Today, the costs of bringing a new drug from target to the market vary between US$500 and 2000 million [1]. A major part of this money is spent on evaluating the safety of a new compound by toxicity tests in preclinical and clinical trials. Notwithstanding, since the year 2000 alone 18 approved drugs were withdrawn from the market by the US FDA due to safety reasons [2]. The number of drug failures in clinical trials is even higher, which clearly points to a shortcoming in the predictability of toxicity test used in preclinical trials before administering compounds to humans. In the preclinical test phase in vitro cell cultures and animal experiments are used to screen drug compounds for potential toxic side effects. While results obtained from animal models are not fully comparable to the human body response, in vitro cell culture tests often do not provide the cells with an environment appropriate for long-term stability of cell phenotype and functionality. Therefore, a new approach towards long-term, stable cell cultures is taken by providing the cells with an in vivo-like environment in so called ‘artificial microorgans’ cultures.

We believe that the following four prerequisites are particularly critical in the design of these ‘artificial microorgans’: the cells have to be cultured in an environment that mimics the in vivo situation by providing:

- An extracellular matrix that is specific for the particular organ;
- Cell–cell interactions between the different cell types present in this organ;
- A 3D arrangement of cells that resembles the structure found in the organ;
- Active perfusion of the culture.

The hypothesis underlying this concept is that culturing cells in an in vivo-like environment will result in an in vivo-like cell functionality and response to stimuli.

Microfluidic device technologies are especially well suited to realizing complex cell cultures as they provide dimensions that match well with the size of cells and blood vessels and, thus, can be used to achieve organ-like perfusion of the culture while also allowing for small diffusion distances. Microfluidic systems also provide new tools for manipulation and positioning of cells by the utilization of microscale phenomena such as laminar flow, dielectrophoresis and effects resulting from large surface-to-volume ratios. As a result, in the past years many microfluidic-device-based approaches have been investigated for the purpose of culturing cells.

The simplest approach to microfluidic cell cultures are 2D, confluent layers of primary cells or cell lines cultured on the bottom of microchambers or channels. This method has been employed by several groups for liver cell cultures [3–5] or even ‘multiorgan’ chips with several cell compartments with different cell types connected by microfluidic channels [6]. This approach represents an improvement over cell cultures in static well systems, as it features better perfusion, smaller size and a reduction of the number of cells required. It has been demonstrated in some applications, such as a lung model developed by Grothberg and Takayama [7,8]. There, epithelial cells were cultured on a permeable membrane in a microfluidic device. One side of the membrane was exposed to air, while the other side was perfused with medium, thus resembling the microenvironment of the lung. However, these 2D systems must fail with respect to in vivo
compatibly when used for culturing cells from organs exhibiting complex 3D structure, such as the kidney, liver or intestines.

An approach towards an artificial gut-on-a-chip was taken by the Allbritton group, who used a microfluidic device to capture isolated colon crypts in a defined orientation in microfabricated holes [9].

"...a new approach towards long-term, stable cell cultures is taken by providing the cells with an in vivo-like environment in so called ‘artificial microorgan’ cultures."

The first 3D liver cell cultures in a microfluidic device were shown by the group of L Griffith. Here, spheroids of hepatocytes and endothelial cells are captured and perfused in microsieves. But despite their 3D nature, the structure of spheroids is a far cry from mimicking the actual fine structure of the smallest functional entity of the liver, the liver sinusoid. Several groups are using mechanical traps in the form of micropillars arranged in an elongated horseshoe formation to capture and culture hepatocytes [10,11] or even different cell types on a multiorgan chip [12]. Although this concept provides a good method for culturing cells in a 3D monoculture, it cannot be used to position different cell types in an organ-like co-culture. Recently however, evidence has mounted that the presence of all cell types relevant for a particular tissue and their cell–cell interactions play a critical role in organ function. It has been shown, for example, that liver cell function in vitro is significantly enhanced by co-culturing with endothelial cells [13].

Dielectrophoresis denotes the manipulation of particles in inhomogeneous electric high-frequency fields by forces resulting from induced dipole moments [14,15]. It provides a powerful tool to manipulate and position cells in microfluidic systems and can be used to build organ-like structures with multiple cell types. The Liu group could show that, by using a special electrode design, cells (HepG2 and human umbilical vein cells) can be manipulated by dielectrophoretic forces to form a planar structure resembling the liver lobule [16]. With their design, however, only a 2D structure may be assembled and organ-type perfusion is not feasible. In addition, this group could not show any results with primary human cells.

Our group recently devised a microfluidic chip – HepaChip® – that comprises 3D microstructures and microelectrodes. In addition, a method was developed allowing for selective coating of cell culture regions by extracellular matrix proteins typical for the liver [17]. Primary cells may be manipulated by dielectrophoretic and hydrodynamic forces to form sinusoid-like cell assemblies [18]. This system therefore meets all the requirements for a truly in vivo-like cell culture, as discussed above. After cell assembly the culture was perfused through the microchannels and cells were shown to adopt a typical hepatocyte morphology.

"...microfluidic technology offers a unique toolbox to build artificial microorgans."

All these examples show that microfluidic technology offers a unique toolbox to build artificial microorgans. However, there are also reasons why microfluidic systems have not yet found broader application outside a small scientific community. A user-friendly interface between micro and macro, especially in fluidic connection, still remains a great challenge. Most microfluidic cell-culture systems are still far from being ‘plug-and-play’, requiring manual fixing of tubing and electrical connections. Therefore new solutions for automation, parallelization and standardization are required and respective technical developments are in fact currently receiving increasing attention in the microfluidics community.

Executive summary

- There is a need for in vitro models with better comparability with respect to human response for use in preclinical toxicity and ADME studies.
- Cells cultured in an in vivo-like environment will show enhanced cell functionality and, thus, a significant response to test compounds.
- Microfluidic technology offers a toolbox to build ‘artificial microorgans’ and first promising results in microfluidic cell culture have already been shown.
- Essential developments in the field of microfluidics are still required to offer user friendly ‘plug and play’ solutions.
- The time has come to develop new gold standards for toxicity and ADME testing and replace unreliable animal models by new in vitro test systems based on microfluidic ‘artificial microorgans’.
Another challenge for artificial microorgans— as for all new models aiming at pharmacological applications—consists in the time-consuming and costly validation processes and the reluctance found in the pharmaceutical industry to adopt novel test schemes.

We believe, however, that after 80 years of reliance on animal testing as the ‘gold standard’ in ADMET testing, considering the lack of significance and precision of these tests as well as the vast increase of knowledge in genomics, proteomics and metabolomics, the time has come to develop a new gold standard based on ‘artificial microorgans’ assembled in microfluidic systems.

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