Basic paper

WHAT IS CELL CULTURE

AND HOW DOES THE 2D-METHOD WORK?

heidolph
research made easy

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1. What actually is cell culture?

A cell culture is the growth of plant and animal cells outside of an organism under controlled conditions. The reason why cell culture is so popular is because by using mammal cells, for example, the use of animal tests can be reduced or completely avoided. In addition, certain hormones important to humans, such as insulin, can be produced quickly and easily from animal cells. Furthermore, particularly in medical research, by using cell cultures it has been possible to find out much about the behavior of cancer cells.

Accordingly, there are a large number of possibilities, which is why more and more researchers are opting for this topic.

This basic paper shows only a small section of this field of research and is intended to give an initial insight into this interesting world. In the following chapters, different types of cell culture, different cell forms, the most important materials, and a short introduction to troubleshooting in the cell culture are presented.

2. Which cell types are used for cell cultures?

There are a large number and a wide variety of organisms from which cells can be isolated and reproduced. Here we limit ourselves to four different forms of animal cells that are used in almost every cell culture laboratory.

a. Fibroblasts

Fibroblasts are autochthonous (resident) cells from the loose connective tissue. The loose connective tissue is a so-called gap filler. It enfolds nerves and tissue and is also used as a support. Fibroblasts form the components of the intercellular substance and are particularly active in the basic framework of the connective tissue. Their main task is the formation of scar tissue in destroyed areas of tissue. The morphology of fibroblasts is spindle-shaped or star-shaped and due to their cytoplasm dendrites, fibroblasts can be in mechanical and communicative connection to each other.

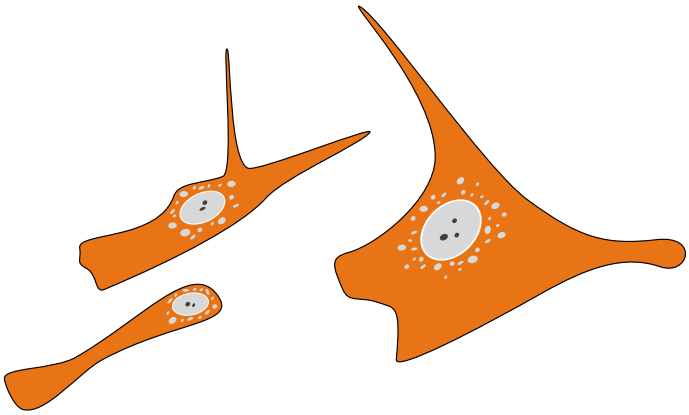

Fig. 1: *Mouse embryo fibroblast*

b. Epithelial cells

Epithelial cells are a cell group that is separated from the connective tissue and does not contain any blood vessels. The main common characteristic of epithelial cells is polarity. Here, polarity means that epithelial cells have an outer (apical) side (skin or organ surfaces) and a (basal) side facing the lumen/interior.

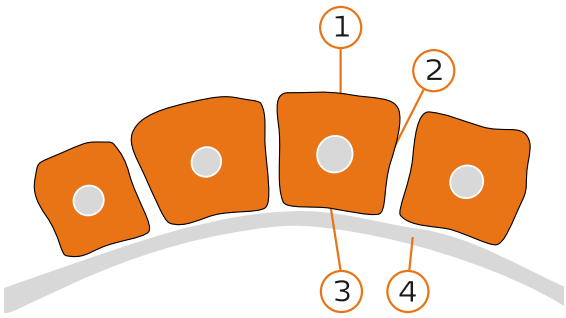

Fig. 2: *Polarity of an epithelial cell in relation to the surface: 1) Apical pole (directed towards the external milieu), 2) side area, 3) basal pole (directed towards the internal milieu), 4) basal membrane*

Epithelial cells are either monolayer or multilayer. The basal side is also connected to the underlying tissues. Epithelial cells have all kinds of different functions in the body, e.g. mechanical protection through the skin's tear resistance, the secretion or the absorption of nutrients in the intestinal tissue. There are naturally morphological differences between epithelial cells. In simple terms, they can be divided into two forms: monolayer epithelia and multirow epithelia.

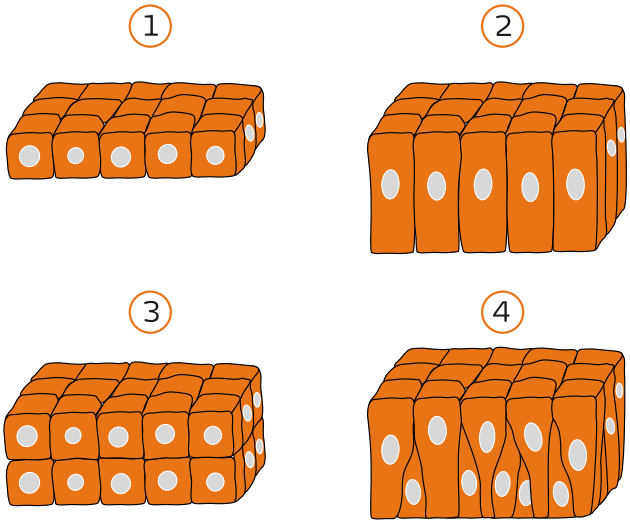

Fig. 3: *Different forms of epithelial cells: 1) Cubic epithelium, 2) cylindrical epithelium, 3) multilayered isoprismatic epithelium, 4) multirow epithelium*

c. Endothelial cells

This cell unit lines the blood vessels in a single layer. They form a sort of barrier between the blood vessels and the adjacent tissue. Due to this "boundary formation", endothelial cells also play a large role in the exchange of materials. Several important functions are, for example, the production of substances for regulating blood pressure (nitrogen monoxide) or regulating the exchange of materials between the blood and tissue. Since endothelial cells grow as a united cell structure and form a kind of tissue mesh between each other, their outward appearance is determined by the degree of confluence. The degree of confluence describes the continuous cover of a surface with an adherent (see chapter 3) cell line. Until the cells cover 100% of a surface, therefore, they tend to resemble fibroblasts.

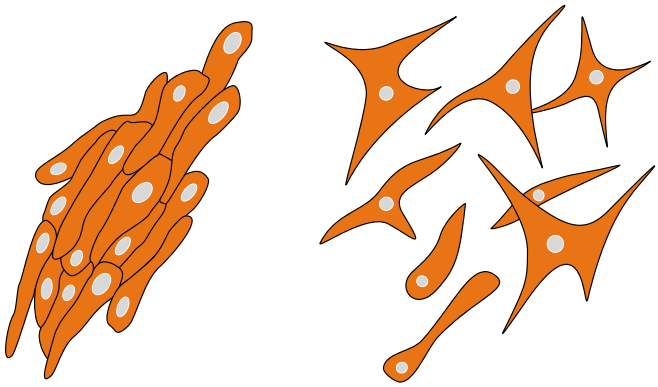

Fig. 4: *Degree of confluence of cells: 1) 100% confluence (100% cover of a surface); 2) 50% confluence (50% cover of a surface)*

d. Stem cells

Stem cells are a much discussed and interesting cell group which are currently mainly used for research. Depending on their environment, stem cells have the ability to develop into different cells or tissue types. These are then differentiated between embryonic stem cells (potentially any tissue) or adult stem cells (specific, differentiated tissue). In addition, stem cells can form identical daughter cells, which can then change further into differentiated tissue types/cell types, depending on the biological milieu.

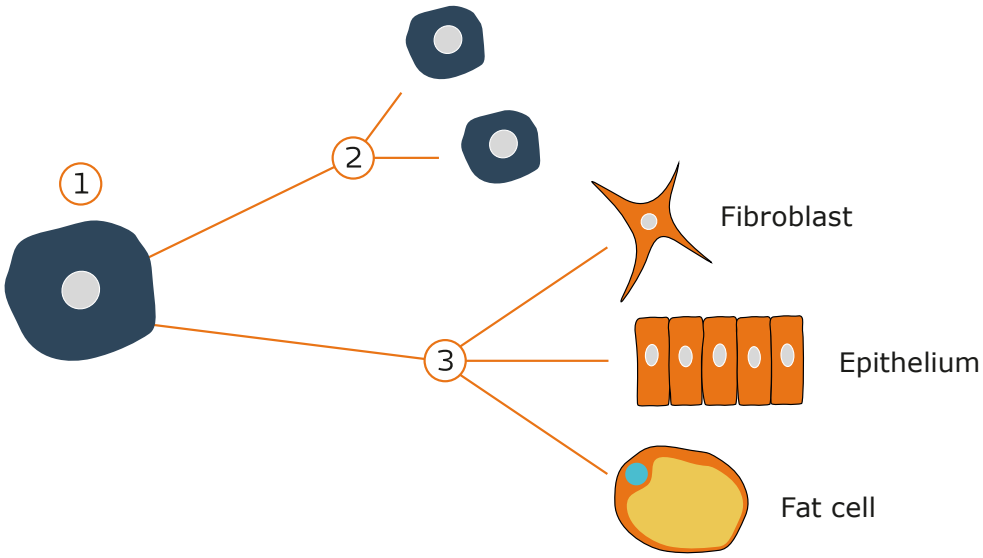

Fig. 5: *Asymmetrical cell division of a stem cell (1) with formation of identical daughter cells (2), and differentiated cell types (3), in this case fibroblast, epithelium and fat cells*

3. Which cell culture methods exist?

Before the cell culture experiment starts, it is advisable to know which cell culture you want to operate, and in which form the chosen cells grow. To this end, a differentiation should be made between the different types of cell culture.

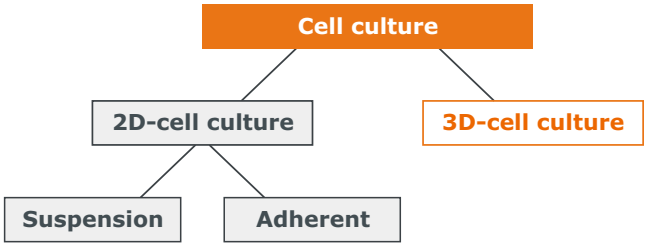

Fig. 6: *Cell culture methods*

2D-cell culture is the method applied the most and with which many cell culture laboratories work. As shown in the diagram above, this can take place in two ways. The physiology of the cells more or less determines whether the culture takes place as a suspension or as an adherent. In the suspension culture the cells are freely suspended in the medium, while in the adherent culture the cells adhere and grow in a layer (monolayer) on the surface of the culture vessel.

In 3D-cell culture, cells are cultivated in three-dimensional structures similar to organs in the body. 3D-cell cultivation considers the structure and orientation of tissues. Three-dimensional cell cultivation is a very distinct topic, which should be considered separately. This introduction focuses on 2D-cell cultivation.

4. Precisely what do I need for a cell culture?

To this end, the human body can be considered first. To stay viable as a human, we need nutrients (in the form of different foods), water, moderate temperatures, and occasional movement. The same points can be roughly transferred to a cell culture. The difference here, of course, is that the human body has to be replaced by an artificial environment. The cell must be provided with everything from the outside, in order to be able to grow sufficiently and survive. The parameters that are important for a successful cell culture are discussed in the following.

a. Temperature / CO_2 / Moisture

Most mammals, from which many of the cells used in the laboratory are cultivated, have a body temperature of approx. 37 °C. To ensure that the cell does not stop growing, it is important to use an appropriate incubator, which precisely simulates this ambient temperature and can keep it stable for long periods.

The pH value in the cell surroundings is another important factor that should be noted. Many of the mammal cells used feel most at home in a pH-range between 7.0 – 7.4. Used in combination with a CO_2 gas supply (5 % by vol.–20 % by vol.), an incubator can also provide the best environment in this respect. Since cells are in cultivated in a medium and this should not evaporate, it is important to set a high humidity (approx. 95 %) at the device.

Fig. 7: *Section of the user interface of an incubator with parameters*

b. Nutrients and medium

The right nutrients and the choice of a suitable medium are an important factor for successful cultivation of mammal cells. Media basically consist of carbon sources, such as glucose, nitrogen and phosphate sources, and oxygen. Vitamins, growth hormones, and minerals are often also added. In a cell culture laboratory, media are often used in different categories. For example, if cell lines only need certain constituents to grow, synthetic media are used that have a defined, predetermined concentration of the constituents. On the other hand, complex media can also be used in which organic constituents are used, such as peptones. However, there is no universal medium and a detailed search of the cell line should be undertaken, or the media recommended by the cell bank should be used for the cultivation. That way you are always on the safe side. A selection of media with the corresponding cell lines is shown in the following table:

Tab. 1: *Common media for the culture of mammal cells*

Cell line	Organism	Medium
CHO-K1 (epithelial cells)	Mouse	F-12 with 10% FKS
HeLa (epithelial cells)	Human	MEM with 10% FKS and NEAA
CV-1 (Fibroblast)	Ape	MEM with 10% FKS
HUVEC (endothelial)	Human	F-12K and 10% FKS and 100 µg/ml Heparin

c. Culture vessels/shakers and sterile workbench

There are a large number of culture vessels. Here the user – depending on the task or cell culture routine – can opt for corresponding vessels. Most vessels used for adherent cell cultures are tissue culture bottles (T-bottles), which are often made of polystyrene and whose surface is hydrophilic due to chemical treatment. In addition, there are screw tops with or without gas permeable membranes. Here the user must note whether or not the cell culture needs CO_2 for growth.

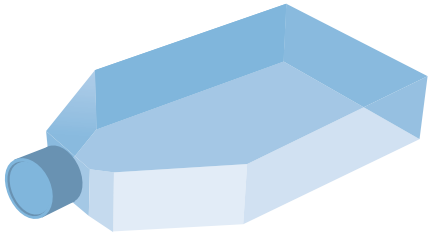

Fig. 8: *Tissue culture bottle (T-bottle)*

Sterile Erlenmeyer flasks, in which the glass has hydrophilic properties, are used to create suspension cultures. Different types of Erlenmeyer flasks are available. In cell culture the main differentiation is the fastening. Depending on the requirements, either a sterile bung with a sterile metal cap are placed on the Erlenmeyer flask or a handy screw top is used. Examples of different flasks are shown in the following illustrations.

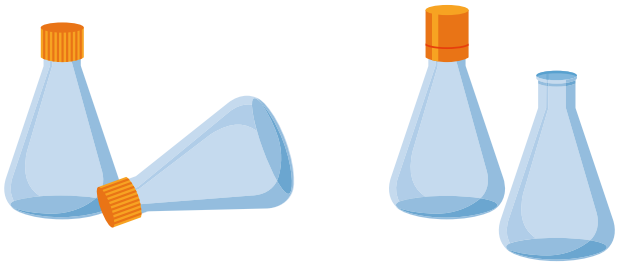

Fig. 9: *Erlenmeyer flask with screw top (left) and with sterile metal cap (right)*

In addition, with a suspension culture it must be considered that the suspended cells must be moved in order to obtain a sufficient quantity of the relevant nutrients. Depending on the stability of the cell lines used, either orbital or platform wave shakers can be used.

Orbital platform shakers are particularly suitable for suspension cultures with higher volumes. Due to the option of most devices of setting a higher speed, the medium and cells are mixed and, if sufficient nutrient supply is available, proper growth occurs. It should be noted that orbital platform shakers should be used for less sensitive cell lines, since high rotational speeds and a large radius cause a hefty turbulence to occur.

The platform wave shaker is a popular choice where very gentle rinsing of mammal cells is needed. Here the movement takes place in combination with a very low speed in three directions. The suspension therefore keeps moving without exerting any “stress” on the cells.

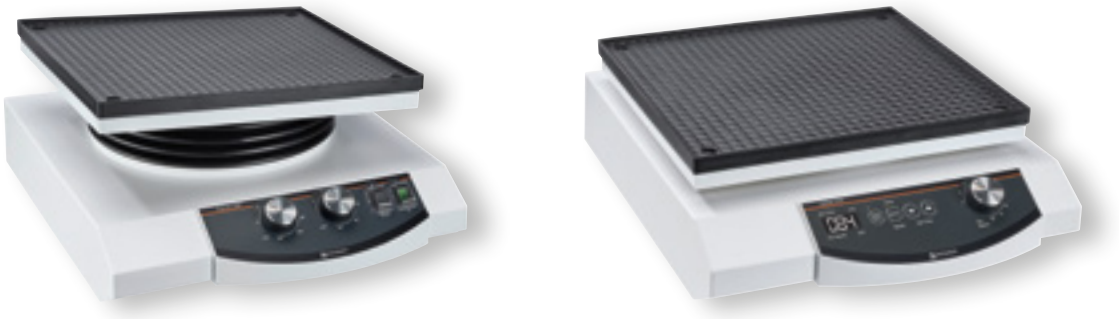

Fig. 10: *Polymax 1040 (left): Platform wave shaker with gentle movement shape and a speed of 2 - 50 rpm; Unimax 1010 (right): Orbital platform shaker for optimal nutrient supply of less sensitive suspension cultures*

Sterile workbenches are the be-all and end-all in every cell culture laboratory. Different types are available depending on the requirements and classification of the laboratories. They are classified according to the air supply and extraction and the filtering of the airflows.

Class I safety workbenches only have a vertical airflow. Here the room air is extracted through an opening above the table, this class is therefore not really suitable for cell culture routines. Class II safety workbenches are more suitable for work with cell cultures, since here the airflow is filtered, and the user is protected from escaping aerosols by an air curtain. Class III safety workbenches on the other hand are completely closed off and work with a negative pressure (vacuum) system. The user can only operate the closed off workspace via two integrated gloves. These workbenches are used above all in the manufacture of medicines.

Fig. 11: *Sterile workbench (laminar flow workbench)*

5. Troubleshooting: What can happen and how to fix it

Having discussed the appropriate equipment, different cell types and the importance of the media, the cell culture experiment can start.
Unfortunately, even with the best preparation of the experiments and work methods, something sometimes goes wrong. There are different problems that can occur during the cell culture, for example, microbial contamination, contamination with mycoplasma, decelerated growth due to physical influences, and much more. This list could be added to endlessly. Three of the more frequent problems are discussed in the following and approaches to controlling contamination are elucidated.

a. Mycoplasma
Mycoplasmas belong to the domain of bacteria and are parasitic for vertebrates. The difference to other prokaryotes, however, is that this type does not have a cell wall. They are also much smaller than their relatives and can pass through sterile filters, which makes these gadflies a real problem in cell culture. Mycoplasmas are often unfortunately not detected until the culture is completely overgrown with them, since mycoplasma cannot be identified by light microscopy in the early stage.

What steps can be taken against infestation? Since mycoplasma are bacteria, a large number of antibiotics can be used for the treatment, for example, gentamycin, tetracycline HCl. As humans are the greatest causers of mycoplasma contamination, because they often populate the upper sinuses, attention should always be paid to clean work methods. If contamination

has nonetheless crept in, apart from using antibiotics, there are also other ways for removing it. These follow other approaches, such as inhibiting propagation, dissolving the cell membranes or inhibiting the ribosome activity. It is important that a polymerase chain reaction is carried out after the end of the treatments to be really sure that there are no longer any mycoplasma in the culture.

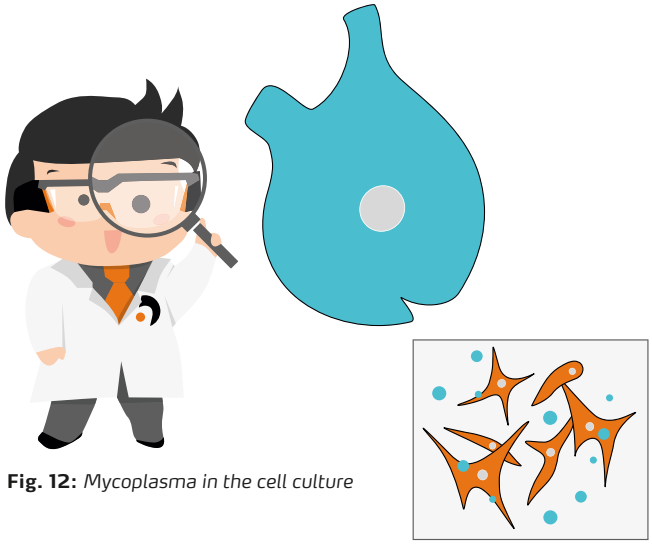

Fig. 12: *Mycoplasma in the cell culture*

b. Microbial contamination

Apart from mycoplasma, other bacteria can of course populate a cell culture. With this form of contamination, it is relatively irrelevant what type of bacteria they are. Similar to a mycoplasma contamination, a large number of antibiotics help to deal with the contamination. However, before these are "wildly" added to the medium, it is necessary to find out what type of bacteria is involved so that specific action can be taken. Furthermore, once again, attention should be paid to extreme personal cleanliness when working, since there are many germs on the skin, such as, staphylococci or streptococci. It also helps to keep the working environment clean and to pay attention to disinfection.

c. Cross contamination

Cross contamination is a case of so-called other immigrated cell lines that have gotten into the actual desired cell line due to unsterile working practices. The most well-known example of undiscovered cross contamination is the HeLa cell line. This has unfortunately been introduced into many other, frequently used cell lines. How do we detect that cross contamination has occurred? Above all, molecular biology techniques help, for example, profiling or DNA fingerprinting. If one of these methods has found a contamination, there are unfortunately few options for rescuing the culture apart from discarding it.

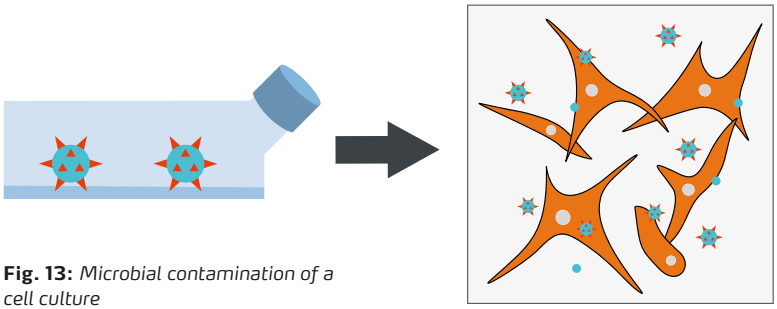

Fig. 13: *Microbial contamination of a cell culture*

Summary

It sometimes is a long way from a single cell or an isolated tissue to a successfully completed cell culture experiment. Even though there are many hurdles to overcome in cell culture, it is worthwhile to continue to practice this interesting field of research. Merely the fact that it is possible to find out more about intercellular processes or, for example, to better understand the origin of tumor cells is one of the most worthwhile feelings for every researcher and young scientist.

Sources:

- *S. Schmitz, Der Experimentator: Zellkultur, 2011, Spektrum Akademischer Verlag, Heidelberg, 3rd edition, https://doi.org/10.1007/978-3-8274-2573-7*
- *L.M/H.L/S.Gä, Lexikon der Biologie: Zellkultur; www.spektrum.de/lexikon/biologie/zellkultur/71584, (Retrieved: 07/11/2022)*
- *Glossar; Helmholtz Zentrum für Infektionsforschung; www.helmholtz-hzi.de/de/wissen/glossar/entry/zellkultur/; (Retrieved: 07/11/2022)*
- *InCelligence: 3D-Zellkultur: Übersicht und Neuigkeiten; www.incelligence.de/zellkultur/3d-zellkultur (Retrieved: 07/11/2022)*

ANY QUESTIONS

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