Short communication

Guidelines for cultivation and preservation of the serum-free cell line McCoy–Plovdiv

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Abstract

In this paper, we offer detailed information concerning manipulations with the novel serum-free cell line McCoy–Plovdiv. Guidelines for procedures as trypsinization of the monolayer, subculturing, as well as freezing and thawing conditions are proposed. Our results give us grounds to assume that this is a cell line entirely serum-independent at any step of the process of culturing and preservation. The serum-free cell line McCoy–Plovdiv enriches the collection of mammalian serum-free cell lines. The easier cultivation, the lower expenses and the higher sensitivity in comparison with the serum-supplemented McCoy cells are discussed as possibilities for broad applications of McCoy–Plovdiv cells in different types of laboratory investigations.

Abbreviations: ATCC – American type culture collection; DMSO – Dimethyl Sulfoxide; HD – Ham's nutrient mixture F-12 Medium/DMEM (Dulbecco's Modified Eagle's Medium 1 : 1).

Introduction

McCoy–Plovdiv cell line was recently reported as a new serum-free strain produced from the McCoy cell line (Draganov et al. 2000).

The McCoy cell line was derived in 1955 from cells of synovial fluid obtained from the knee joint of a patient with degenerative arthritis (Pomerat et al. 1957). The cells are scattered in labs all over the world as human synovial cells. In 1960, Defendi et al. examined the McCoy cells taken from two laboratories and identified them as McCoy A subline (human cells) and McCoy B subline (mouse cells). In fact, the cells that are distributed nowadays, are mouse fibroblasts (ATCC). In our laboratory, we confirmed the McCoy cell line as being a McCoy B subline-mouse cells (Draganov et al. 2002).

The McCoy cell line is employed in biological experiments examining adaptation of animal cell cultures to different media and influence of various factors on cell growth (Hassell et al. 1991; McDermott and Butler 1993; Harper et al. 2000), apoptosis (Perotti et al. 1990) and *in vitro* cytotoxicity studies of diverse substances and extracts (Shrivastava et al. 1992; Devienne et al. 2002).

McCoy cells are widely applied in medical research including culturing of microorganisms like chlamydia (Black 1997), viruses (Nogueira 1998), trichomonads (Garber et al. 1987), *Helicobacter pylori* (Hua-Xiang et al. 1999), etc.

The McCoy cell line was obtained in our laboratory in 1997 from the National Bank of Industrial Microorganisms and Cell Cultures (NBIMCC) – Sofia, Bulgaria, originally transported from the

Institute of Microbiology in Johannesburg, South Africa in 1990. In cell line documentation there were no deposited details regarding the number of passages. McCoy cells were cultivated in EMEM medium + 10% FCS and supplements according to Kluytmans et al. (1993).

Material and methods

The McCoy cell line has been purposefully adapted, stabilized and cultured in completely serum-free and protein-free medium since the second passage. The optimal medium is a mixture of Ham's F-12/DMEM 1:1, further on called HD-medium (Draganov et al. 2000). Both original media have strictly defined composition according to the Producer company: from SERVA (Ham's F-12, 47501 and DMEM, 47304) and from SIGMA (Nutrient mixture Ham's F-12, N-4388 and DMEM, D-1152), and are a HEPES modification (25 mM). Only sodium bicarbonate was added (1.2 g l^{-1} to Ham's F-12 and 3.7 g l^{-1} to DMEM). No other supplements were used when the serum-free cells from the strain McCoy-Plovdiv were grown in HD-medium in a Heraeus incubator (Germany) at 37°C , 5% CO_2 and high humidity. The same medium (HD) with addition of 7% DMSO could be used for cell culture preservation in liquid nitrogen, too.

The serum-free culture McCoy-Plovdiv could be plated in a broad scope of initial plating density: from $0.8 \times 10^4 \text{ cells cm}^{-2}$ to $8.0 \times 10^4 \text{ cells cm}^{-2}$. Five to seven days following plating the cells reach a confluent state and are ready for subculture. The following procedures are applied.

Trypsinization with centrifugation

1. Put 2.5% trypsin in the culture medium at final concentration of 0.005%.
2. Remove the medium as the cells grow round (after 30 s). Shake the flask gently two or three times to detach the cells.
3. Add fresh HD-medium and transfer the suspension into a sterile plastic centrifuge tube (Falcon 50 ml).
4. Spin the cell suspension for 10 min at 200 g.
5. Remove the supernatant and resuspend the pellet in fresh HD-medium.

Trypsinization without centrifugation

In case centrifugation is not performed after trypsinization, step 2 is followed by:

1. Place the tissue culture flask vertically. Add fresh HD-medium (5 ml for a 25 cm^2 flask) carefully dropping it on the wall opposite to the one to which the cells are attached. Gently place the flask horizontally for 10–15 s.
2. Gently put the flask upright without shaking. Remove the medium. Repeat carefully step 3.
3. Remove the medium after 15 s, shake the flask several times and add fresh HD-medium).

Freezing

1. Trypsinize the confluent cells. Collect the trypsinized suspension in a centrifuge tube.
2. Spin for 5–7 min at 200 g. Resuspend the pellet in a cold (4°C) freezing medium (HD 1:1 + 7% DMSO).
3. Distribute the cell suspension at a concentration of $2\text{--}5 \times 10^6 \text{ cells ml}^{-1}$ in cryotubes (precooled in ice-cold water bath). Put the tubes in a penoplast box in a freezer (Ultra Low, Model 192, Sanyo) at -70°C for at least 48 h. Transfer the cells into a cryocontainer with liquid nitrogen at -196°C .

Thawing

1. Draw the tubes out from the liquid nitrogen and immediately put them into a hot water bath at $37\text{--}45^\circ\text{C}$ till the entire thawing of the medium.
2. Transfer the cells carefully into a preheated (37°C) culture medium and place them in a CO_2 incubator.
3. Discard half of the medium one or two hours later and replace with a fresh medium aliquot.

Results and discussion

The procedures, proposed for the serum-free McCoy-Plovdiv cells, are a result of hundreds of manipulations for their maintenance, preservation and application in various experiments. During the

last five years following the establishment of the serum-free culture, the cells have been permanently subcultured for more than 200 times.

The kinetics of cell proliferation varies between serum-supplemented McCoy cells and serum-free McCoy–Plovdiv (Draganov et al. 2001). McCoy–Plovdiv cells easily adhere and spread on the substrate but the lack of exogenous stimulators in the HD-medium prolongs their doubling time to 28.3 ± 1.5 h for a period of 24 to 48 h after plating (in contrast to this McCoy cells' doubling time is 20.6 ± 2.2 h for the same period). There are also differences in the cell morphology between these cell lines – McCoy–Plovdiv cells' shape is more epitheloid-like in comparison to McCoy cells (Draganov and Murdjeva 2003). This is obvious even in the first minutes after seeding but is more pronounced when the culture is in a monolayer state. Changes in morphology and proliferation kinetics of McCoy–Plovdiv cells reflect their adaptive plasticity to the new, serum-free conditions of culturing in HD-medium.

Growth curves of McCoy cells in serum-containing medium and McCoy–Plovdiv cells in serum-free medium are shown in Figure 1. The monolayer confluent stage is slightly prolonged for McCoy–Plovdiv cells in comparison to the McCoy cells.

The time for monolayer formation and the number of population doublings depend on the initial plating density (Draganov and Murdjeva 2003). The higher initial plating density of McCoy–Plovdiv cells shortened the time for monolayer formation and vice versa. At initial plating density 0.8×10^4 – 3.2×10^4 cells cm^{-2} the culture reached its confluent stage 24–72 h after plating. When the initial plating density was 2.4×10^4 , 1.6×10^4 or 0.8×10^4 cells cm^{-2} the monolayer confluent stage was observed on the 96th, 120th and 144th h, respectively.

The plating efficiency and its effect on the number of population doublings are presented in Table 1.

The development of McCoy–Plovdiv cell culture reflects the great potential of serum-free cells to develop steadily and reproducibly from seeding till confluent monolayer formation. Other investigations on cell growth of other cell lines (BHK-21 and Vero) in serum-free medium showed that cells grew similarly to standard serum-containing media (Merten et al. 1994).

Figure 1. Kinetics of cell proliferation of McCoy and McCoy–Plovdiv cells. McCoy (●) and McCoy–Plovdiv (■) cells were plated with an initial density 1.04×10^4 cells cm^{-2} in 6-well plates and 2.5 ml/well of serum-containing and serum-free culture medium, respectively. Cell density was measured every 24 h in a hemocytometer using the Trypan blue exclusion test. Results are derived from three independent experiments with three repetitions each and are presented as mean values with standard deviation.

The McCoy–Plovdiv cell line is entirely serum-independent not only for the cell culturing process but also for the storage in liquid nitrogen. After having been stored in liquid nitrogen, cells can be successfully thawed, preserving a cell viability of 95.8% and can grow steadily in serum-free medium (Draganov et al. 2000). As it was shown by Merten et al. (1995) DMSO was enough as a cryoprotector to provide cell survival in a serum-free freezing medium.

The absence of serum in the HD-medium facilitates enormously the laboratory procedures referred to McCoy–Plovdiv cells. The advantage of manipulating without serum renders the cultural system more than 20 times cheaper compared to the traditional one (Draganov et al. 2000).

The McCoy–Plovdiv cells have been used for the following applications:

In vitro cytotoxic studies (Docheva et al. 1999) showed that the McCoy–Plovdiv cells were more sensitive to the tested substances than the McCoy cells.

Our parallel investigations on McCoy and McCoy–Plovdiv cells for detection of *Chlamydia trachomatis*

Table 1. Plating efficiency of McCoy–Plovdiv cell line.

Number of the sample	Total NPC	NPC		Total FPD	FPD		NPDs
		cells cm ⁻²	cells ml ⁻¹		cells cm ⁻²	cells ml ⁻¹	
1	20 × 10 ⁴	0.8 × 10 ⁴	2.5 × 10 ⁴	469 × 10 ⁴	18.76 × 10 ⁴	88.50 ± 6.56 × 10 ⁴	4.56
2	40 × 10 ⁴	1.6 × 10 ⁴	5 × 10 ⁴	574 × 10 ⁴	22.98 × 10 ⁴	108.40 ± 14.16 × 10 ⁴	3.85
3	60 × 10 ⁴	2.4 × 10 ⁴	7.5 × 10 ⁴	628 × 10 ⁴	25.12 × 10 ⁴	118.5 ± 9.16 × 10 ⁴	3.40
4	80 × 10 ⁴	3.2 × 10 ⁴	10 × 10 ⁴	615 × 10 ⁴	24.60 × 10 ⁴	116.0 ± 13.51 × 10 ⁴	2.95
5	100 × 10 ⁴	4.0 × 10 ⁴	12.5 × 10 ⁴	631 × 10 ⁴	25.24 × 10 ⁴	119.0 ± 6.08 × 10 ⁴	2.66
6	120 × 10 ⁴	4.8 × 10 ⁴	15 × 10 ⁴	623 × 10 ⁴	24.92 × 10 ⁴	117.5 ± 6.08 × 10 ⁴	2.38
7	200 × 10 ⁴	8.0 × 10 ⁴	25 × 10 ⁴	636 × 10 ⁴	25.44 × 10 ⁴	120.0 ± 10.07 × 10 ⁴	1.67

IPD – Initial plating density, FPD – final population density, NPDs – number of population doublings. McCoy–Plovdiv cells were plated with gradually increasing cell densities (from 0.8 to 8.0 × 10⁴ cells cm⁻²) and were grown for 168 h in 25 cm² cell culture flasks in 8 ml of culturing medium. Cell density was measured in a hemocytometer. NPD values for each culture are presented. Results are derived from three independent experiments with three repetitions each.

in genital samples from patients showed that the serum-free cells had the same sensitivity and specificity as the McCoy cells (Murdjeva and Draganov 2002).

Conclusion

It can be stated that the serum-free McCoy–Plovdiv is a stable cell line with unlimited abilities for *in vitro* culture in HD, serum- and protein-free medium, according to the proposed guidelines.

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