Immunology
Biol 465

Lab #3: Introduction to hematopoietic cell lines

In today's lab, we will practice the handling of human cells growing in culture. Much of what we know about the function of specific cells of the immune system (and virtually all eukaryotic cell biology) comes from studying cells/cellular activities *in vitro* (outside the organism). All the techniques used to maintain and manipulate cells in vitro are referred to as *tissue culture* or *cell culture*. Two major goals of growing cells in culture are to:

1) Provide the necessary nutrients and environment for cells to divide regularly, but NOT to change in major characteristics. (inhibit differentiation)
2) Maintain sterility (prevent bacterial and fungal contamination)

Cell lines are established from tissues by dissociating the cells from the tissue and providing them the proper conditions for their growth. Dissociated cells can divide for some period of time, but then cease to divide or *senesce*. True cell lines require some genetic change to become immortal (divide forever). This can occur spontaneously (cancer cells), or be induced with chemicals or cancer-causing animal viruses. *Hematopoietic* cell lines are established from blood cells, often from individuals with leukemia or lymphoma. In this case the cells have already undergone the genetic changes that make them grow indefinitely while in the person having leukemia or lymphoma.

Cell lines usually require specific nutrients formulated in a specific *media*. Other factors called *growth factors* are provided in serum. Fetal calf/bovine serum (FBS) is often used as a supplement to the specific medium providing growth factors, some of which are still undefined. Cell culture media often has an additive to monitor the pH. As cells become more crowded, waste accumulates, lowering the pH. pH changes can be seen as a change in color of the media with these additives.

Cell lines grow in one of 2 ways, either *attached* to a substrate (such as a plastic flask, or in *suspension* (floating around). Hematopoietic cells such as lymphocytes grow in suspension, whereas macrophages tend to attach to a substrate. Fibroblast cells (e.g. from skin) tend to grow as attached cells.

Today we will practice basic cell culture techniques using a cell line derived from a leukemia patient. The cell line is called *THP-1* and I use it in my research.

Today's goals:

1. Become familiar with *THP-1* cell morphology and in vitro growth characteristics.

2. Practice sterile techniques for passing cells to fresh media.

3. Determine cell concentration (# cells/ml culture media) and viability.

4. Set up new cultures for a growth experiment.

Materials

Flask of *THP-1* cells
Sterile transfer tube
Sterile pipettes/pipettor
Phosphate buffered saline PBS (to dilute cells for counting)
Trypan blue solution
Tubes for preparing/diluting cells
Hemacytometer
Clean flasks for new cultures
Cell culture media (20% and 1% FBS)

Procedure

1. Make observations of *THP-1* cells growing in plastic flasks. Dr. Super will have a flask of cells that were transferred to fresh media yesterday. Note shape/morphology and relative density of the cells, using the inverted microscope.

2. Dr. Super will have an additional flasks of cells. These cells are in need of transfer of to a new flask, with new media, at a lower cell concentration. Note differences between this flask and the *fresh* flask. What are the clues that these cells are not as "happy"?

3. **Begin passage of cells---** First you will transfer some cells from the overcrowded flask to a tube (for counting). Later you will set up 2 flasks of cells for monitoring over the next week. Cell transfer will take place in the laminar flow hood in room 216. Use all sterile plastic pipettes and take care not to touch any surfaces with the tips. Dr. Super will demonstrate and advise.
    a. Thoroughly sterilize all surfaces with the 70% ethanol.
    b. Prepare a counting tube with your name; loosen the cap.
    c. Loosen the cap on your flask of cells.
    d. Carefully, partially unwrap a 5 ml pipette. Insert the top into the pipettor, keeping the tip in its wrapper.
    e. Carefully pull off the wrapper, and transfer 2 ml of cells from the flask to the sterile tube. Cap the tub.
    f. Tighten the cap on the flask of cells and allow the next students to use the flask, if necessary. The last group will return the flask to the CO_2 incubator. Loosen the cap one quarter turn, keeping the cap connected by the threads.

4. **Take the counting tube to lab 318 to determine of cell concentration (# cells/ml) and viability. Each member of the team should take a sample to count. See how close your counts are---use an average as your final count?**
   Trypan Blue (TB) is an indicator of dead cells. Dead cells, with compromised membranes, take up trypan blue, whereas live cells exclude it.
    a. Start by diluting cells in the cell dye Trypan Blue. (100 µl TB + 100 µl cells).
    b. Charge the hemacytometer and count cells in 4 of the big ($0.1mM^3$) squares.
    c. If cells are too concentrated to count accurately, dilute with the PBS buffer and repeat the trypan blue staining. (again 100 µl TB + 100 µl diluted cells.)

(continued)

d. Keeping track of all dilution steps, determine:
   1. the concentration of cells in your flask (#cells/ml media)**You will need to recall the volume of the hemacytometer.
   2. the total number of cells you started with or that remain in your flask downstairs
   3. the viability (number of live cells/total).

5. **Set up 2 new flasks of cells.**
   a. In a clean flask seed cells at **0.1 X10^6 cells/ml** in 10 ml media. Use the media containing 20% FBS. (Mark the flask appropriately).

   b. In a second clean flask, seed cells at **0.1 X10^6 cells/ml** in 10 ml media. Use the media containing 1% FBS.

5. Starting Monday, one member of the groups will need to count the cells every 24 hrs. Keep track of your concentration, and graph the growth of the cells in each flask.

*We will compare data of the groups.

Tumor Cell Lines by Disease

Disease	Source	Species	Name	ATCC® No.
Interscapular tumor	possibly basal cell	bat, mouse-eared	Mvi/lt	CRL-6012*
Keratoacanthoma	skin	human	Hs 892.T	CRL-7630*
Keratoacanthoma, malignant acanthocytosis	skin	human	Hs 898.T	CRL-7641*
Leiomyoblastoma, renal	kidney	human	G-402	CRL-1440
Leiomyosarcoma	muscle	human	TE 149.T	CRL-7751*
Leiomyosarcoma	connective tissue	human	Hs 5.T	CRL-7822*
Leiomyosarcoma	smooth muscle, ductus deferens	hamster, Syrian golden	DDT_1 MF-2	CRL-1701
Leiomyosarcoma	uterus	human	SK-UT-1	HTB-114
Leiomyosarcoma	uterus, endometrium	human	SK-UT-1B	HTB-115
Leiomyosarcoma	vulva	human	SK-LMS-1	HTB-88
Leukemia	B lymphocyte	mouse	CW13.20-3B3 (clone of BCL 1)	CRL-1669
Leukemia	basophil, peripheral blood	rat	RBL-1	CRL-1378
Leukemia	basophil, peripheral blood	rat	RBL-2H3	CRL-2256
Leukemia	erythroblast, spleen	mouse	BB88	TIB-55
Leukemia	erythroblast, spleen	mouse	D1B	TIB-56
Leukemia	lymph node	bovine	2FLB.Ln	CRL-6045*
Leukemia	myelomonocyte, macrophage-like	mouse	WEHI-3	TIB-68
Leukemia	spleen	mouse	T27A	TIB-57
Leukemia	spleen	mouse	D2N	TIB-58
Leukemia	spleen	mouse	BC16A	TIB-59
Leukemia	spleen	mouse	BC3A	TIB-60
Leukemia, acute lymphoblastic	B lymphoblast	human	SUP-B15	CRL-1929
Leukemia, acute lymphoblastic	B lymphoblast, peripheral blood	human	CCRF-SB	CCL-120
Leukemia, acute lymphoblastic	B lymphoblast, peripheral blood	human	8E5	CRL-8993†
Leukemia, acute lymphoblastic	bone marrow, myeloblast	human	KG-1	CCL-246
Leukemia, acute lymphoblastic	bone marrow, myeloblast	human	KG-1	CRL-8031†
Leukemia, acute lymphoblastic	bone marrow, promyeloblast	human	KG-1a	CCL-246.1
Leukemia, acute lymphoblastic	T lymphoblast	human	TALL-104	CRL-11386†
Leukemia, acute lymphoblastic	T lymphoblast	human	MOLT-4	CRL-1582
Leukemia, acute lymphoblastic	T lymphoblast, peripheral blood	human	CCRF-CEM	CCL-119
Leukemia, acute lymphoblastic	T lymphoblast, peripheral blood	human	CCRF-HSB-2	CCL-120.1
Leukemia, acute lymphoblastic	T lymphoblast, peripheral blood	human	MOLT-3	CRL-1552
Leukemia, acute lymphoblastic	T lymphoblast, peripheral blood	human	CEM/C2	CRL-2264
Leukemia, acute lymphoblastic	T lymphoblast, peripheral blood	human	CEM/C1	CRL-2265
Leukemia, acute lymphoblastic t(16;20) translocation	T lymphocyte, peripheral blood	human	Loucy	CRL-2629
Leukemia, acute lymphoblastic, t(4;11) translocation	bone marrow	human	RS4;11	CRL-1873
Leukemia, acute lymphocytic (non-T, non-B)	unknown	human	Reh	CRL-8286†
Leukemia, acute monocytic	monocyte	human	THP-1	TIB-202
Leukemia, acute monocytic	monocyte, peripheral blood	human	AML-193	CRL-9589†
Leukemia, acute myeloblastic	peripheral blood	human	Kasumi-1	CRL-2724
Leukemia, acute myeloblastic	peripheral blood	human	Kasumi-3	CRL-2725
Leukemia, acute myelogenous	peripheral blood, B lymphoblast	human	BDCM	CRL-2740

* Part of the NBL collection; see page 12. † Patent item; see page 12.
See the ATCC online catalog for the complete description of a cell line.
These products are for laboratory research use only. Not intended for use in humans, animals or for diagnostics.

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Cell Biology

ATCC® Number:	**TIB-202™** Order this Item	**Price:**	**$244.00**
Designations:	THP-1	**Depositors:**	S Tsuchiya
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	suspension
Organism:	*Homo sapiens* (human)	**Morphology:**	monocyte

Source: **Organ:** peripheral blood
Disease: acute monocytic leukemia
Cell Type: monocyte;

Cellular Products: lysozyme [58053]

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Applications: transfection host(technology from amaxa
Roche FuGENE® Transfection Reagents)

Receptors: complement (C3), expressed [58053]
Fc, expressed

Antigen Expression: HLA A2, A9, B5, DRw1, DRw2 [58053]

DNA Profile (STR): Amelogenin: X,Y
CSF1PO: 11,13
D13S317: 13
D16S539: 11,12
D5S818: 11,12
D7S820: 10
THO1: 8,9.3
TPOX: 8,11
vWA: 16

Age: 1 year infant

Gender: male

Comments: The cells are phagocytic (for both latex beads and sensitized erythrocytes) and lack surface and cytoplasmic immunoglobulin. [58053]
Monocytic differentiation can be induced with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA). [22193]

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: 2-mercaptoethanol to a final concentration of 0.05 mM; fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C