Microfluidic Multi-Tissue Platform for Use with Spherical Microtissues

A Proof-of-Concept Study using a Liver-Tumor Microtissue Model

The use of microphysiological systems is often considered a next step towards more comprehensive and representative \textit{in vitro} testing systems. Microphysiological systems comprise conventional or more advanced, three-dimensional cell cultures representing a specific organ type and a fluidic routing scheme, which allows for applying controlled perfusion protocols. On a next level of complexity, different organ models are then combined in the same platform and are fluidically interconnected which enables physiological metabolic communication. The interest in and relevance of these systems is mainly based on their potential to better predict the impact of compounds on processes in the human body and to better understand - in a more systemic way - how different organs interact with each other under different conditions. Microphysiological systems are, therefore, ideally suited to bridge the gap between conventional cell cultures and clinical trials and considered as potential alternative to animal trials.

Pro & Contra of Different Cell Culture Models

3D \textit{ex vivo} cell culture models are of increasing interest, as they seemingly better reproduce tissue morphology and functionality, and feature dynamic mechanical properties and a biochemical environment that resemble those of native three-dimensional phenotypes of living organs [1]. The combination of 3D tissue engineering approaches with microfluidics technology, however, poses some challenges. The increasing complexity may entail limited reproducibility and, therefore, larger variation of tissue responses upon compound testing. This aspect becomes even more important when different 3D tissue types are handled in the same device and are interacting through the same circulating liquid phase. Reproducibility and predictability of cell or tissue behavior in complex experimental scenarios is a pivotal issue in all current initiatives towards more biomimetic \textit{in vitro} models, including multi-organ devices or so-called \textit{body-on-a-chip} configurations.
Any chosen technological approach will be a trade-off between complexity and resemblance to the *in vivo* situation on the one hand, and ease of use, reproducibility, and potential for parallelization on the other hand.

**Multi-Cellular Spheroids as 3D Tissue Model System**

Multi-cellular spheroids have emerged as a promising 3D tissue model system, which can overcome some of the observed limitations of 2D culture systems in mimicking *in-vivo-like* environments [2].

Spheroids are scaffold-free, self-assembled cell clusters that have proven to feature tissue-like phenotypes and functionality [3]. These spherical microtissues can be produced at reasonable costs in mono- or co-cultures and can be generated from a variety of cell sources within a few days. They can reliably be produced in well-controlled sizes at high throughput in an automated way. Owing to their compact constitution that makes them easy to handle with conventional cell culture equipment, microtissues have become a popular choice for use with microfluidic culturing setups (fig. 1). Most important, a large fraction of organs of the human body can be represented by spheroids, since they represent fully functional tissue units that can readily be combined on chip. This feature entails a substantial reduction in system complexity and greatly improves reliability and reproducibility of the obtained experimental results.

**Microfluidic Culturing Platform**

In a joint effort between ETH Zurich (Basel, Switzerland) and InSphero AG (Schlieren, Switzerland), we recently developed a microfluidic culturing platform that fully capitalizes on the outstanding advantages of microtissue spheroids [4,5]. The platform consists of 96-well-compatible culturing plates and an automated tilting system for liquid perfusion control (fig. 2). The microfluidic plate comprises of 10 separate straight perfusion channels or lanes, and each of these lanes...
features 6 serially aligned spheroid compartments. Open medium reservoirs are located at both ends of each channel, which can host up to 150 µl medium. Preformed spheroids are introduced into the plate through funnel-like loading ports. A subsequent slight tilting of the plate transfers the spheroids to the culturing compartments that are located at the intersection of the loading channels and the perfusion channel. The optimized design of those culturing compartments obviates direct flow onto the spheroids in order to minimize shear forces and, at the same time, ensures good medium exchange. The low height of the perfusion channel (100 µm) prevents spheroids from leaving the respective compartments upon media perfusion during the experiments. Both, medium reservoirs and loading ports are arranged at 9 mm pitch and match the positions of conventional 96-well plates, which enables easy loading of multiple spheroids and convenient media exchange using conventional multi-channel pipettes and 96-well-plate equipment. All spheroid compartments are placed between two loading ports of neighboring channels. This arrangement enables the gravity-based reliable loading and good optical accessibility for transmission light microscopy. Up to 60 interchangeable spheroids can be accommodated in a single culturing plate with a high degree of flexibility in arrangement and medium compositions.

For experiments, the microtissue-loaded plate is placed on an automated tilting system, the tilting frequency, tilting angle, and tilting speed of which can be programmed. The whole set-up can be operated in a 5% CO₂, 37 °C humidified incubator. Upon tilting the plate by a certain angle, the two open reservoirs will be at different heights, which induces medium flow from the higher reservoir through the perfusion channel to the lower reservoir. Upon continuous tilting of the plate back and forth by a defined angle, medium flows back and forth, which enables continuous inter-tissue metabolic exchange and interaction through the liquid phase. The tilting concept enables bubble-free perfusion without the need of additional tubing and external pumps. The open reservoirs provide sufficient gas exchange and allow for direct access to the medium for sampling. Finally, throughput can be readily increased to over 100 parallel multi-tissue experiments, as multiple channels are integrated in one plate, and as many plates can be stacked on one tilting system without increasing system complexity.

Results with Primary Rat Liver Spheroids

Experiments with primary rat liver spheroids evidenced a microtissue viability after 8 days of culturing in the microfluidic plate that was comparable to that of microtissues that have been cultured conventionally under non-perfusion conditions in a static well plate. The ATP content of the spheroids remained constant as compared to initial values measured at day 0, before the spheroids were loaded into
the system. Albumin secretion was also measured to monitor the metabolic activity of the primary rat liver spheroids. Under static conditions in the well plate, albumin concentration in the supernatant remained constant over time, while it increased during the 8 days of culturing under perfusion conditions. This increase indicates a higher functionality of liver tissues in the tilting device, since albumin constitutes a specific biomarker to assess metabolic activity of hepatocytes in liver tissue [1].

As a proof-of-concept study, primary rat liver microtissues were cultured over a timespan of 8 days in combination with colorectal tumor microtissues (fig. 3). The relevance of the possibility to interconnect liver and tumor tissues was demonstrated by applying cyclophosphamide, a pro-drug, which requires activation by the liver to become effective. The impact of cyclophosphamide on tumor growth was simultaneously assessed under static culture conditions by discrete liquid transfer between separate liver and tumor wells and under perfusion conditions with interconnected tissue compartments on the tilting system. Remarkably, under static culture conditions hardly any effect on tumor growth was observed, whereas in the case of direct and continuous fluidic coupling in the tilting system, a significant reduction of growth of the colorectal microtumors was detected after treatment with cyclophosphamide [5]. These results illustrate the importance of continuous interaction between different tissue types in order to mimic bio-activation effects seen in vivo. Small fully functional tissue units (spheroids), low complexity in tissue handling, possibility to interconnect different tissue types, and straightforward parallelization of the overall setup constitute key features and advantages of the presented microphysiological platform concept. Recent studies have shown similar effects for a fully human system using InSphero’s 3D human microtissues. InSphero and ETH Zurich are currently developing a commercial solution of the multi-tissue platform.

Authors
Kasper Renggli¹, Andreas Hierlemann¹ and Olivier Frey¹,²

Affiliation
¹ Department Biosystems Science and Engineering, ETH Zürich, Bio Engineering Laboratory, Basel, Switzerland
² InSphero AG, Schlieren, Switzerland

Contact
Kasper Renggli
Department Biosystems Science and Engineering
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