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3D mammalian cell culture models in toxicology testing

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3D mammalian cell culture models in toxicology testing

3D cell culture can be successfully used as an alternative to laboratory animals, and as a cost-effective and time-saving tissue culture technique, which also reduces the trial period for drug testing.

Toxicology testing is performed to understand the adverse effects of drugs and chemical substances on humans and other living organisms. In the EU, the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation (EC 1907/2006) applies a "no data, no market" rule, and responsibility has been placed on industry to manage the risks from new chemicals and to provide safety information on them.

What is 3D cell culture?

3D cell culture (**Figure 1**) describes a number of techniques to grow cells in three dimensions, such as in a spheroid, using an artificially created microenvironment. Cells in 3D cell culture have physiological cell-cell interactions and cell-extracellular matrix component interactions, which allow cells to grow *in vitro* in an environment that closely resembles *in vivo* conditions. For example, spheres possess a hypoxic (oxygen-deprived) core resembling solid tumours with cells at the centre of the sphere having extremely low oxygen and nutrient concentrations. Spheres also show complex transport dynamics by creating diffusion gradients of drugs, oxygen, nutrients and waste, and also show resistance and drugs/chemical substances show low potency as an *in vivo* condition. The neighbour geometry and cellular support found in 3D cell cultures can improve gene expression, cellular communication, migration, differentiation, survival and growth similar to *in vivo* tissues, which provide better representation for toxicological testing. Mixed-cell populations can also be cultured in 3D to closely model human tissues. Hence, 3D cell culture plays a vital role in measuring biological responses to new chemicals.

Why should I use it?

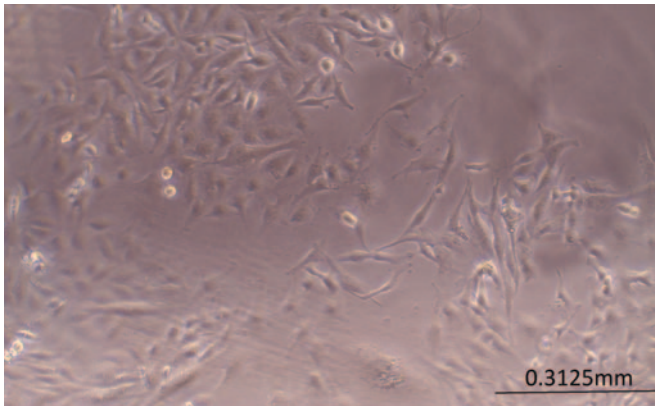
Use of animals in toxicological research has been reduced due to the ethical concerns, expense, time consumption, and misleading results due to differences between human and animal physiology. The REACH regulation's stated aim is: "to ensure a high level of protection of human health and the environment from effects of hazardous chemicals. It strives for a balance: to increase our understanding of the possible hazards of chemicals, while at the same time avoiding unnecessary testing on animals" (European Chemicals Agency, 2020). 3D cell culture is a much better technique, which supports the '3Rs' of animal research (replacement, reduction and refinement) and the REACH recommendation to perform humane animal toxicology research.

Conventional 2D cell cultures (**Figure 1**) are unable to detect organ-specific toxicity and have inadequate representation of cell migration, differentiation, signal transduction, survival and growth. A 2D cell culture does not reveal toxicological resistance (**Figure 2**), architecture as *in vivo* tissues, accurate depiction of cell polarisation or gene expression. It also provides unreliable predictions of *in vivo* drug efficiency and toxicity, which leads to low success rates in clinical trials. 3D cell culture can overcome the disadvantages of *in vivo* animal testing and conventional 2D cell culture by providing a more accurate platform for short- and long-term studies, demonstrating the long-term effects of the drugs.

How is it done?

Different 3D culture techniques such as anchorage independent, anchorage dependent, hydrogels based and specialised culture platforms can be used.

Anchorage-independent/scaffold-free techniques rely on non-adherent cell-to-cell aggregation to form spheroids, which show cell-cell interactions and secrete their own extracellular matrix. These spheroids are formed without a physical support resulting in consistency of shape and size, which provides better understanding about cellular cytotoxicity. Low-adhesion plates



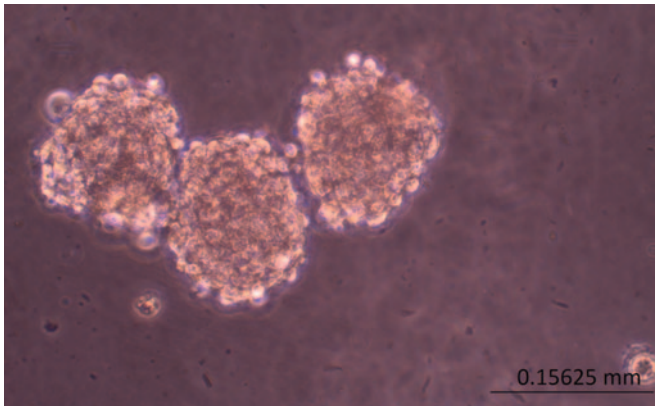


FIGURE 1: 2D (left) and 3D (right) cell cultures of U373MG cells.

are specialised culture plates with ultra-low attachment coating (polyhydroxyethylmethacrylate/agarose), which promote cell aggregation to form spheroids. Hanging drop plates are open bottomless wells, which promote the formation of droplets of media that provide space to form spheroids. In magnetic levitation, cells are preloaded with magnetic nanoparticles and external magnetic fields to provide non-adhesion, plate-like properties to form uniform tumourspheres. A rotational stirrer has a container to hold cells and impeller, stirring continuously. Liquid flow prevents cell adhesion and distributes nutrition and oxygen uniformly to form tumourspheres.

The anchorage-dependent technique provides physical support by using engineered porous membranes, polymeric fabric meshes called 'scaffolds'; these scaffolds can be made of natural or synthetic components. This physical support can provide structures from simple mechanical up to extracellular matrix-like structures. Cells are embedded in extracellular components and are able to initiate cell-cell and cell-matrix interactions, and physical support for cell growth, adhesion and proliferation. Natural scaffolds have higher biocompatibility and lower toxicity when compared to synthetic polymers.

Hydrogels can be defined as water-swollen networks of a polymer, which is a liquid at room temperature and forms a gel-like structure at 37°C. Cells can be embedded inside hydrogels and provide a similar microenvironment to an extracellular matrix. Animal- and plant-derived hydrogels can be used in 3D cell culture. Specialised 3D cell culture platforms, such as microfluidic devices, can equally distribute oxygen and nutrients, while removing waste to facilitate spheroid formation. Micro-patterned plates are micro-space, low-adhesion plates that promote the formation of spheres.

Conclusion

3D cell culture can be successfully used as an alternative to laboratory animals, as a cost-effective and time-saving tissue culture technique, and also to reduce trial periods for drug testing. Moreover, they can be used as an alternative to 2D cell culture, since they give an accurate outcome for toxicological testing. 3D cell culture reduces the gap between *in vitro* and *in vivo* drug testing and its effects at the clinical level.

Acknowledgment

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DMSO cytotoxicity assay for U373MG

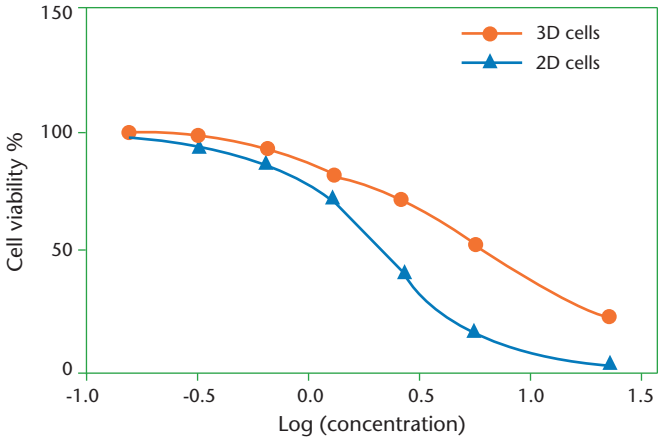

FIGURE 2: Different concentrations of dimethyl sulfoxide (DMSO) cytotoxicity towards 3D and 2D cell culture.

Reference

European Chemicals Agency. (2020). 'Animal testing under REACH – ECHA)'. [Accessed February 23, 2020]. Available from: https://echa.europa.eu/animal-testing-under-reach.

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