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Hot from the press!

TEDD – Innovation Network for 3D Cell Cultivation

Switzerland has the potential to lead the way in cutting-edge tissue engineering processes, for instance in the automation of three-dimensional (3D) cell cultivation. For this purpose, interested parties from academia, hospitals and industry have joined together in the TEDD (Tissue Engineering of Drug Development) expert network to the benefit of all involved.

In June 2011, Professor ***Ursula Graf-Hausner***, Head of Section, Tissue Engineering and Cell Culture Technology at the ZHAW Wädenswil, launched the TEDD network together with Schlieren-based InSphero AG, a leading supplier of organotypic biological micro-tissues for biomimetic drug testing.

Stronger together

In the meantime, about 50 partners actively take part in this competence center, often making unique contributions, like the BioFactory® developed by regenHU in conjunction with the ZHAW team. This cell-friendly 3D bio-manufacturing instrument patterns cells, biomolecules and a range of soft and rigid materials in desirable 3D composite structures so as to mimic biomimetic tissue models. The magic gel is BioInk™, a semi-synthetic hydrogel that supports the growth of different cell types by providing cell adhesion sites and mimicking the natural extracellular matrix.

Synthetic hydrogels are the speciality of Cellendes GmbH, another TEDD member, which works closely with the ZHAW. It supports the testing of active substances as they are used in the pharmaceutical industry. Its hydrogels facilitate a more natural cell environment and can be used to cultivate cells within a precisely defined matrix.

Today, assays are still based on systems where cells are grown in a monolayer, although many researchers confirm an improvement in proliferation, morphology, gene and protein expression levels when cells are grown in 3D. In order to simplify the adoption of 3D techniques in drug discovery, the researchers from the ZHAW, Cellendes and Tecan, who specialize in instruments and automated workflow solutions for laboratories, combined their complementary technologies in a TEDD project. Adopting a Freedom EVO® liquid handling robot from Tecan as their platform, they succeeded in automating the production, maintenance and application of scaffold-based 3D tissues in a reproducible and reliable way. As the results prove, the approach produces results that are fully comparable to manually produced tissues.

Impression from the TEDD Annual Meeting 2013: Visitors enjoy the opportunity to exchange ideas and network in the greenhouse of the ZHAW campus.

3D Cell Culture is Ready for Drug Development

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In the drug development and substance testing industry the screening process of new compounds is mainly performed with cells growing in two dimensions (2D) on flat polystyrene surfaces. Although for a long time three-dimensional (3D) cell culture is recognized as being more biologically relevant than the standard 2D cell culture setup the 3D culture is still mostly entrenched in basic research.[1] However, in recent years research groups have started to evaluate the potential of 3D cell culture for drug screening processes. Kostadinova *et al.*[2] developed an *in vitro* liver model containing different cell types. With their 3D co-culture model they could better predict *in vivo* drug-induced toxicity compared to a 2D monolayer hepatocyte culture. Many different 3D cell culture systems are commercially available. 3D cell culture systems are divided into scaffold-free and scaffold-based systems whereas the scaffold-based ones are further divided into hydrogel-based and rigid scaffolds.[3] Scaffold-free microtissues are either produced in hanging drops by gravity force or by cell cultivation in non-adhesive cell culture dishes to foster cell–cell contacts. The automated processing of such scaffold-free 3D systems is already performed in 96- and 384-well plates. Drewitz *et al.*[4] developed a robust process of microtissue production of the human colon carcinoma cell line HCT-116 in hanging drops using a liquid-handing robot in a 96-well format. They showed narrow microtissue size distribution and reproducible IC_{50}-values with the reference compounds Staurosporine and Chlorambucil, which is a prerequisite for a standardized drug screening process.

In order to complement the recent advances of automating scaffold-free 3D cell culture for drug development we were interested to translate this process to a scaffold-based system. The complete study was recently published in *JALA*.[5] We used a synthetic hydrogel-based system, which shows high flexibility in incorporating bioactive molecules due to its modular composition. The backbone of this hydrogel consists of biocompatible cell-inert dextran and polyethylene glycol (PEG). Chemical crosslinking of these polymers occurs within seconds and thus keeps cells in 3D space. After culture and drug treatment incorporated cells are released from the hydrogels under mild conditions without adverse effects and can be used for downstream processing. In order to provide cell adhesion sites inside the hydrogel a peptide containing the receptor binding amino acid sequence RGD was incorporated covalently. We seeded HCT-116 cells in these dextran-based hydrogels in a 96-well plate format either manually or automated with a liquid handling robot. The cells were observed microscopically over time to verify whether they behaved similar in the automated compared to the standard manual process. In Fig. 1, optical analysis of automated versus manually produced hydrogels are shown at days 2, 5 and 8 after seeding. For both preparation methods the cells formed typical multicellular spheroids over time. The automated processing of cells and encapsulation into dextran-based hydrogels did not show any optical difference compared to the manual encapsulation procedure. In order to verify whether this 3D format has the potential for a real industrial application we automated the whole process of drug screening. We used the reference compound Taxol, a well-known anti-tumor agent to generate dose response curves in order to calculate IC_{50}-values. The automation included cell incorporation into dextran hydrogels, media exchange, serial Taxol dilution and application as well as ATP measurements. As a control experiment HCT-116 cells were seeded in standard 48-well plates in 2D to analyze the Taxol dose response in comparison with the 3D hydrogel-based setup. In Fig. 2 Taxol dose responses are shown for 2D and 3D cell cultivation. HCT-116 cells are approximately 7× more sensitive to Taxol when cultivated under standard 2D conditions

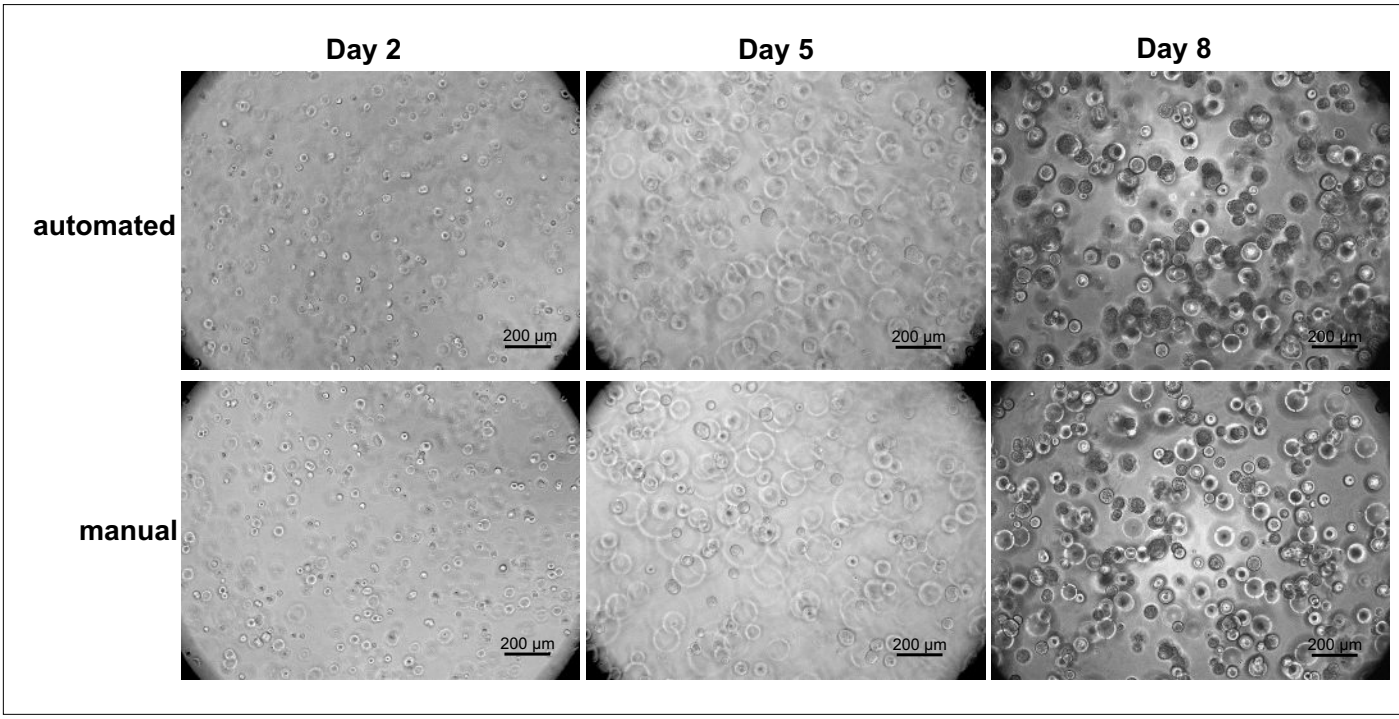

Fig. 1. Optical images of HCT-116 cells grown in 3D dextran-based hydrogels over time. In the upper panel cells were encapsulated automated using a liquid handling robot whereas in the lower panel, for comparison, cells were manually encapsulated. Cell seeding density was 1×10^4 cells per 50 µl hydrogel and well (96-well plate). Images were taken at day 2, 5 and 8 of cultivation. Picture adapted from ref. [5].

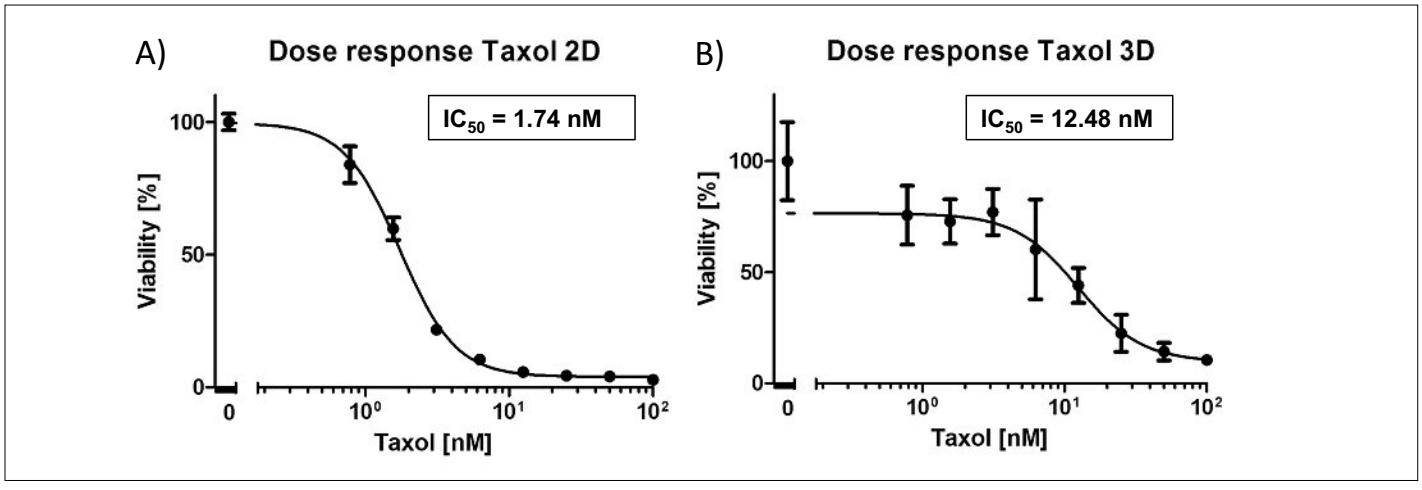

Fig. 2. Taxol dose responses of HCT-116 cells grown in 2D versus 3D. In A) HCT-116 cells were grown in 2D in standard polystyrene cell culture plates (48 well plate), manually seeded with 1×10^3 cells per well. In B) HCT-116 cells were grown in 3D dextran-based hydrogels in a fully automated process, which included cell seeding, media exchange, Taxol dilution and addition and ATP measurements to determine cell viability. In both cultures different Taxol concentrations were added at day 3 of cultivation and at day 7 for 2D and day 8 for 3D cell viability was determined using an ATP assay (n = 4 in A; n = 8 in B, per data point). From the obtained Taxol dose response curves the IC_{50}-values of cells grown in 2D and 3D were calculated and shown in the graph. Picture adapted from ref. [5]

(IC_{50} = 1.74 nM) compared with cells cultivated in 3D dextran-based hydrogels (IC_{50} = 12.48 nM). The former data is in good accordance with the Taxol IC_{50}-value of HCT-116 cells cultivated in 2D published by Rose and coworkers[6] (IC_{50} = 1.7 nM).

The difference between the drug responses of cells cultivated in 2D versus 3D has been shown in many publications. Furthermore, the number of data demonstrating that 3D cell-based assays produce consistent and reliable data in a high-throughput setup is steadily increasing. Our study exemplifies that even complex scaffold-based systems like our hydrogel *in vitro* model are amenable for automation. This process leads to a robust read-out and therefore justifies the initial investment into the novel 3D technology. This approach will pave the way for more efficient drug development.

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