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Guidelines for Maintaining Cultured Cells

This lecture provides guidelines and general procedures for routine sub culturing, Note that cell culture conditions

sub culturing

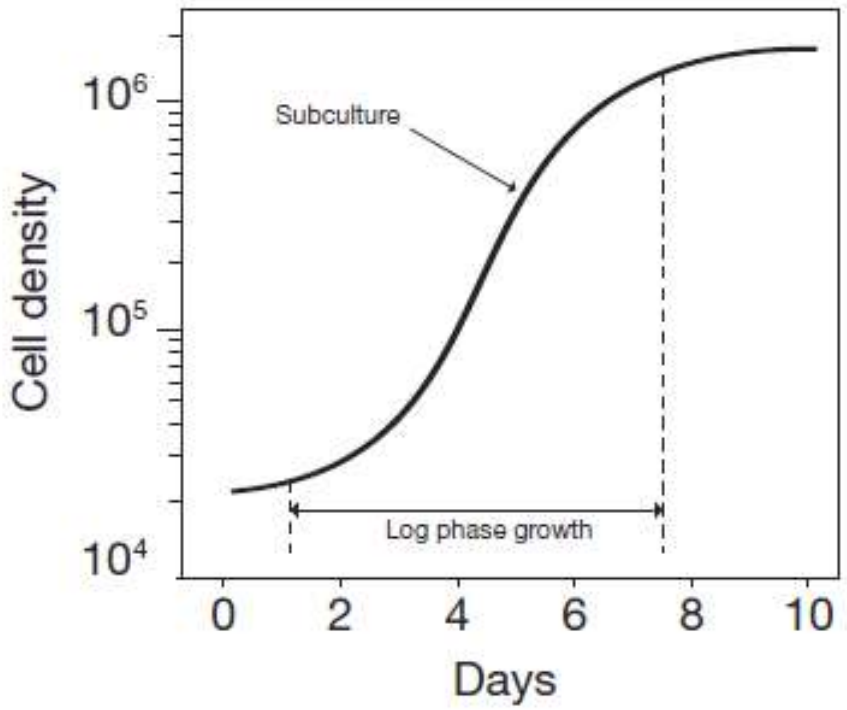
What is Subculture?
Subculturing, also referred to as passaging, is the removal of the medium and transfer of cells from a previous culture into fresh growth medium, a procedure that enables the further propagation of the cell line or cell strain.

The growth of cells in culture proceeds from the lag phase following seeding to the log phase, where the cells proliferate exponentially.

When the cells in adherent cultures occupy all the available substrate and have no room left for expansion, or when the cells in suspension cultures exceed the capacity of the medium to support further growth, cell proliferation is greatly reduced or ceases entirely (see Figure).

To keep them at an optimal density for continued growth and to stimulate further proliferation, the culture has to divided and fresh medium supplied.

When to Subculture?
The criteria for determining the need for subculture are similar in adherent and suspension cultures; however, there are some differences between mammalian and insect cell lines.



Cell Density

• Mammalian cells: Adherent cultures should be passaged when they are in the log phase, before they reach confluence. Normal cells stop growing when they reach confluence (contact inhibition), and it takes them longer to recover when reseeded. Transformed cells can continue proliferating even after they reach confluence, but they usually deteriorate after about two doublings. Similarly, cells in suspension should be passaged when they are in log-phase growth before they reach confluency. When they reach confluency, cells in suspension clump together and the medium appears turbid when the culture flask is swirled.

• Insect cells: Insect cells should be subcultured when they are in the log phase, before they reach confluency. While tightly adherent insect cells can be passaged at confluency, which allows for easier detachment from the culture vessel, insect cells that are repeatedly passaged at densities past confluency display decreased doubling times, decreased viabilities, and a decreased ability to attach. On the other hand, passaging insect cells in adherent culture before they reach confluency requires more mechanical force to dislodge them from the monolayer. When repeatedly subcultured before confluency, these cells also display decreased doubling times and decreased viabilities, and are considered unhealthy

Exhaustion of Medium

• Mammalian cells: A drop in the pH of the growth medium usually indicates a build up of lactic acid, which is a by-product of cellular metabolism. Lactic acid can be toxic to the cells, and the decreased pH can be sub-optimal for cell growth. The rate of change of pH is generally dependent on the cell concentration in that cultures at a high cell concentration exhaust medium faster than cells lower concentrations. You should subculture your cells if you observe a rapid drop in pH (> 0.1–0.2 pH units) with an increase in cell concentration.

• Insect cells: Insect cells are cultured in growth media that are usually more acidic that those used for mammalian cells. For example, TNM-FH and Grace's medium used for culturing Sf9 cells has a pH of 6.2. Unlike mammalian cell cultures, the pH rises gradually as the insect cells grow, but usually does not exceed pH 6.4. However, as with mammalian cells, the pH of the growth medium will start falling when insect cells reach higher densities.

Subculture Schedule

Passaging your cells according to a strict schedule ensures reproducible behavior and allows you to monitor their health status. Vary the seeding density of your cultures until you achieve consistent growth rate and yield appropriate for your cell type from a given seeding density. Deviations from the growth patterns thus established usually indicate that the culture is unhealthy (e.g., deterioration, contamination) or a component of your culture system is not functioning properly (e.g., temperature is not optimal, culture medium too old).

they strongly recommend that you keep a detailed cell culture log, listing the feeding and subculture schedules, types of media used, the dissociation procedure followed, split ratios, morphological observations, seeding concentrations, yields, and any anti-biotic use. It is best to perform experiments and other non-routine procedures (e.g., changing type of media) according to your subculture schedule. If your experimental schedule does not fit the routine subculture schedule, make sure that you do not passage your cells while they are still in the lag period or when they have reached confluency and ceased growing.

Media Recommendations

Many continuous mammalian cell lines can be maintained on a relatively simple medium such as MEM supplemented with serum, and a culture grown in MEM can probably be just as easily grown in DMEM or Medium 199. However, when a specialized function is expressed, a more complex medium may be required.

Information for selecting the appropriate medium for a given cell type is usually available in published literature, and may also be obtained from the source of the cells or cell banks. If there is no information available on the appropriate medium for your cell type, choose the growth medium and serum empirically or test several different media for best results.

In general, a good place to start is MEM for adherent cells and RPMI-1640 for suspension cells.
The conditions listed below can be used as a guide line when setting up a new mammalian cell culture. Insect cells are cultured in growth media that are usually more acidic that those used for mammalian cells such as TNM-FH and Grace's medium

Mammalian Cell Culture				
Cell Line	**Cell Type**	**Species**	**Tissue**	**Medium***
293	fibroblast	human	embryonic kidney	MEM and 10% FBS
3T6	fibroblast	mouse	embryo	DMEM, 10% FBS
A549	epithelial	human	lung carcinoma	F-12K, 10% FBS
A9	fibroblast	mouse	connective tissue	DMEM, 10% FBS
AtT-20	epithelial	mouse	pituitary tumor	F-10, 15% horse serum, and 2.5% FBS
BALB/3T3	fibroblast	mouse	embryo	DMEM, 10% FBS
BHK-21	fibroblast	hamster	kidney	GMEM, 10% FBS, or MEM, 10% FBS, and NEAA
BHL-100	epithelial	human	breast	McCoy'5A, 10% FBS
BT	fibroblast	bovine	turbinate cells	MEM, 10% FBS, and NEAA
Caco-2	epithelial	human	colon adeno carcinoma	MEM, 20% FBS, and NEAA
Chang	epithelial	human	liver	BME, 10% calf serum
CHO-K1	epithelial	hamster	ovary	F-12, 10% FBS
Clone 9	epithelial	rat	liver	F-12K, 10% FBS
Clone M-3	epithelial	mouse	melanoma	F-10, 15% horse serum, and 2.5% FBS
COS-1, COS-3, COS-7	fibroblast	monkey	kidney	DMEM, 10% FBS
CRFK	epithelial	cat	kidney	MEM, 10% FBS, and NEAA
CV-1	fibroblast	monkey	kidney	MEM, 10% FBS
D-17	epithelial	dog	osteosarcoma	MEM, 10% FBS, and NEAA
Daudi	lymphoblast	human	blood from a lymphoma patient	RPMI-1640, 10% FBS
GH1, GH3	epithelial	rat	pituitary tumor	F-10, 15% horse serum, and 2.5% FBS

* **BME**: Basal Medium Eagle; **DMEM**: Dulbecco's Modified Eagle Medium; **FBS**: Fetal Bovine Serum; **GMEM**: Glasgow Minimum Essential Medium; **IMDM**: Iscove's Modified Dulbecco's Medium; **MEM**: Minimum Essential Medium; **NEAA**: Non-Essential Amino Acids Solution.

Thank you