Empa
Materials Science and Technology

Culturing and differentiating THP-1 cells

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1 Introduction

The THP-1 cell line (ATCC: TIB-202) is a human monocytic cell line derived from the peripheral blood of a one year old human male with acute monocytic leukemia (Tsuchiya S. et al., 1980). The cells are phagocytic (for both latex beads and sensitized erythrocytes) and lack surface and cytoplasmic immunoglobulin (www.atcc.org). THP-1 cells can be differentiated into macrophage-like cells using e.g. phorbol 12-myristate 13-acetate (PMA) (for some examples see: Kurosaka et al. 1998; Takashiba et al. 1999; Song and Phelps 2000; Antonicelli et al. 2004; Tang et al. 2005; Park et al. 2007; Daigneault et al. 2010).

2 Principle of the Method

THP-1 cells are grown as single cell suspensions in T75 cell culture flasks under standard growth conditions (see 6 Procedure). Subculturing is done by replacement of medium and necessary when cell concentrations reach $8x10^5$ cells/ml. Cell concentrations should not exceed $1x10^6$ cells/ml.

3 Applicability and Limitations

After thawing THP-1 cells tend to grow in cell aggregates instead of single cells. Before starting any experiment assure that THP-1 cells grow in single cell suspension. This should be the case after four to five subculturing cycles after thawing. Furthermore a maximum of 20 passages (after thawing) must not be exceeded.

4 Related Documents

Table 1: Documents needed to proceed according to this SOP and additional NM-related interference control protocols.

Document ID	Document Title

5 Equipment and Reagents

5.1 Cell culture type

THP-1 cells are supplied by American Type Culture Collection (ATCC); ordering number: TIB-202.

5.2 Equipment

- Cell freezing container
- Centrifuge (for cell pelleting; able to run 15 ml as well as 50 ml tubes at 200 x g)
- Conical tubes (15 ml and 50 ml; polypropylene or polystyrene; e.g. from Falcon)
- Cryotubes
- Hemocytometer
- Laminar flow cabinet (biological hazard standard)
- Light microscope (for cell counting and cell observation)
- Microreaction tubes (1.5 ml; e.g. from Eppendorf)
- Pipettes (volumes of 10 µl up to 20 ml)
- T75 cell culture flasks
- Water bath

5.3 Reagents

For cell culturing and differentiation:

- Dimethyl sulfoxide (DMSO) *[CAS number: 67-68-5]*
- Fetal Calf Serum (FCS)
- L-glutamine
- Neomycin[1)]
- Penicillin[1)]
- Phorbol 12-myristate 13-acetate (PMA) *[CAS number: 16561-29-8]*
  Note: Carcinogenic! Handle with special care! Special waste removal (see chapter 8)
- Phosphate buffered saline (PBS)
- Roswell Park Memorial Institute medium (RPMI-1640)
- Streptomycin[1)]
- Trypan blue solution (0.4%) *[CAS number: 72-57-1]*

[1)] bought as a 100x concentrated mixture of Penicillin, Streptomycin and Neomycin (PSN) e.g. from Gibco.

For waste treatment:

- HCl (smoking) *[CAS number: 7647-01-0]*
  Note: Corrosive and Irritating! Handle with special care! (see chapter 8)
- NaOH *[CAS number: 1310-73-2]*
  Note: Corrosive! Handle with special care! (see chapter 8)

5.4 Reagent Preparation

5.4.1 Complete cell culture medium

Basic medium:

- RPMI-1640

supplemented with:

- 10% FCS
- 1x PSN, which results in final concentrations of:
  - 50 µg/ml Penicillin
  - 50 µg/ml Streptomycin
  - 100 µg/ml Neomycin
- 0.2 mM L-glutamine

5.4.2 Freezing medium

The stock solution of the freezing medium has to be freshly prepared and pre-cooled on ice for at least one hour before usage. It is prepared as a 2x concentrated stock and will finally be diluted with the cell suspension.

Stock:

- 60% complete cell culture medium
- 20% FCS
- 20% DMSO

Final concentration after mixing cell suspension (in complete cell culture medium) with freezing medium at a 1:1 ratio:

- 80% complete cell culture medium (containing cells)
- 10% FCS
- 10% DMSO

5.4.3 PMA stock solution

Prepare a 1 mM stock of PMA in DMSO. Therefore resuspend the 1 mg (standard packaging size) PMA powder in 1.62 ml DMSO. Aliquote and freeze at -20°C. Can be stored for years.

Note: Carcinogenic! Handle with special care! Special waste removal. (see chapter 8)

5.4.4 NaOH

Prepare a 5 M solution of NaOH for PMA waste treatment.

- Dissolve 200 g NaOH pellets in 1 l ddH_2O.

Note: Be careful, exothermic reaction, gets HOT. NaOH is corrosive, wear protective clothing (especially eye protection).

6 Procedure

6.1 General growth conditions

THP-1 cells are grown in T75 cell culture flasks in a total volume of 20 ml of complete cell culture medium. They are kept at 37°C, 5% CO_2 in humidified air in an incubator (hereafter referred to as "standard growth conditions").

6.2 Cell thawing

Remove cryovial from the liquid nitrogen storage. Thaw cells in a 37°C-water bath until only a small frozen piece is visible. Decontaminate by spraying the vial with 70% Ethanol and transfer cryovial to a sterile hood. All following steps are performed under sterile conditions.

- Transfer entire content of the cryovial into 20 ml pre-warmed (37°C) complete cell culture medium.
- Spin cells down for 5 min at 200 x g at room temperature (RT).
- Discard supernatant, resuspend cells in 20 ml pre-warmed complete cell culture medium and transfer cells into one T75 cell culture flask.
- Incubate at standard growth conditions overnight (ON).
- Check cell concentration the next day. Cells have to be subcultured (see 6.4 below) starting from $8x10^5$ cells/ml. Less concentrated cultures stay under standard growth conditions for additional 24 h.
- Culture cells as described below. Subculture until cells grow as single cell suspension without apparent aggregate formation (at least four subculturing cycles) before usage in any experiment.

Note: This cell line suffers from freezing and thawing more than other cell lines (e.g. A549 cells). This results in the following observations:

- Cells grow slowly and in aggregates during the first weeks of culturing. Therefore subculturing becomes necessary only once a week.
- Cell concentrations during the first (approximately four) weeks should not be lower than $1x10^5$ cells/ml.
- Cells can be used for experiments after the fourth subculturing cycle (this will take up to four weeks after thawing).

6.3 Cell freezing

To prepare a liquid nitrogen stock THP-1 cells are expanded until growing in single cell suspension (at least passage four after thawing). At least four T75 flasks are recommended to obtain enough cells for freezing. Grow THP-1 cells in these 4 T75 flasks until a concentration of approximately $1x10^6$ cells/ml is reached and harvest and freeze them as follows:

Prearrangements:

- Prepare freezing medium (see 5.4.2) and cool down on ice at least one hour before cell harvesting.
- Make sure to have a freezing container on hand.

Cell harvesting:

- Merge content of all 4 T75 flasks in one flask.
- Count cells as described in 6.5 “Cell counting” and calculate the number of cryovials needed considering that each vial has to contain $1x10^6$ cells.
- Label each cryovial with cell name, passage number, date and cell number.
- Adjust cell concentration to $2x10^6$ cells/ml in complete cell culture medium. If the initially calculated concentration is higher the suspension is diluted with an appropriate amount of complete cell culture medium. If the initially calculated concentration is lower (e.g. $5x10^5$ cells/ml) cells have to be centrifuged for 5 min at 200 x g at RT and resuspended in the correctly calculated volume of complete cell culture medium.
- Mix cell suspension ($2x10^6$ cells/ml) at a 1:1 ratio with pre-cooled freezing medium (e.g. 10 ml cell suspension + 10 ml freezing medium). This results in a final cell concentration of $1x10^6$ cells/ml.
- **Cells have to be kept at 0°C (e.g. on ice) from now on!**
- Mix well by gently inverting the tube. A homogenous distribution of DMSO as well as cells is important to assure proper freezing and cell viability after thawing.
- Distribute 1 ml of the cell suspension in freezing medium into each cryovial.
- Place cryovials in a freezing container and place this into a freezer (-70°C to -80°C) for 24 h. This leads to a freezing rate of approximately 1°C/min.
- After 24 h place the frozen cryovials into liquid nitrogen for long term storage.

6.4 Subculturing

Routinely THP-1 cells are cultured in T75 flasks in 20 ml complete cell culture medium. They are subcultured every three to four days (twice a week) at a concentration of $8x10^5$ to $1x10^6$ cells/ml. This situation is achieved by seeding $5x10^5$ to $1x10^6$ cells per flask (equaling $2.5x10^4$ to $1x10^5$ cells/ml, respectively).

- Pre-warm complete cell culture medium at 37°C.
- Count cells as described in 6.5 “Cell counting”.
- Take $5x10^5$ cells directly from the cell suspension (volume calculated as described in 6.5 “Cell counting”). Add up to 20 ml with complete cell culture medium and transfer into one T75 flask.

6.5 Cell counting

Note: Cell counting can be performed according to each laboratories regular practice. The Neubauer counting chamber is described in brief as an example below.

- Harvest cells as described above (6.4 “Subculturing”).

- Take 10 µl of the cell suspension and pipette underneath the coverslip of the hemocytometer (see Figure 1, sample introduction point).

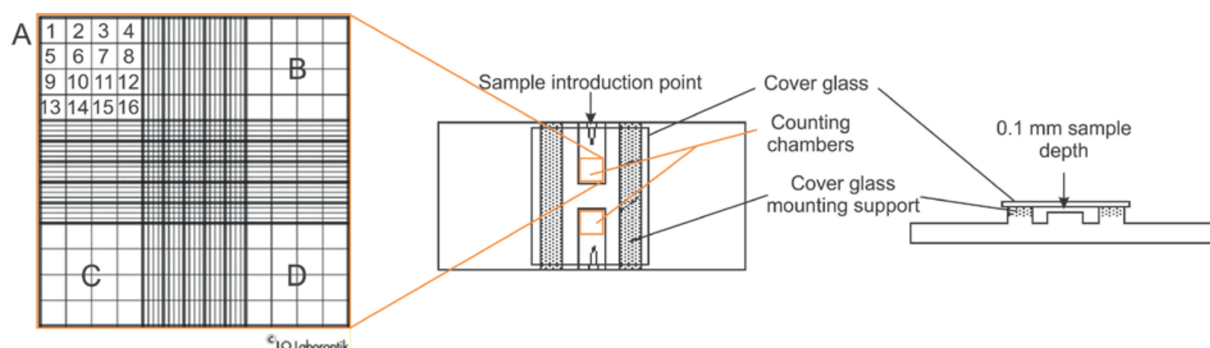

Figure 1: Schematic illustration of a hemocytometer.
Picture modified from: www.ruf.rice.edu/~bioslabs/methods/microscopy/cellcounting.html

- Count all cells in areas A, B, C and D (each area made up of 16 squares as detailed for area A in Figure 1).
- Calculate the average cell count per area [(A+B+C+D)/4].
- Calculate the number of cells per unit volume (cells/ml) using the following equation.

Number of cells/ml = average cell count x 10^4 x dilution factor[1]

- Resuspend the required number of cells (taken directly from the cell suspension) in the required volume of complete cell culture medium and seed into appropriate cell culture flasks (e.g. T75 flasks for subculturing, multi-well plates for certain experiments (see respective SOPs)).

6.6 Determination of cell viability by Trypan blue

Trypan blue is a diazo dye and cell impermeable. Viable cells are thus not stained by Trypan blue while dead cells (with disintegrated plasma membranes) appear blue in the light microscope.

To determine cell viability by Trypan blue, harvest cells as described in 6.4 “Subculturing” and proceed for cell counting as follows:

- Take 30 µl of the cell suspension and add 30 µl of 0.4% Trypan blue solution in a microreaction tube (dilution factor = 2). Mix well by pipetting 3-5 times with a 100 µl pipette.
- Take 10 µl of the cell suspension and pipette underneath the coverslip of the hemocytometer (see Figure 1, sample introduction point).
- Count the total number of cells in areas A, B, C and D (Figure 1) as well as the number of dead cells (blue) in the same areas.
- Calculate the cell viability [%] using the following equation.

Cell viability = (number of dead cells/total no of cells)*100

[1] Only necessary if you dilute your sample before counting.

- Calculate the number of viable cells per unit volume (cells/ml) using the equation in 6.5 “Cell counting”.

Note: Here you need to calculate a dilution factor of 2.

- Seed the required number of viable cells as described above.

6.7 Cell differentiation

To differentiate THP-1 cells into macrophage-like cells 200 nM phorbol 12-myristate 13-acetate (PMA) is used for a differentiation period of three days. During this time, cells attach to the bottom of the cell culture plates and develop macrophage-like morphology.

Note: PMA is carcinogenic and has to be handled with special care! Special waste removal is required (see chapter 8)!

- Dilute PMA in complete cell culture medium (1:5000 dilution from 1 mM stock).
- Count cells as described in 6.5 “Cell counting”.
- Harvest the desired amount of cells by centrifugation (5 min at 200 x g at RT).
- Resuspended the pellet at the desired concentration in PMA containing medium and seed into desired multi-well plates for experiment.
- Incubate under standard growth conditions in the presence of PMA for three days (72 h; e.g. cell seeding on Monday, differentiation till Thursday).
- Remove PMA containing medium on day three.

  Toxic! Discard this supernatant separately! See Chapter 8.

- Wash cells twice with pre-warmed PBS.
- Add complete cell culture medium (WITHOUT PMA) and start desired experiment.

7 Quality Control, Quality Assurance, Acceptance Criteria

THP-1 cells grow as single cells in suspension. Aggregate formation is possible during the first period after thawing but should no longer occur after four to five subculturing cycles. It is important to use only cultures that grow as single cells for experiments.

THP-1 cells show an approximate population doubling time of 26 h. This can be calculated from total cell counts during each subculturing procedure and monitored over time as a quality criterion. If this value changes or fluctuates over time cell viability can be checked as an additional quality control parameter. A healthy culture should contain at least 80% viable cells.

8 Health and Safety Warnings, Cautions and Waste Treatment

All procedures have to be carried out under sterile conditions in a laminar flow cabinet (biological hazard standard). Only sterile equipment must be used in cell handling. Operators should wear laboratory coat and gloves (according to laboratory internal standards). During handling of cryovials in liquid nitrogen, a full-face mask and appropriate gloves must be worn. Special care has to be taken during PMA handling (carcinogenic potential of the substance!).

PMA waste treatment: use a separate exhaust extraction system with a collecting flask containing already 20 ml 5 M NaOH to neutralize PMA. The resulting non-toxic solution is very alkaline and has to be neutralized using HCl before final disposal in the sink.

NaOH is **corrosive**. It causes severe burns. Wear especially eye/face protection. Dissolution of NaOH is an exothermic reaction, the solution will get fairly hot – be careful! It is strongly recommended to wear eye protection when handling 5 M NaOH.

HCl is **corrosive and irritant**. It is very hazardous in case of skin contact, of eye contact and of ingestion. It is slightly hazardous in case of inhalation. Therefore avoid inhalation as well as contact with skin and eyes and avoid exposure in general.

Discard all materials used to handle cells (including remaining cells themselves) according to the appropriate procedure for special biological waste (i.e. by autoclaving).

9 Abbreviations

ATCC	American Type Culture Collection
DMSO	dimethyl sulfoxide
FCS	fetal calf serum
g	constant of gravitation
ON	overnight
PBS	phosphate buffered saline
PMA	phorbo 12-myristate 13-acetate
RPMI	Roswell Park Memorial Institute medium
RT	room temperature

10 References

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