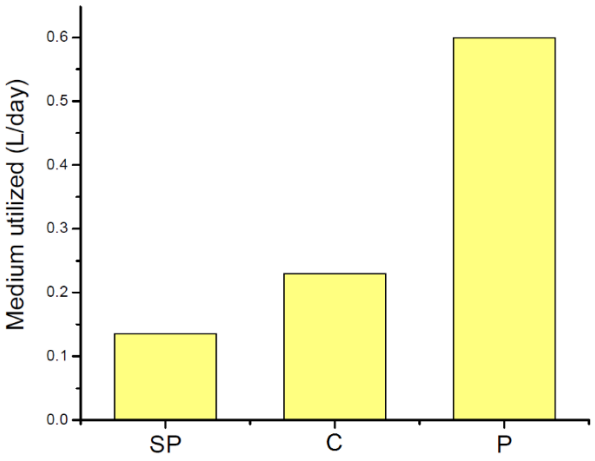

Figure 5. Medium Consumption Rate (L/day)

6 Summary

The result shows that hybridoma OKT 3 cell cultured both in CelCradle-500 and CelCradle-500P perform better cell growth and production than spinner flask. Near 9x109 cells could be cultivated in CelCradle-500P, compared with $3.7x10^9$ cells in CelCradle-500 bottle and $3.0 x10^9$ cells in spinner flask. This is due to continuous nutrient supply and less disturb in CelCradle-500P culture. Therefore, it is very important not to disturb the bottles during culture. Otherwise, cells will dislodge and be discarded during medium exchange. This is essential for all suspend cell lines' culture in CelCradle system. Therefore, for medium exchange inCelCradle-500, only vacuum aspiration is allowed; for CelCradle-500P, the bottle and tubing is set at the first day without disturbing the bottle until the end of the culture.

Note1: Do not move or disturb CelCradle-500P during cell culture.

Note2: Exchange culture medium by vacuum aspiration in CelCradle-500

6 VacciXcell Technical Support

For queries and comments, please contact the VacciXcell Technical Support team.

Email: mail@vaccixcell.com

Address: 21 Changi South Street 1, Singapore 486 777

Telephone: +65 6542 0833

Website: http://vaccixcell.com/