Advanced Drug Delivery Reviews 69 (2014) vi

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Preface

3D — A new dimension of in vitro research☆

Cell culture is prone to artifacts. Cells live — and as any living being, they react to survive. Survival of the most adaptable one: a principle well known in the evolution of organisms holds true also for cell populations under the selection pressure of culture conditions.

The closer the cellular environment mimics the physiological situation, the less cells need to move away from tissue-type differentiation. And we have first examples that show organo-typic cultures to provide more relevant results. They might help us to make better predictions of the organism's response to treatment, disease agents and chemical exposure. We need to admit that the current systems are of limited use, e.g. to decide on developing agents with higher probability of success in clinical trials. In this context, 3D is a key aspect to get to organotypic cultures. Traditional 2D cultures look like pan-fried eggs "sunny side up". Their environment: half plastic, half culture medium, and a little bit of other cells. They typically have less than 1% of both cell density per volume and cell-to-cell contacts when compared to native tissue. Intra-cellular communication is difficult, whether by contact or paracrine mediators, which are instantly diluted by cell culture medium. Most cultures do not result in polarization of cells, as especially epithelial cells show in the organism.

This ADDR special theme issue demonstrates a number of technical solutions to achieve 3D cultures and their benefits: cell differentiation, reduced variability, long-term stability, etc. — not all in each and every set-up, but in many cases.

But everything comes with a price: more work, more costs, slower growth, and heterogeneity; cells on the surface of our 3D cultures are not the same as those in the inside. And there is a nutrition problem; if not combined with perfusion, medium supply for the inner cells is limited by diffusion and cell barriers. Some hundred micrometer diameter is a typical limit, before lack of oxygen and nutrients leads to necrosis at the center.

But 3D alone is not yet organo-typic. The challenge starts with the choice of cell types. If tumor lines are used, 3D cannot restore their genetic make-up; thousands of point mutations, chromosomal multiplications, rearrangements and losses cannot be turned back. There is tremendous hope that we can increasingly use stem cells to obtain quasi-primary human cells, but the differentiation protocols still have major limitations. So we will often have to use primary cells, but their supply is challenging if we look for human ones. Often 3D culture will slow down dedifferentiation, the loss of specific cell functions typical for primary cells brought into culture, but again reliable protocols are only emerging. We are left with time windows between establishing the 3D culture and critical loss of differentiation. Adding the fourth dimension, time, to make long-term exposures and long-term reactions of our tissue equivalents possible is the next challenge.

There is more to do in order to make a 3D culture organo-typic. Perfusion can make culture more homeostatic, if we are not recirculating the culture media. Each medium change is the most drastic change of environment for a cell that we can imagine. In an instance, all waste is gone, and nutrients are replenished. To adapt, cells need to stay flexible, i.e. they have to avoid terminal differentiation. But an organ consists of many cell types, which we can model in co-cultures — and they are organized in structures, often form functional units. This is very challenging to recreate in vitro. Moreover, there is an extracellular matrix to add. We are only starting to engineer all of this, including mimicking the impact of physical factors — stretch, pressure, peristaltic, and more.

The current excitement for 3D in cell culture technology is fueled by the increasing awareness, how much we miss with traditional approaches. In a collaborative R&D funding initiative by the US agencies NIH, DARPA and FDA as well as a similar program by DTRA, $200 million over 5 years have been made available, aimed at creating 3-D chips with living cells and tissues that model the structure and function of human organs. Such tools not only are expected to help develop medical countermeasures for chemical and biological warfare and terrorism, but also are equally important in the general drug discovery and development area for predicting more accurately how effective a therapeutic candidate would be in clinical studies. European programs are more dispersed, less coordinated, but certainly not of much smaller dimension. They bring bioengineers and the in vitro testing community together. We can learn from alternative methods and their validation, which have addressed quality assurance for in vitro tests over the last decades, most explicitly with the development of the *Good Cell Culture Practice* (GCCP) guidance. If we want to become more predictive, we have to follow this avenue, and going 3D is among the first of many steps of our journey to meaningful models of tissues and organs.

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☆ This preface is part of the *Advanced Drug Delivery Reviews* theme issue on "Innovative tissue models for drug discovery and development".

http://dx.doi.org/10.1016/j.addr.2014.04.003