THE INDUSTRIAL SCALE PRODUCTION OF BHK_{21} CLONE 13 CELLS IN SUSPENSION IN FMD LABORATORY IN THAILAND

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ABSTRACT

The production of baby hamster kidney cells (BHK_{21} C 13) on an industrial scale was carried out on two sets of fermenter tanks. The first set was 3 of 300 liters chemoferm tank producing cells only, the second was 2,000 liters Marubishi tank producing cells and FMD virus. Cell start on both sets in each cycle was 0.4-0.5 $\times$ 10^6 cells/ml, after 48 hours incubation, the cell concentration was slightly different; an average was 2 $\times$ 10^6 cells/ml.

INTRODUCTION

In 1962, Macpherson and Stoker (1962) described the origin of the baby hamster kidney cell strain 21 (BHK_{21}). In the same year, the susceptibility of this strain to the virus of Foot and Mouth Disease (FMD) was established by Mowat and Chapman (1962). Furthermore, Capstick *et al.* (1962) proved that BHK_{21} Cloning 13 cells grown in suspension culture remain susceptible to FMD virus. At this stage, the production of FMD vaccine on a large scale by BHK_{21} C13 cell line was introduced to many FMD vaccine-producing laboratories.

The work of Telling and Ellsworth (1965) gave the opportunity to develop production of BHK_{21} C13 cells and FMD virus on an industrial scale. Their work led to development of bigger and better adapted fermentors.

The FMD Laboratory in Laboratory in Nhongsarai, Pakchong was started in 1978 under Thai-Japan Co-operation. The project is to produce FMD vaccine by suspension method. This report explains the results achieved on an industrial scale production of the BHK_{21} C13 cell line in suspension in 300 litres chemoferm and 2000 litres Marubishi fermentor.

MATERIALS AND METHODS

1. Baby Hamster Kidney Cells (BHK_{21} Clone 13)

The BHK_{21} Clone 13 cells were received from Dr. Jensen, State Veterinary Institute for Virus Research, Lindholm, and were maintained in the FMD Laboratory in Nhongsarai, Pakchong using Modified Eagles' medium supplement with 0.25 % Lactalbumin hydrolysate, 0.2 % Tryptose Phosphate Broth (TPB) and 0.1 % Peptone.

Stock cells are stored at -80°C in 10 % Dimethylsulfoxide (DMSO) growth medium. The cells are stored in 20 ml plastic cap bottles in a concentration of approximately 1×10^7 cells/ml. Viability of the cells is checked by growing a randomly selected bottle of cells six months after initial freezing and then one year. Cells are discarded after two-year storage.

2. Culture Medium

The medium used consisted of Eagle's basal medium containing the same concentration of amino-acids and vitamins as used by Telling and Radlett (1969) The medium supplement with 0.25 % Lactalbumin hydrolysate, 0.2 % TPB and 0.1 % Peptone. Antibiotics were added to a final concentration per ml 100 Mg of Neomycin (base), 100 Mg of Streptomycin (base). The growth medium contains the following constituents :

Growth Medium 10 L. (Eagle, 1955)

Earles' Balanced Salt Solution (BSS) × 10	1.00 litre
L-Glutamine	2.40 grams (g)
$NaHCO_3$	24.00 g.
Amino acids × 50	200.00 ml.
Vitamin × 250	40.00 ml.
Lactalbumin Hydrolysate	25.00 g.
T.P.B.	20.00 g.
Peptone	10.00 g.
Neomycin Sulfate	1.00 g.
Streptomycin Sulfate	1.00 g.
Bovine Serum	1.00 litre
Distilled water to make up	10.00 litres

Final pH : 7.3 - 7.4
Sterilized by filtration through Seitz EK and Pall catridge filter.

Earles' BSS × 10 (Earle *et al.* 1943)

$CaCl_2$ anhydrous	16.00 g.
NaCl	512.00 g.
KCl	32.00 g.
$MgSO_4$ $7H_2O$	16.00 g.
NaH_2PO_4 $2H_2O$	11.20 g.
Glucose	360.00 g.
Fe $(NO_3)_3$ $9H_2O$ (Sol 0.1 %)	5.00 ml.
Phenol red (Sol 0.4 %)	150.00 ml.
Distilled water to make up	10.00 litres

Filtration of Culture Medium (Growth Medium) :

Large quantities of medium with serum were filtered through a horizontal multi-pad Seitz filter. Twenty pads measuring 40 × 40 cm. were used to filter 650 litres of growth medium. The Seitz EK filter is used for prefilter as clarification. The Pall filter set composed of three catridges, the first is 0.45 - 1 Mm, the second and the third are 0.22 Mm in pore-size. All filters were sterilized by a current of steam at 121°C, 1.2 kg/cm^2 for at least 1 hour. Before filtration of any medium, the filter was prewashed. The filtered medium passed directly from the filter to the chemoferm and Marubishi fermentor tank.

3. Bovine Serum

Blood was collected from two to three years old cattle from the slaughterhouse in 10 litres aluminium cans, and allowed to clot in 4°C gauze. The resulting serum was centrifuged by Alfa Laval separator and then clarified through K-5 Seitz filter, serum were pooled together and precipated by 8 % Polyethylene glycol (PEG) 6000 (Barteling, S.J., 1975) and distributed in plastic containers of 10 litres and stored at - 20°C until used. Each batch was tested for toxicity and its effects on supporting BHK_{21} C 13 cells grown in suspension, before use in vaccine production.

4. Fermentor Tank

In production line, we have three sets of fermentor tank : (Figure 2)

1) 30 litres Marubishi tank
2) 100 to 300 litres chemoferm tank
3) 2000 litres Marubishi tank

All fermentors have been designed to conform to all requirements for cultivations of BHK cells. The fermentors made from stainless steel except the window part consists of glass. All fermentors connected to each other with stainless pipe controlling by valves. Atmospheric requirements of the cells are provided by surface aeration or deep sparging with the preferred gas. The rate of gas (O_2 or CO_2) can be monitored or controlled through flow measuring valves fitted on the tank. The temperature are controlled by automatic system. Cells are maintained in suspension by rotating impellers within the tank. Sterilization of piping and fermentor by current steam at 121°C for 30 minutes under pressure.

5. Cell Count and Viability

The cell number is determined by counting in a modified Rosenthal Chamber.

Cell viability is determined by using vital stain of 0.05 % Trypan Blue. The dead cells are stained blue, while the living ones remain unstained.

6. pH Control of the Culture

The pH is controlled by deep sparging O_2 or CO_2 from the control panel and determined by the external set pH meter.

7. Cell Growth

Figure 2 represents the procedure used for preparation of cell cultures in suspension. This is explained as follows :

1) Cells from the Revco -80°C deep freezer are rapidly thawed, centrifuged, washed and resuspended incomplete growth medium. Cell concentration is adjusted to 0.4 - 0.5 $\times$ 10^6 cells/ml.

2) The pyrex bottle containing cells is incubated at 37°C in water bath and stirred by magnetic stirror. Good cell growth is indicated by an increase of 100 % or more in cell number in a 24 hours period of incubation.

3) After 48 hours of incubation, the viable cell number should reach

2.0×10^6 cells/ml or more if exponential growth has been maintained.

4) At a concentration of 2.0×10^6 cells/ml or more, the cells are harvested by centrifugation and resuspended in growth medium. This cell suspension transfer into 30 litres Marubishi tank by adjusted cells start at concentration $0.4 - 0.5 \times 10^6$ cells/ml.

5) Ater 48 hours, the number of cells will reach 2.0×10^6 cells/ml or more. The cells are harvested by centrifugation and resuspended in growth medium. This cells suspension transfers into 100 litres chemoferm tank by adjusted cell start at concentration $0.4 - 0.5 \times 10^6$ cells/ml.

6) After 48 hours, the number of cells will reach 2.0×10^6 cells/ml or more. This cells suspension transfers into 1-300 litres chemoferm tank, the remained cells in 100 litres tank resuspended in growth medium to make the cell concentration $0.4\text{-}0.5 \times 10^6$ cells/ml and cooling down 4 - 5°C for new cycle.

7) In 1-300 litres chemoferm fermentor after 48 hours, the number of cells will reach 2.0×10^6 cells/ml or more, 1/3 of cells suspension transfers into 2-300 and 3-300 litres chemoferm tank, the remained cells in 1-300 litres tank resuspended in growth medium to make the cell concentration $0.4 - 0.5 \times 10^6$ cells/ml and cooling down 4-5°C for new cycle.

8) In 2-300 and 3-300 litres chemoferm fermentor after 48 hours, the number of cells will reach 2.0×10^6 cells/ml or more. Transfer 600 litres of cells from both fermetors into 2000 litres Marubishi fermentor tank, resuspended maintenance medium to be infected with the FMD virus.

9) Sterility check and viable cell count were determined every step.

RESULTS

Cell cultures have been obtained continuously in 2-300 and 3-300 litres capacity of chemoferm fermentor tank in 1979-1981 and in 2000 litres capacity of Marubishi fermentor tank in 1981-1985. The results of the development of each cycle of cell culture in a year was different. In Table 1, demonstrates the final cell concentration per each fermentor tank and the average of the cells in 48 hours incubation per each cycle. The final average cell concentration in the total culture cycles range between $1.80 - 2.60 \times 10^6$ cells/ml. Cells viability in all culture cycles range between 90 - 95 % as demonstrated by Trypan Blue.

Table 1. Development of cell cultures in Chemoferm and Marubishi fermentor.

Year	No.of Cycles	Average Cell Count $\times 10^6 \times$ ml.		Final Average Cell count.
		Chemoferm 2&3-300 L.	Marubishi. 2,000 L.	
1979	9	2.14-3.69	-	2.60
1980	9	2.15-3.03	-	2.46
1981	16	1.60-3.02	-	2.05
1982	14	-	1.70-3.40	2.22
1983	20	-	1.70-3.49	2.26
1984	36	-	1.50-3.50	2.49
1985	16	-	1.30-2.26	1.80

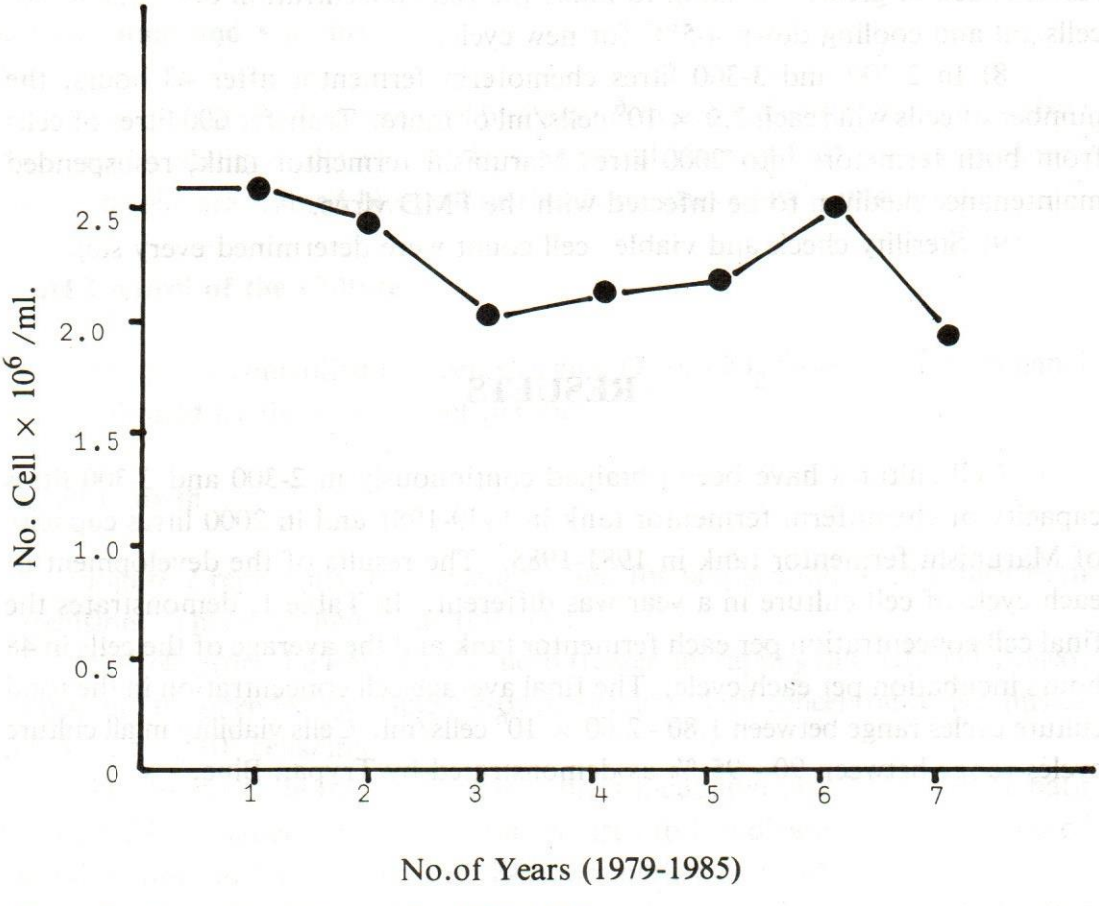

Figure 1. Average cell count in 1979-1985

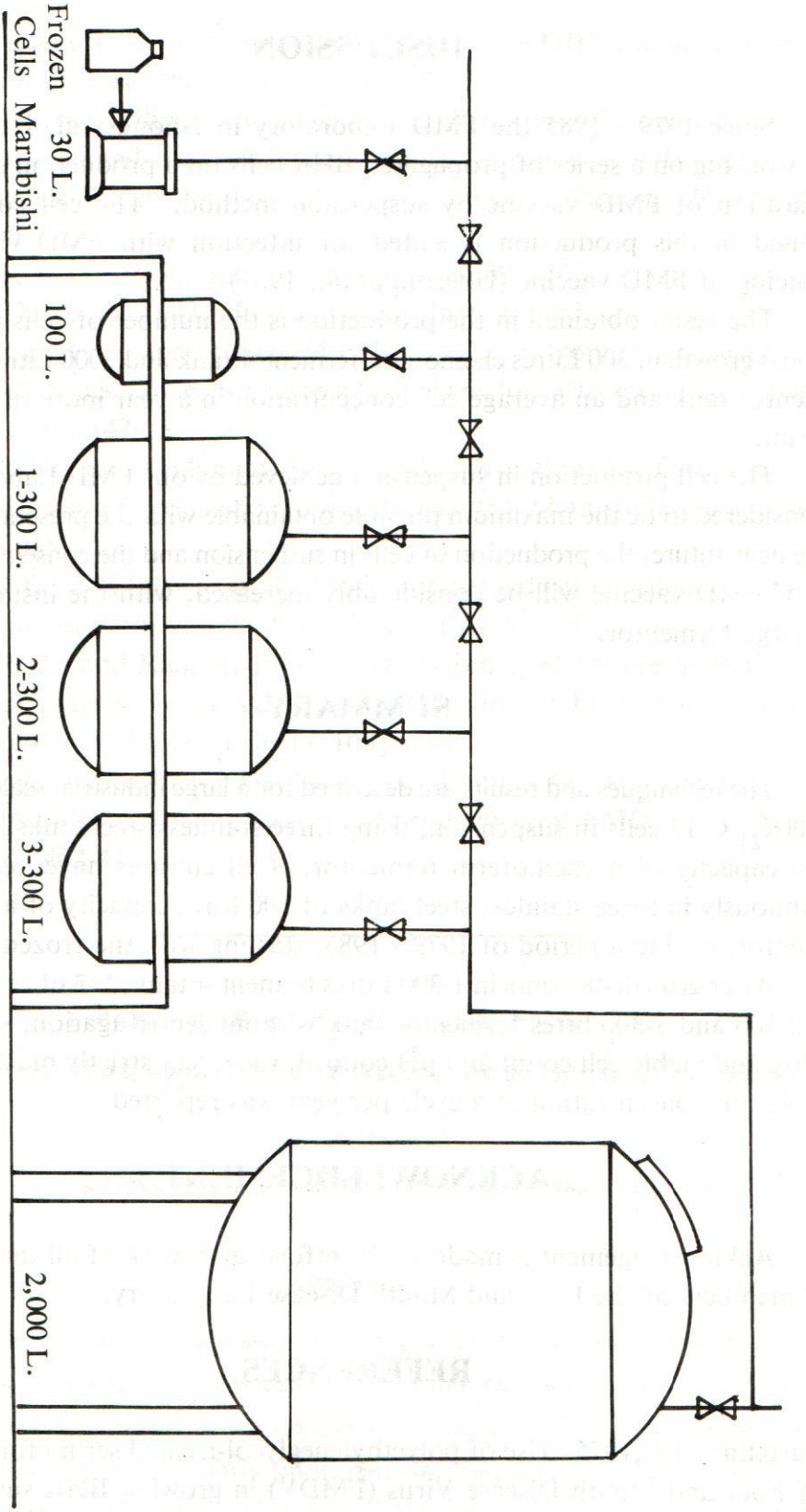

Figure 2. Diagramatic scheme of cell production in suspension using chemoferm and Marubishi fermentors.

DISCUSSION

Since 1979 - 1985 the FMD Laboratory in Nhongsarai, Pakchong has been working on a series of propagated BHK cells on a production scale for the preparation of FMD vaccine by suspension method. The cell concentration obtained in this production is suited for infection with FMD virus for the producing of FMD vaccine (Ubertini *et al.*, 1967)

The result obtained in the production is the number of cells per ml after 48 hours growth in 300 Litres chemoferm fermentor tank and 2000 Litres Marubishi fermentor tank and an average cell concentration in a year more than 2 million cells/ml.

The cell production in suspension achieved by our FMD Laboratory may be considered to be the maximum possible obtainable with the present equipment. In the near future, the production of cells in suspension and the consequent production of FMD vaccine will be considerably increased, with the installation of a new large fermentor.

SUMMARY

The techniques and results are described for a large industrial scale production of BHK_{21} C 13 cells in suspension, using three stainless steel tanks, each of 300 Litres capacity of a chemoferm fermentor. Cell cultures have been obtained continuously in three stainless steel tanks of 300 litres capacity of a chemoferm fermentor, during a period of 1979 - 1985, starting with the frozen cells.

After growth 48 hours in 1-300 Litres fermentor tank, 1/3 of cells transfered into 2-300 and 3-300 litres fermentor tank without centrifugation, sampling for sterility and viable cell count and pH control, were very strictly maintained. An average cell concentration in a cycle per year was reported.

ACKNOWLEDGEMENT

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การผลิตเซลล์ BHK_{21} C 13 แบบ Suspension ในระดับอุตสาหกรรมที่ศูนย์ผลิตวัคซีนโรคปากและเท้าเปื่อยในประเทศไทย

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บทคัดย่อ

การผลิตเซลล์ *baby hamster kidney cells (BHK_{21} C 13)* ในระดับอุตสาหกรรม ทำการผลิตในถังเพาะ *(fermentor)* 2 ชุด ชุดแรกเป็นถังเพาะขนาด 300 ลิตร ยี่ห้อ *Chemoferm* จำนวน 3 ถัง ใช้ในการเพาะเซลล์อย่างเดียว ชุดที่สองเป็นถังเพาะขนาด 2,000 ลิตร ยี่ห้อ *Marubishi* ใช้ในการเพาะเซลล์และผลิตไวรัสโรคปากและเท้าเปื่อย จำนวนเซลล์เริ่มต้นในถังทั้งสองชุดในแต่ละรอบเท่ากับ 0.4-0.5 $\times 10^6$ เซลล์/ซี.ซี. หลังเพาะ 48 ชั่วโมง ปริมาณความเข้มข้นของเซลล์แตกต่างกันเล็กน้อย โดยเฉลี่ยมีปริมาณเท่ากับ 2 $\times 10^6$ เซลล์/ซี.ซี.