Basic paper

WHAT IS

3D CELL CULTURE

AND HOW DOES THE 3D METHOD WORK?

heidolph
research made easy

Contents

1. Introduction

Cell culture is an important research area which receives increasing attention. Here, animal cells offer the basis for the different researches as well as the examination and effect of new medicines on the cell. In addition, it is possible that animal tests can be considerably reduced. But also in the cell culture, different approaches can be applied. In the 2D cell culture, the animal cells are either cultivated in a suspension culture (free-floating in the medium) or as adherent cells (monolayer on a surface). However, regarding the 2D cell culture, you can reach certain limits as many interactions, especially between the cells, are ignored. To avoid these limitations, the 3D cell culture can be taken into consideration. The context for the 3D cell culture is actually relatively simple. Due to the fact that the world and all organisms are three-dimensionally structured, of course it makes sense to apply them also to the cell culture

With the 3D cell culture, the natural environment of an organ or an organism can better be traced. This has also a positive effect on the research results as for example the interactions of active agents in a 3D cell culture can better be observed and traced.
In this Basic paper, the focus is on the topic "3D cell culture".

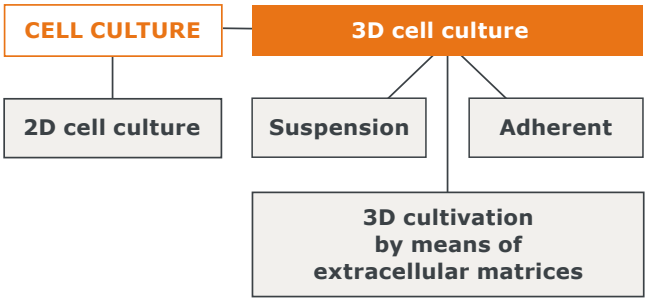

Fig. 1: *3D cell culture methods*

2. What exactly is 3D cell culture?

As mentioned earlier, the most natural form of cell clusters and organs is the three-dimensional one. If even better in *vivo* conditions shall be created, it is advantageous to trace them accordingly. But what exactly is needed for the 3D cell culture and what is the general difference to the conventional two-dimensional cell culture? The 2D cell culture is limited as this form of cell culture can only simulate a part of the in vivo situation of cell clusters. This is because in the standard 2D cell culture, cells grow on a surface as monolayer. The growth stops as far as the cells get "too close" within the cell cluster and the whole surface of the vessel is covered. For a three-dimensional approach and depending on the requirements, you need an extracellular scaffold or a matrix which gives the cell cluster the opportunity to grow out in all directions. Other possibilities are the cultivations of spheroids or organoids to understand tumor formations and medicinal influences even better.

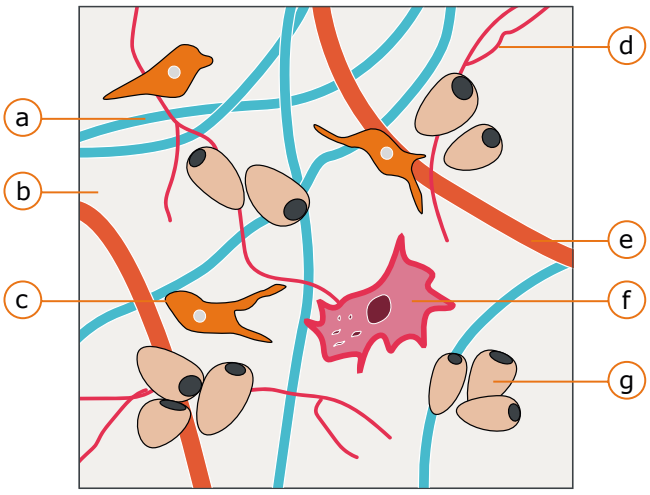

Fig. 2: *Example of a set up of an extracellular matrix with a: collagen fiber; b: basic substance; c: mesenchymal cell; d: elastic fibre; e: blood vessels; f: makrophage; g: fat cell*

a. The extracellular matrix

The extracellular matrix (ECM) exists in animal tissues and is the tissue part located between the cells in the intercellular space. The main functions are for example to ensure the water content of the tissues, to carry out signaling in tissues and to affect wound healing processes.

This is basically the totality of all macromolecules which are located in tissues and organs outside the plasmamembrane of cells. The basic function is the cell fixation. Here, it should be noted that it is not a rigid surrounding structure. The ECM and cells are in equilibrium with each other. The vast majority of the ECM components are produced in cells, separately fixed via further bindings and subsequently degraded outside or inside the respective cell.

The structure of the ECM can be divided in two groups: Basic substance and fibers

Depending on which culture technique you, as researcher, finally choose, it is important to understand the interactions of the cells with the extracellular matrix.

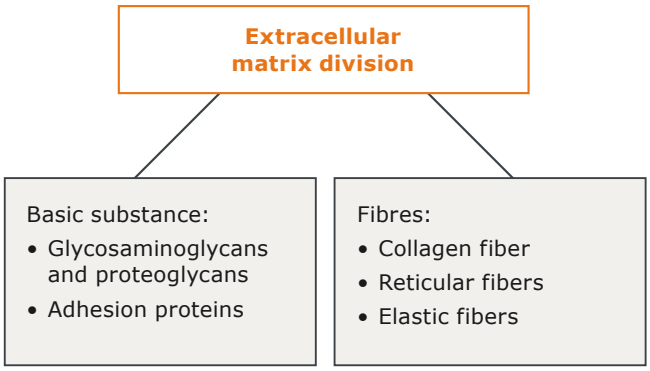

Fig. 3: *Division of the extracellular matrix*

b. Organoids, spheroids
Organoids or spheroids can also be cultivated without extracellular matrix, thus they can be processed easier and faster in the laboratory. To understand the difference of both forms, the following chapter takes a closer look at them.

Spheroids
Spheroids are cell aggregates which can be produced by aggregation and organization of thousands of cells to a 3D sphere. Spheroids are preferably used in the tumor research. Spheroids can be generated from different cell types, for example from Embryoid Bodies, hepatocytes, tumor tissues and mammary cells (breast cancer). Within the spheroids, cells produce a heterogeneous cell distribution by interactions with the ECM or with each other. In addition, a metabolic gradient is created in the "cell sphere" which means that the cells absorb the vast majority of the nutrients outside the cell and form a kind of "rest area" inside the cell. This behavior could also be observed in vivo for tumors, thus the cultivation of spheroids is particularly suitable for tumor research. Moreover, spheroids are used for the screening in the medicine efficacy and toxicity.

Organoids
Organoids are improved spheroids which have functions of specific organs. Unlike spheroids, organoids are complex tissues which consist of several cells. As a starting point for the cultivation of organoids, pluripotent cells are used. Puripotent cells are stem cells which can evolve completely undifferentiated. By the cultivation of organoids, the meaning of pharmacology and toxicology assays can be improved as organoids mirror the in *vivo* situation better.

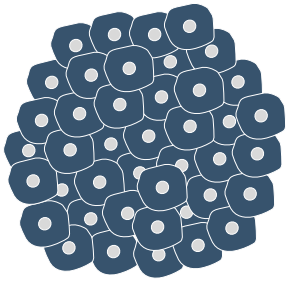

Fig. 4: *Spheroid*

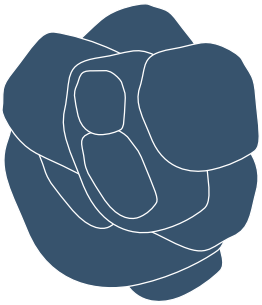

Fig. 5: *Organoid*

3. What formats are available for 3D cell cultures?

What format is used for the culture depends on the desired result and the used cell line. If you want to observe the interactions of new active agents with tumors, the culture of spheroids would be a good idea to do so. If, on the other hand, complex interactions of several cells are researched, the choice falls on scaffold-supporting cell cultures. Hereinafter, different methods with which you can produce spheroids easily are described. In addition, different possibilities of the scaffold supporting 3D cell culture are elucidated.

Methods for the formation of spheroids

a. Pellet culture

A very simple method to form spheroids or to manage spheroid culture, is the pellet culture. Here, the centrifugal force is used to collect the cells in suspension as pellet in the conical bottom of the used vessel. The spheroid formation is enhanced by the cell-cell interaction. However, a disadvantage of this method is that the individual cells can be damaged by the acting centrifugal forces.

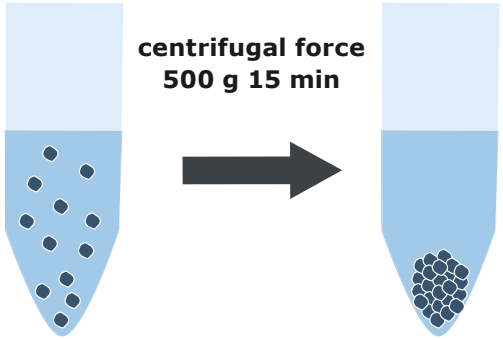

Fig. 6: *Spheroid formation by using the force of gravity during centrifugation.*

b. Hanging Drop

The Hanging Drop culture form uses the surface voltage of the medium. The suspension culture is put in the form of a drop on a surface, preferably the Petri dishes' cover, the construct is then slipped over a Petri dish. In the Petri dish, you often put a small amount of liquid, for example a phosphate-buffered solution to prevent the drops from drying out during the culture period.

By the surface voltage, the drop gets stuck "upside down" on the cover. The gravity forces the cells to the bottom end of the drop. In this way, by cell-cell interactions, they can join together again to a spheroid . Due to the fact that you can control the concentration of the used suspension culture and the used samples, with this method you can form spheroids with uniform cell number. This ensures a certain reproducibility within the individual experiments.

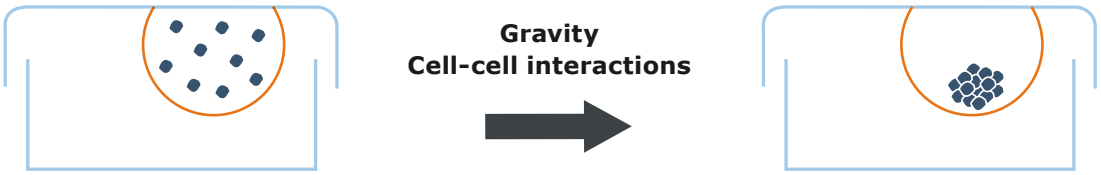

Fig. 7: *Hanging Drop method; cells in suspension are put as drops on Petri dish's cover. By inverting the cover, cells can form spheroids at the bottom of the drop.*

c. Spheroid formation in multi-well plates

The formation of spheroids is just as easy in specially designed multi-well plates. Here, the cavities are such that they have conical recesses on the bottom so that the spheroids can be formed by the predetermined geometry. Here, gravity also helps so that the cells find their way to each other and can join together as aggregate. The good thing about this method is that, being a researcher, you do not have to deviate from the existing protocol already finished as you only have to put the cell suspension in the multi-well plate.

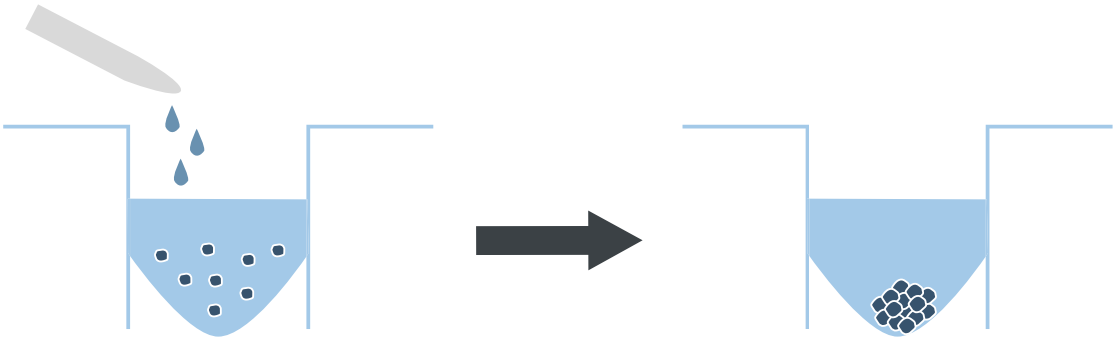

Fig. 8: *Multi-well plates with conical cavities*

4. Scaffold-based method technique

In addition to the formation of spheroids or organoids, it is also possible to grow cells three-dimensionally by means of scaffolds. These scaffolds reproduce the extracellular matrix. Depending on which material is used for the scaffold, you have to pay additional attention to which growth factors and proteins are used for the growth. Furthermore, it is important to know the pore size of the respective material. This is mainly because it is basically interesting to know how the cells embed in the respective matrix and which growth behavior they present in the respective scaffold. There are different approaches for the implementation of an extracellular matrix. Some examples are listed as follows:

- Preassembled matrices
- Hydrogels
- Tissues

a. Preassembled matrices

The matrix composition can be completely adapted to the respective cell. The production can be done via several processes: Separation of the polymer phases, Lyophilization or 3D print for example Many of these production methods exert an excessive pressure on the cells or the trace elements inside the material are defective. However, there are already relatively new approaches to avoid this by using fibrins which are extremely biocompatible. By using fibrins, the growth of the used cells in the preassembled matrices could be significantly improved. Thus, the use of additional stabilizing and beneficial proteins for the cells should be taken into consideration when choosing this matrix form.

b. Hydrogels
Hydrogels are very advantageous as they have a very similar structure to tissues. The main component of these hydrogels are biopolymers. A major advantage of hydrogels is that they can vary according to the cell structure so that there is more space for internal interactions and bio-activity. The way the grown cell structures have to be removed from the hydrogel after finishing the experiment is particularly important to note. In the meantime, research is so advanced that enzymatically degradable biopolymers can be used.

c. Tissues
This approach is often used for the growth of organs or complex tissues. Instead of very complex scaffolds which have to be printed before via 3D printing, several cell layers are stacked on top of each other. This approach has shown that it is not always absolutely necessary to use very complex scaffolds for the 3D cell culture or the production of tissues.

Summary

The final choice of the 3D cell culture strongly depends on the respective research project. Generally speaking, the cultivation of spheroids is more suitable to the tumor research as spheroids show a similar physiology. Here, the disadvantage is that an interaction with the extracellular matrix cannot be observed.
Thus, if the desired final result is the observation of complex cell mechanisms and cell physiology as a whole, the culture possibilities by means of the artificial extracellular matrices are ideal. This approach follows the reproduction of the natural environment and let the cells grow in clusters as in the case of animal or human organisms. In this way, you can represent internal cell processes better.
All in all, the 3D cell culture offers many possibilities to learn more about cellular processes. As a result, mechanisms that were hidden until now, become comprehensible. These knowledge is extremely important as it is used in different areas such as in medicine or drug discovery and thus it can protect many people from diseases in the future.

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ANY QUESTIONS

Contact us:

Heidolph Instruments GmbH & Co. KG

+49 9122 9920-0
redaktion@heidolph.de
www.heidolph.com

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