TOWARDS A "BODY ON A CHIP" USING SPHERICAL MICROTISSUES IN A MICROFLUIDIC NETWORK

Olivier Frey[1], Soumyaranjan Mohanty[1], Wolfgang Moritz[2] and Andreas Hierlemann[1]
[1]ETH Zurich, Dept. of Biosystems Science and Engineering, Bio Engineering Laboratory, Basel, Switzerland
[2]InSphero AG, Zurich, Switzerland

ABSTRACT

Combining 3-dimensional spherical microtissues and microfluidic technologies lays the foundation for a modular pharmacological platform for drug development, toxicity testing, and investigations of complex compound-tissue interactions. In this contribution, we present the basic concept of our idea and illustrate how simple 3D tissue structures can be integrated in a continuous-perfusion environment by using basic microfluidic structures. First validations have been performed by optical means and by measuring tissue-specific viability markers.

KEYWORDS

Body on a chip, 3D tissue, drug development, perfusion, HepG2

INTRODUCTION

In-vitro cell-based assays play a key role in the overall process of drug discovery and can provide essential information on the efficacy and toxicity of a new compound. In order to increase the predictability of such assays, 3-dimenstional tissue constructs receive more and more attention, as their organotypic nature is better suited to study complex physiological processes than that of 2-dimensional cell cultures. This includes aspects of morphology, mechanical properties, and biochemical functionality [1]. Such an in-vivo-like behavior is not only dependent on the fabrication method of 3-dimensional tissue structures, as the utilized microenvironment also strongly influences long-term viability and functionality.

In this contribution, we focus on spherical microtissues, produced by the hanging drop technology [2], as they offer two key advantages over other cell culture formats that have been used in conjunction with microfluidic networks for cell-based assays [3]: First, they are comparably simple and reproducible to fabricate, and possess organotypic functionality and biomimetic morphology. Second, their spherical shape and compact constitution, as well as their precisely controllable size make them ideal candidates for handling in microfluidic structures in contrast to 3D-hydrogel or scaffold-based cell-cultures.

Combining the advantages of spheroids and the technical capabilities of microfluidic engineering offers the possibility to develop a modular platform that accommodates multiple tissues of different cell types (e.g. tumor, liver, heart). Dedicated culturing compartments host the microtissues, and fluidic interconnections between these compartments allow for mimicking the physiological context and conditions of the human body. Such a "Body-on-a-Chip" system will potentially allow for obtaining more predictive and reliable data at an early stage in the drug development process.

EXPERIMENT

The technological approach is presented in Figure 1. 3D-microtissues of different human cell types (e.g. HepG2) are reliably formed off-chip in hanging drops using the commercially available GravityPLUS^{TM} plate of InSphero AG, Switzerland. With a defined number of cells in suspension (500-1000), drops of 40 µl are generated. After 3-4 days in culture, spheroids are formed, which continuously grow. Upon reaching a defined diameter, they are directly transferred into the tissue inlet of the microfluidic chip, which is aligned underneath the specific drops. The collected spheroid(s) is/are moved to the respective compartments by using gravitational force (Fig. 1a). A defined channel layout (400 µm high, 600 µm wide) and tilting sequence fully exploit the tissue shape and allow for positioning of different microtissue types at different sites. This can either be achieved in parallel (b1) or in series (b3) and provides a physiologically relevant arrangement to mimic conditions in the human body (Body-on-a-Chip). The microtissues can also be used as building blocks to create larger tissue structures on chip by merging tissues of the same or different cell types (b2).

The tissue sites are fluidically interconnected to simulate blood-flow-conditions and metabolite-exchange. A lower height (100-150 µm) of these perfusion channels ensures that the microtissues will not be dragged away from their culturing site and are not directly exposed to the flow stream, which could generate excessive shear-stress at the tissue surface.

The operation of this gravity-based approach is very simple, especially under the required sterile working conditions; the robust and parallel loading of tissues is scalable towards the GravityPLUS^{TM} 96-well plate format for higher-throughput testing.

Microfluidic structures have been fabricated in PDMS, casted from a multi-layer SU-8 mold, fabricated by standard photolithographic processes. The PDMS has been subsequently bonded to a glass slide using O_2-plasma activation. Inlet ports for liquid and tissues are vertically punched into the PDMS. Two additional reservoirs (PDMS ring structure) are placed at the fluidic in- and outlet. PDMS chips are stored at room temperature for at least two days to recover the hydrophobic surface. Before use, the microfluidic chips are degassed, sterilized using an ethanol dilution series,

978-0-9798064-5-2/µTAS 2012/$20©12CBMS-0001

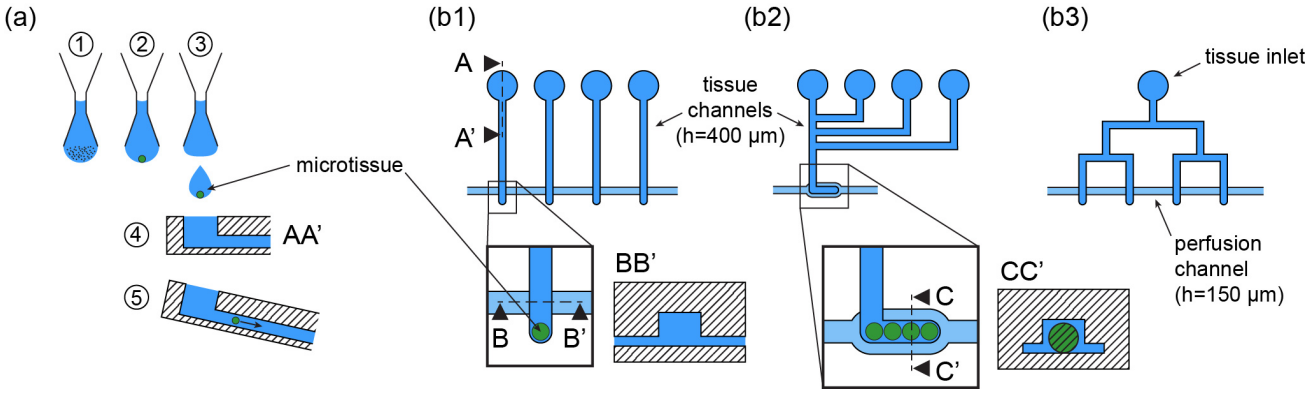

Figure 1: (a) Sequence for off-chip microtissue fabrication using the hanging-drop technique in a GravityPLUS™ plate of InSphero AG, Switzerland and direct transfer into the microfluidic chip. The tissue (diameter ~300 µm) is guided through dedicated channels to trapping sites by using a defined tilting sequence of the chip. Different tissue loading channel layouts allow for reliable arrangement of different tissue types for dedicated drug screening experiments: (b1) single tissues and individual inlets, (b2) parallel inlet and tissue accumulation and merging, and (b3) serial inlet into different trapping sites. Lower-height fluidic interconnection channels are used for continuous perfusion and metabolite transfer.

thoroughly rinsed with sterile de-ionized water, and filled with culture medium. After tissue loading, inlet ports are closed with stainless steel pins (Fig. 2a). The perfusion flow-rate is controlled by bi-directional tilting of the microfluidic chip at specific angles generating a hydrostatic pressure difference ($\Delta p = \rho \cdot g \cdot \Delta h$) between the two media reservoirs (Fig. 2b). A custom-designed, automated tilting device is used and directly placed into a conventional incubator. No tubing connections, low media-volumes (~100 µl), simple reliable long-term perfusion and no bubble-generation constitute the decisive advantages of this approach. The tilting frequency is adapted with respect to the applied angle. For a flow-rate of 20 µl/min, a tiling angle of 30 degrees is used, and the rotation is inversed every 10 min.

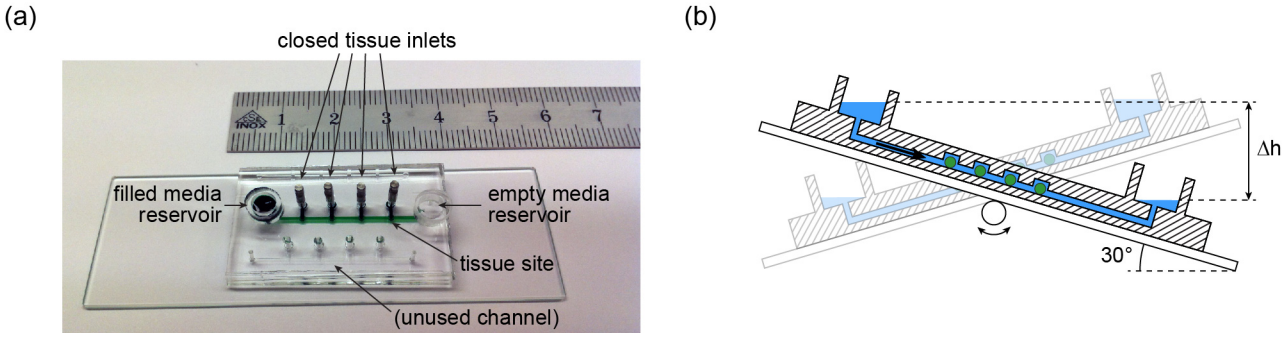

Figure 2: (a) Photograph of the PDMS chip, loaded with green food dye for visualization. The tissue inlets have been closed with stainless steel pins after loading. Two media reservoirs are used for perfusion. The flow-rate is controlled by automated tilting of the device at specific angles generating a hydrostatic pressure difference between the two media reservoirs (b).

Suspended HepG2 human hepatocytes have been seeded into 40-µl drops (500 cells/drop) of RMPI 60 medium supplemented with penicillin/streptomycin (100 U / 100 µg/ml) and 10% FCS. The hanging drop was cultivated for 3-4 days at 36 °C for microtissue formation in a standard cell culture incubator (95% relative humidity, 5% CO_2). For control, microtissues were transferred to GravityTRAP™ (InSphero AG) and custom-designed PDMS plates of similar geometry. Albumin secretion – a liver-specific biological functionality indicator – and tissue growth were monitored over 2 weeks. No substantial differences of albumin concentration in the PDMS and GravityTRAP™ plate were observed (results not shown), indicating only minor protein adsorption of PDMS as compared to non-adhesive-coated polystyrene of the plates.

After transfer into the chip, single microtissues have been continuously perfused with medium over more than 6 days. During this time, the microtissues preserved their morphology, and no cell attachment to the PDMS chip surface was observed However in some cases, microtissues have been displaced along their loading channel due to the tilting and fluid flow. This can be reduced by further optimizing the layout of the trapping site.

The tissue diameters have been measured, and media has been exchanged every 2 days. Albumin and urea content in the supernatant has been determined using a commercially available ELISA (Bethyl Laboratories Inc., USA) and a Urea Assay Kit (BioVision Inc., USA).

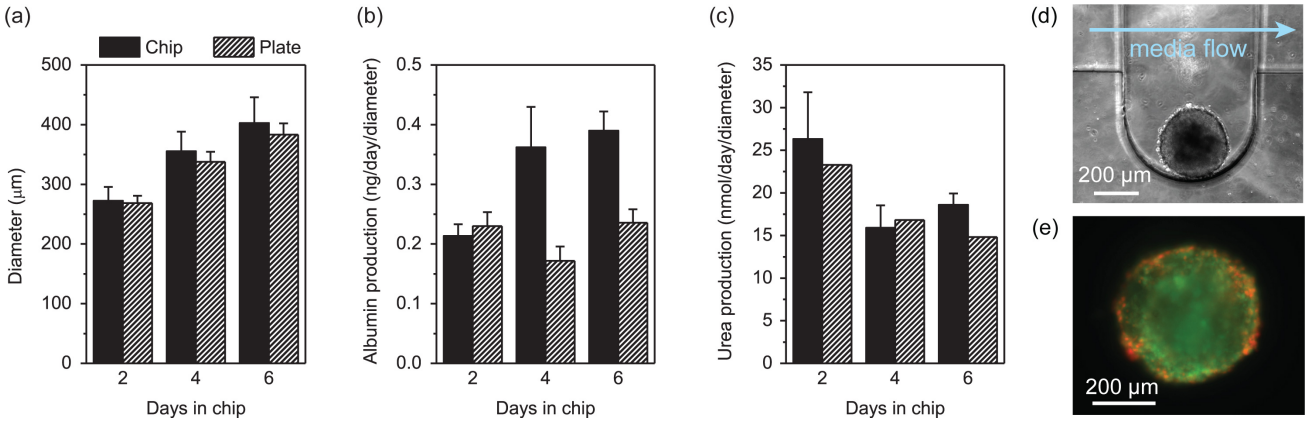

Figure 3: Comparison of (a) tissue growth, (b) albumin secretion and (c) urea secretion of HepG2 microtissues, cultured in a GravityTRAP™ plate and a continuously perfused microfluidic chip. (d) Bright-field and (e) fluorescence image of a HepG2 microtissue in the trapping pocket.

Microtissues, cultured in microfluidic chips, show similar growth-rates in comparison to microtissues grown in the conventional 96-well plate format (Fig. 3a). Albumin (3b) and urea (3c) production have been normalized with respect to the tissue diameter. First measurements show that HepG2 microtissues under constant perfusion show higher albumin and similar urea secretion as compared to static cultures. Bright-field as well as fluorescence images of the microtissues in the trapping pocket at day 6 evidence the high tissue viability, as they show a compact and healthy tissue morphology and a large number of living cells in the microtissue (Fig. 3d and 3e). Live/dead staining has been performed directly in the chip using a propidium iodide (PI)/fluorescein diacetate (FDA) solution.

In another experiment, always two HepG2 microtissues in five parallel-perfused microfluidic chips have been exposed to different liver-toxic Diclofenac concentrations. Again, albumin secretion has been used as a first toxicity indicator. Figure 4 shows a comparable albumin production in all five chips proving similar initial conditions after 10 hours in culture. At this time point, the media have been removed and replaced by new media containing Diclofenac at concentrations from 0 to 5 mM. The graph illustrates an expected substantial viability decrease for microtissues exposed to concentration above 500 µM. These values will have to be further validated in future experiments and compared to results achieved with commonly used read-out methods, such as confocal imaging combined with live/dead cell staining, lactatdehydrogenase (LDH) measurements, and other cell viability assays.

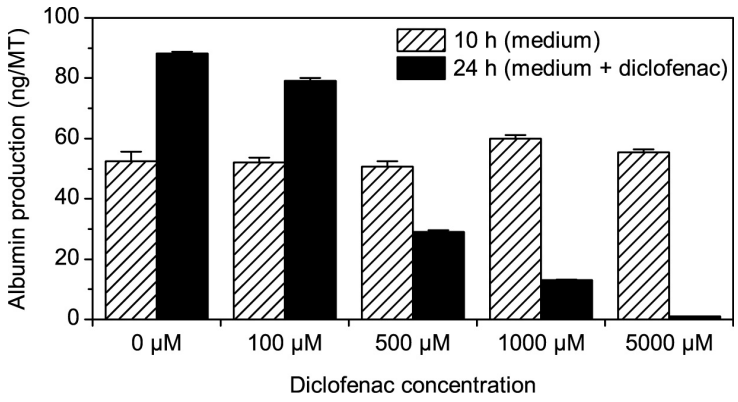

Figure 4: Albumin production rates in supernatants of HepG2 3D microtissues, exposed to the indicated Diclofenac concentrations for 24 hours in a continuously perfused microfluidic chip.

ACKNOWLEDGEMENT

This work was financially supported by the FP7 of the EU within the project "Body on a chip", ICT-FET-296257.

REFERENCES

[1] A. Abbott, *Cell Culture: Biology's New Dimension*, Nature, 424 (6951), pp. 870-872, (2003).

[2] J. M. Kelm and M. Fussenegger, *Microscale Tissue Engineering Using Gravity-Enforced Cell Assembly*, Trends in Biotechnology, 22 (4), pp. 195-202, (2004).

[3] M. B. Esch, T. L. King and M. L. Shuler, *The Role of Body-on-a-Chip Devices in Drug and Toxicity Studies*, Annual Review of Biomedical Engineering, 13, pp. 55-72, (2011).

CONTACT

Olivier Frey 41-61-387-3344 or olivier.frey@bsse.ethz.ch