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Introduction

1.1
Introduction and Usage of This Book

To culture living cells in the laboratory and to keep them proliferating have become a revolutionary part in the Life Sciences. For more than 60 years now researchers are using permanent cell lines and in recent years the so-called primary cell lines. Within this time frame the number of these cell lines has increased tremendously since the first cell line (the mouse fibroblast cell L-929) has been established in 1943. When the first human cell line (HeLa) was introduced in 1952, a boom in the development of such cell lines started and continues until today.

During this development the increasing knowledge regarding the establishment of human and animal cell lines has influenced the culture of cell lines; however, the scientists suffered from various setbacks and problems which could not be reduced to cell's biology alone but rather to the cell culture practice. This started with the definition of the meaning of "cell line" which has not been defined as uniformly as it may be desirable for the biological scientific research.

Both cell lines mentioned above, L-929 and HeLa, have been cloned originally, it means these cell lines originate from one single cell. This basic principle of uniformity or clonality of cell lines has not been followed strictly within the last 50 years. Furthermore, the problem of cross-contamination, that is, the mixing of different cells with each other still poses a serious problem that is not overcome completely.

In the last couple of years a movement within the area of cell culture has established, which makes a point of a more stringent and careful maintenance of the cell lines regarding all the steps in cell culturing and the general handling of the cells. Strict rules of handling cell lines in particular were established (GCCP-Good Cell Culture Practice), and along with the application of these rules a reproducible and transparent work will be possible in the future.

This "Good Cell Culture Practice" should have been basic routine from the beginning, but 60 years ago cell culture work has not been as good resulting in mistakes not only during sterile handling of the cells. Also, the diagnostic instrumentation in the analysis of cells and cell lines in these early times of cell handling have not been present to be able to recognize any modification of a particular cell line on the molecular basis during cultivation such as a switch of the number of passages.

Atlas of Living Cell Cultures, First Edition. Toni Lindl and Rosemarie Steubing.

In the very beginning the analysis of vital cells was restricted to watching them in the microscope (without phase contrast at first); this represented the only possibility besides the analysis of the chromosomes. Still today, a relatively simple inverted microscope equipped with phase contrast and a digital camera is sufficient to visualize the viable cultures routinely. The distance between the light source and the object table should be large enough to be able to watch cells which are kept in large culture flasks such as roller bottles.

However, the microscope being equipped with the phase contrast is necessary to efficiently evaluate the morphology *in vitro*. A modern inverted microscope is fitted with an ocular tube and a second tube which is connected to a digital camera or a CCD camera together with a monitor.

Another useful tool for an inverted microscope is an object table with a coordinating device for exactly locating the cell colonies unambiguously. Special object clamps at the microscope table may facilitate working with the various culture flasks and petri dishes. Inverted microscopes equipped with a fluorescent device are available; however, it is recommended to purchase a conventional upright microscope with fluorescent device together with an inverted microscope to achieve maximum sensitivity and accuracy through the higher magnification and better light yield for maximal performance of the fluorescence technique.

The analysis of specific isozymes as diagnostic tools has been introduced for the first time in the 1960s and 1970s. Within the last decade the diagnostics of cells changed dramatically, at first DNA hybridization emerged to be followed by DNA-fingerprinting and today the DNA profiling in the characterization of cells has become almost routine testing.

1.2 General Remarks

All efforts to characterize human and animal cells and cell lines unequivocally rise and fall with the knowledge of the morphology of the cells. This oldest, most direct and simplest way to visualize and characterize the cells is based on the histology of the cells existing in the body of human beings and animals, how they arrange and appear.

It is important to distinguish between the situations "*in vivo*" and "*in vitro*", which is evident and manifold; therefore simple extrapolation of cell pictures from a histological textbook can be misleading. Thus, observing the vital morphology by phase-contrast microscopy in routine cell culture life is highly recommended.

The environment and the development of the cells *in vitro* are not the same as they are *in vivo*, and these specific characteristics *in vitro* regarding the cellular morphology have to be taken into account and have to be observed and followed up intensely.

Normal epithelial cells cultured "*ex vivo*" as primary cells "*in vitro*" have almost all characteristics of epithelial cells; however, most cell lines may loose defined properties (of molecular kind) if they are transformed or transfected for example, which they may express in a different morphology under the microscope.

Culturing animal tissue cells on a chemically inert but charged material results in large differences to the situation "*in vivo*", which poses a serious problem regarding this type of the morphological characterization. Culture of adherent cells results in the formation of a monolayer on the substrate. The image of a cell line, which can spread out on the bottom

of the cell culture flask when seeded at low density may reflect best the morphological image of the cells in the "*in vitro*" environment.

If the optimum cell density "*in vitro*" is exceeded, the cells are being pushed together as soon as confluency is reached. At this stage formations and structures may arise that are less characteristic. It is evident that the morphology of the cells under the phase contrast microscope are studied best when the cells have not reached confluence yet; then, their origin can be defined as epithelial or fibroblastoid. However, as mentioned above, this conclusion is not always unambiguous.

An obvious discrimination between epithelial cells and fibroblasts in the microscope is as follows: cells are defined as being fibroblastoid if their length is more than twice their width. This structure is also called spindle-like. Epithelial cells in culture appear polygonal and plane. Furthermore, the characteristics of the division process of these two main cell types are differing. Following cytokinesis, the daughter cells of fibroblasts move away from each other and find their position on the substrate. Epithelial cells keep contact with their daughter cells via specific epithelial complexes such as tight junctions. Colonies of growing epithelial cells may arise.

Other environmental factors besides the substrate may play a major role in the formation of cellular morphology, such as the composition of the medium or the presence or absence of serum. The transformation of the cell line in question is an important criterion for the morphology. Diploid, that is, nontransformed cell lines, can be characterized much better than those whose status of ploidy differs from the original tissue.

In addition, the number of diploid cell lines is restricted, as almost all healthy tissue cells are subject to apoptosis. This means that the passage number is constrained, and therefore not many non-transformed lines exist which are useful for *in vitro* culturing compared to the majority of transformed cell lines. Therefore, the number of passages in the case of diploid, nontransformed cell lines is always required. A passage number of about 30–35 in human diploid fibroblasts, for example, MRC-5 or Wi-38, is sufficient to induce apoptosis. These apoptotic cells cease their proliferation and have to be substituted with cells of a lower passage number.

In this case the creation of a "Master Cell Bank" as a prohibitive strategy is very helpful, as nearly all healthy diploid cell lines possess a limited life span *in vitro* as well as *in vivo*. Regarding the maintenance *in vitro*, transformed cell lines can be cultured much easier than diploid cells but still this transformation process represents a dramatic change of the biology of the cell. This holds for the situation *in vivo* as well as *in vitro*. As transformed cells have been and are still widely used, a few remarks regarding the observation and analysis of the cellular morphology follow:

1) Transformed cell lines do not undergo apoptosis, because many of the events that induce a transformation of cells are part of the cell cycle control which is affected.
2) Transformed cell lines mostly, but not always, loose many of the characteristics of the *in vivo* topology.
3) Transformed cell lines can loose their original morphology in many cases, preventing an unequivocal classification to their original tissue.
4) Transformed cell lines are most likely aneuploid, that is, the chromosome set is not euploid or the set of chromosomes switches during the process of culturing and transformation as

does the morphology in dependence of culture conditions, such as serum-free cell culture, change of medium or pH.

5) Recently introduced transformation techniques may keep the diploid stage within the mechanism of senescence. Such cells can undergo many divisions and can be induced to differentiate *in vitro* into cells very similar to the former tissue origin.

Our whole set of pictures represents viable cells cultured as monolayers or as suspension cells. The adherent cells attach to the respective surface or substrate, that is plasma-treated polystyrene with negative charges. No special treatments of the surface nor any other conditioning with, for example, collagen, extracellular matrices were used unless specified. No attempts were made to fix and/or to stain the cells and no three dimensional constructs were used for the pictures.

The pictures were made with a professional equipment (inverted microscope with phase contrast and a digital camera), no further retouch or improvements by digital processing were made. This guarantees that pictures taken in the laboratories of the readers may be comparable to our pictures without any manipulations or "improvements."

Last but not the least, this book is not a textbook nor will give any detailed and special guidelines or protocols how to treat and process the respective cell lines in culture. Please refer to the many textbooks in this field and even the growing number of protocols and procedures of cell culturing appearing in the World Wide Web.

This book may be dedicated mainly to people with previous knowledge in cell culture techniques working in the laboratory.