CellME Berlin 2023

International Forum on Cell Manufacturing & Engineering

7-8 November 2023. Berlin, Germany

www.cellme.de

Organized by:

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Berlin, Germany

CELL MANUFACTURING & ENGINEERING FORUM. Berlin

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INDEX

EXHIBITOR

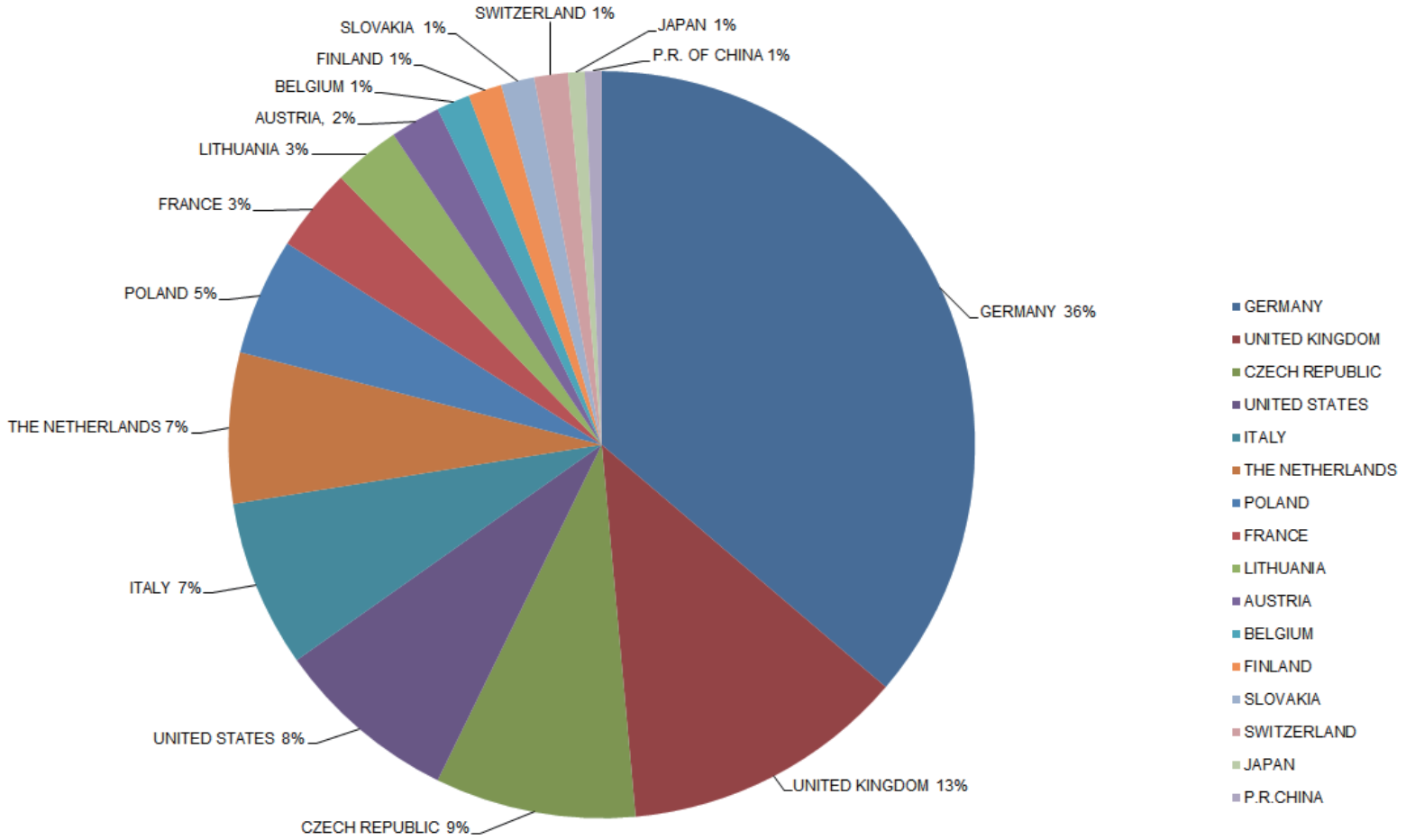
SPEAKER

Last updated: November 03, 2023

COUNTRIES OF ATTENDEES

CellME Berlin 7.-8.11. 2023. Berlin, Germany

Last updated: 3 November 2023. 16:00 CET



CellME Berlin 2023
Conference Schedule

The conference language is English

Day1: Tuesday, November 7, 2023

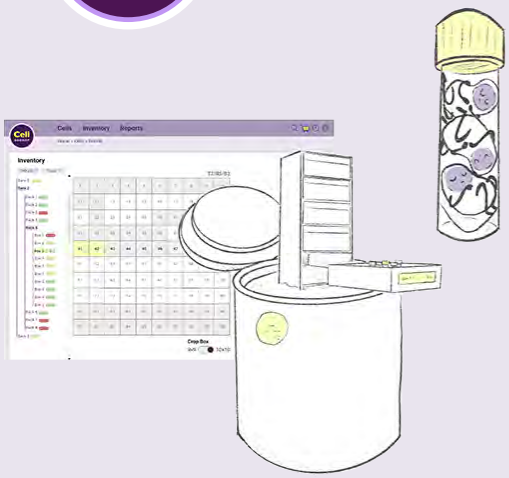
TIME	TOPIC	PRESENTER	COUNTRY
10:00	*Opening Remarks*		
10:10	Peptigels: a technological platform for the design of fully defined synthetic 3d hydrogel scaffolds for cell engineering applications.	THE UNIVERSITY OF MANCHESTER	UNITED KINGDOM
10:35	Agrostemmoside e: a potent tool for transfection assays	FREIE UNIVERSITÄT BERLIN	GERMANY
10:50	Spheroid culture of murine primary chondrocytes and atdc5 chondrocyte cell line for musculoskeletal research	INSTITUTE OF MUSCULOSKELETAL MEDICINE, UNIVERSITY OF MUENSTER	GERMANY
11:05	*Coffee Break*		
11:35	Development of an organoid-based cell transformation assay for intestinal carcinogenicity screening of chemicals	STONY BROOK CANCER CENTER, STONY BROOK UNIVERSITY, NEW YORK	UNITED STATES
11:55	3-D hollow fiber perfusion bioreactors for recapitulation of the in vivo microenvironment: a better way to grow cells.	FIBERCELL SYSTEMS INC	UNITED STATES
12:25	*Lunch Time*		
14:00	Cellbox – the shipment solution for living cells, organoids and sensitive probes.	CELLBOX SOLUTIONS	GERMANY
14:30	Novel automated picodroplet-based technology to streamline cell line development and cell engineering workflows	SPHERE FLUIDICS LTD.	UNITED KINGDOM
15:00	Vital light 3d – towards next breakthrough in bioprinting	VITAL3D TECHNOLOGIES	LITHUANIA
15:25	New non-invasive, label-free monitoring approach for 2D and 3D cell culture	LUDWIG-MAXIMILIANS-UNIVERSITY MUNICH, PHIO SCIENTIFIC GMBH	GERMANY
15:50	Networking		
17:00	*End*		

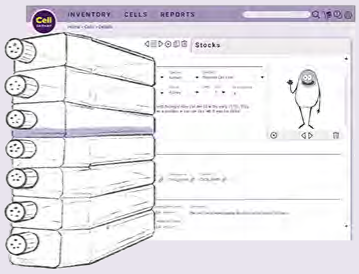
Day2: Wednesday, November 8, 2023

TIME	TOPIC	PRESENTER	COUNTRY
9:30	*Opening Remarks*		
9:40	Creation of skin cell spheres and improved methods for measuring cell viability in the 3D model	WARSAW UNIVERSITY OF TECHNOLOGY	POLAND
10:05	AI-powered analysis in robot-assisted three-dimensional tissue models production	UNIVERSITÄTSKLINIKUM WÜRZBURG - FRAUNHOFER ISC	GERMANY
10:30	*Coffee Break*		
11:00	Single use disposable inclined settling bioreactor for gentle expansion, concentration & harvest of cell and gene therapies	SUDHIN BIOPHARMA	UNITED STATES
11:25	RPMotion - A novel bioreactor for rapid organoid production	ORGONEX	THE NETHERLANDS
11:55	*Lunch Time*		
13:30	Animal-free and chemically defined coating solution for biologically relevant in vitro assays	FACELLITATE GMBH	GERMANY
14:00	From microenvironments to automation: supporting reproducibility of cell manufacturing and organoid research	OXFORD OPTRONIX LTD.	UNITED KINGDOM
14:30	Patient-derived cancer cell models	MEDICAL UNIVERSITY OF GRAZ	AUSTRIA
14:55	Networking		
16:30	*End*		

/Be aware that the start and end times for each session are tentative. /This schedule is subject to change without prior notice. Check back for updates on www.cellme.de. (Lasted updated: 03.11.2023)

Organize your cryostocks





www.cellseeker.org

INVENTORY

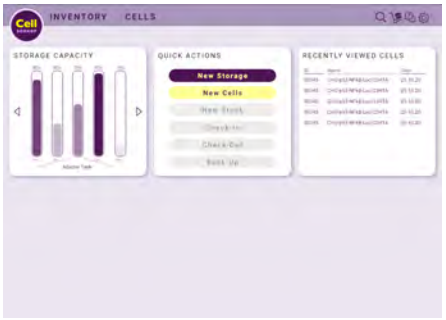
Create a virtual inventory and track the location of every single vial in your cell collection.

CELLS

Create data sheets of your cell lines and link the information to your cell stocks.

Cellseeker GmbH

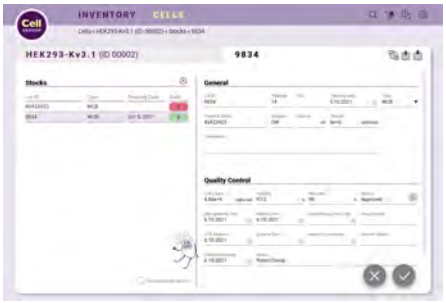
support@cellseeker.org
+49 160 624 060 7
Osterfeldstraße 12- 14
22529 Hamburg
Germany



Manage your lab's cell collection.

Assign users and permissions.

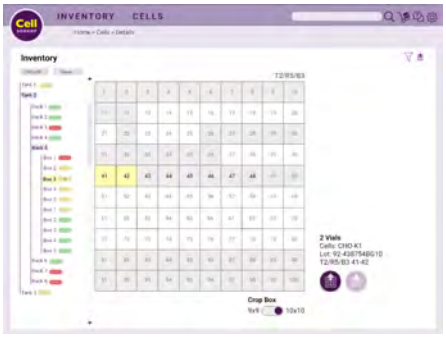
Give selected access to external partners.



Review the quality status of your cell stocks.

Control and track cell usage.

Trace back histroy of cell banks.



Track location and movement of your stocks.

Check-in & check-out vials from a virtual tank.

Import exsixting databases.

cellBOX™ SHIPPER

THE NEW GENERATION FOR LIVE CELL SHIPPERS

Made in Germany

cellBOX Flight 2.0

The **Cellbox Flight 2.0** makes it possible to perform long distance transport by air. It was uniquely engineered to use dry ice as a CO_2 source to comply with airline regulations. The user-programmable CO_2 and temperature values allow you to optimize the conditions in the incubation chamber for your precious biological material.

Benefits of live cell transport

- No pH-shift or use of metabolically active cryoprotectants
- No altered protein expression; no mechanic or osmotic stress
- Intelligently maintains internal environment for up to 32 hours
- High cell viability and accurate continuous data logging
- No freezing, no thawing - Cells arrive ready-to-use

CO_2 SUPPLY
Provides regulated CO_2 environment

TEMPERATURE
Maintains temperature between 28-38°C

APP
Data logging and export via Cellbox App

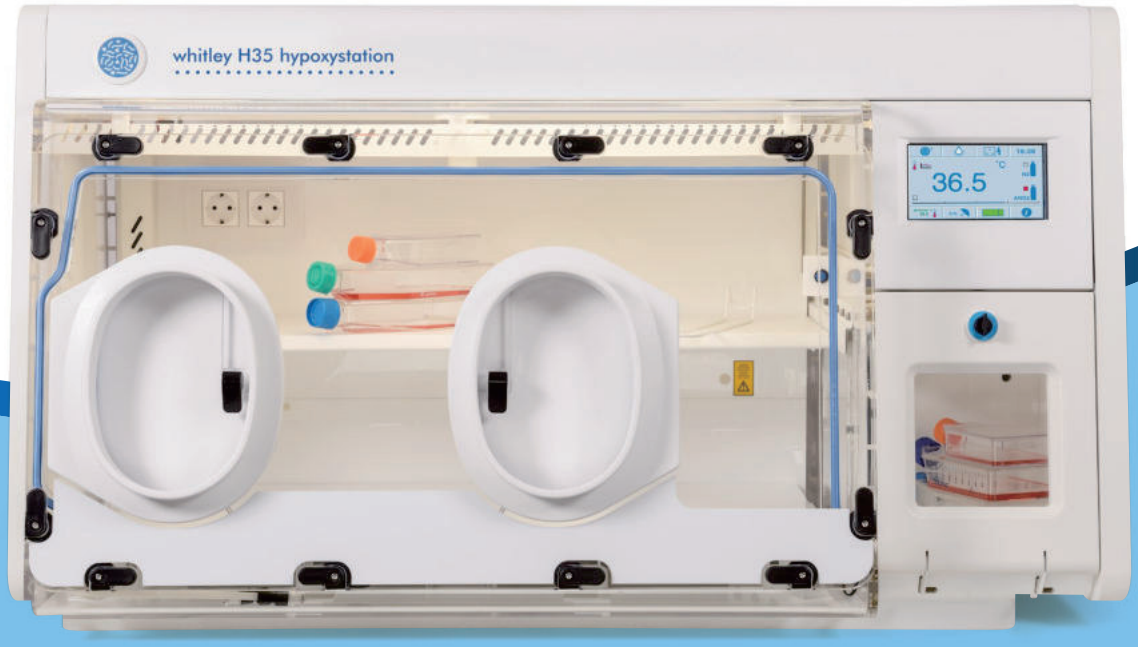
RECHARGEABLE
Li-ION battery with external 100 - 230V power supply

CONTACT

+49 (0) 40 226 316 4-10 enquiries@cellbox-solutions.com www.cellbox-solutions.com

Englische Planke 8 20459 Hamburg | An der Bahn 5 22844 Norderstedt | Germany

don whitley scientific
culturing innovation



Whitley H35 Hypoxystation

- Precise control of O_2 and CO_2
- Precise control of temperature and humidity
- Colour touchscreen control panel for ease of use
- Easy, accurate, 2-point oxygen sensor calibration
- Integral 12 litre airlock
- HEPA option available

www.dwscientific.com

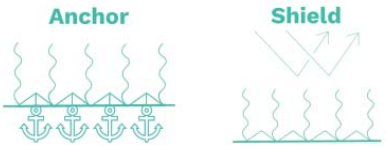
Animal free and chemically defined coating solution for perfect 3D cell cultures

Reliable cell-based assays are urgently needed to develop predictable model and testing systems

BIOFLOAT™

Current challenges

- Model systems mimicking cell natural microenvironment are required
- Rapid and reliable generation of disease models is needed for translational research
- An animal-free environment for human cell cultivation is preferred
- Optimal assay systems for stem cell or cancer research, pharmacology or toxicology are urgently needed



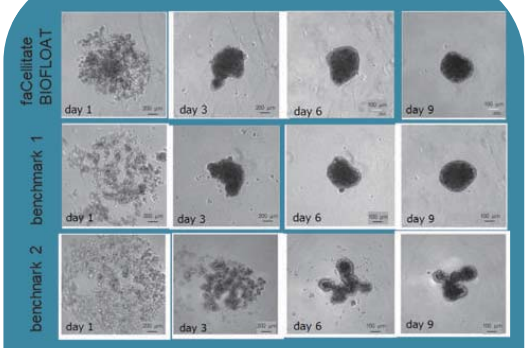
BIOFLOAT™ – features

- DIY coating of polystyrene, glass and PDMS by simple rinsing process – no chemical treatments
- Stable anti-adhesive layer
- Coating prevents attachment of proteins and cells
- Generation of cell instructive surfaces by biological tags

Our solution

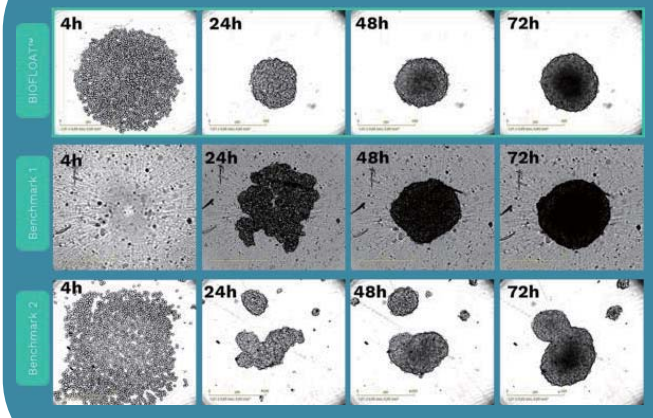
- Rapid and round spheroid generation allowing cell-cell contacts to resemble natural environment
- Cell and protein attachment reliably avoided for optimal cell analysis
- Applicable to various complex systems, including bioreactors and microfluidic devices

BIOFLOAT™ – spheroids with challenging cells



- **Round** uniform hepatocytes spheroids. No satellites
- CYP activity measurable -> **vital** hepatocytes spheroids
- Human hepatocytes are **highly relevant** models for liver diseases/ damage in toxicological studies

BIOFLOAT™ outperform current competitors



- **Round** and **reproducible** spheroids. No satellites
- **Rapid** and **reliable** formation of vital spheroids within few hours. No centrifugation needed
- **FACILITATES** the development of *in vivo* similar model and testing systems for research and industry application

BIOFLOAT™
Order your free sample

faCellitate GmbH
Franz-Volhard Straße 5,
68167 Mannheim
Germany

www.facellitate.com
info@facellitate.com

Contact: valentina.fermi@facellitate.com

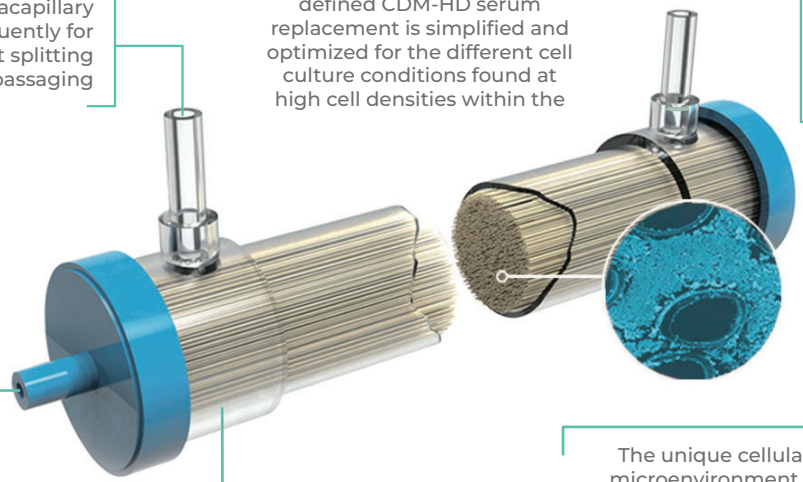
Hollow Fiber Bioreactors: A Better Way to Grow Cells

A closer look at the FiberCell Systems cartridge

Cells seeded in the extracapillary space grow post-confluently for many months without splitting or passaging

FiberCell System's chemically defined CDM-HD serum replacement is simplified and optimized for the different cell culture conditions found at high cell densities within the

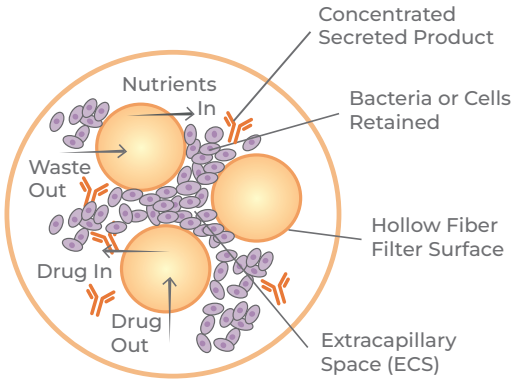
Ideally suited for continuous collection of secreted products at high concentrations



The Duet pump continuously recirculates medium through the hollow fiber bioreactor cartridge (HFBR) to provide nutrient and oxygen while removing waste and CO_2

The closed, bio-safe system is cGMP compliant

The unique cellular microenvironment is generated by the cells themselves and facilitated by the molecular weight cut off of the fibers



APPLICATIONS

3-D Cell Culture

The medium-sized cartridge offers 3,000 cm^2 of surface area, equivalent to 40 T-75 flasks, but can support up to $2x10^9$ cells

Monoclonal Antibody Production

In the HFBR, hybridomas can yield up to 100 mg of antibody every two days, for months of culture

Recombinant Protein Production

Typical protein harvests from FiberCell Systems' cartridges contain 100 µg/mL/day or higher. Daily harvests are 2 mg to 10 mg a day.

Exosome Production

Gram quantities of exosomes can be produced in HFBRs

Cell Co-culture

The PVDF fiber allows various matrix proteins to be bound to its surface

PK/PD

Mimic human bioavailability of antibiotics

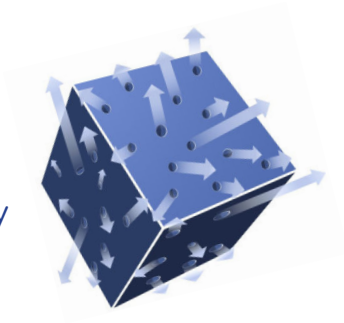
Learn more at **www.fibercellsystems.com**

FiberCell Systems®
A BETTER WAY TO GROW CELLS

HiSS Diagnostics

Growth Factors

PODS® slowly release their growth factor cargo through nanopores, generating steady-state bioavailability (near zero-order kinetics)



100% protein | Slow-releasing | Versatile

PeptiGels

synthetic peptide hydrogels (PeptiGels®) and sustained release growth factors (PODS®) - to provide a reproducible and highly controlled environment for 3D cell culture

Animal-free option | Highly-cited | Reproducible

Matrix Proteins

rigorously controlled for quality and functionally tested for optimal performance in cell culture

Cell Counting

Orangu™ a non-cytotoxic, sensitive, colorimetric assay for the determination of viable cell numbers in cell proliferation and cytotoxicity assays

Non-cytotoxic | Sensitive | Easy-to-use

produced by

CELL guidance systems

provided by

HiSS Diagnostics
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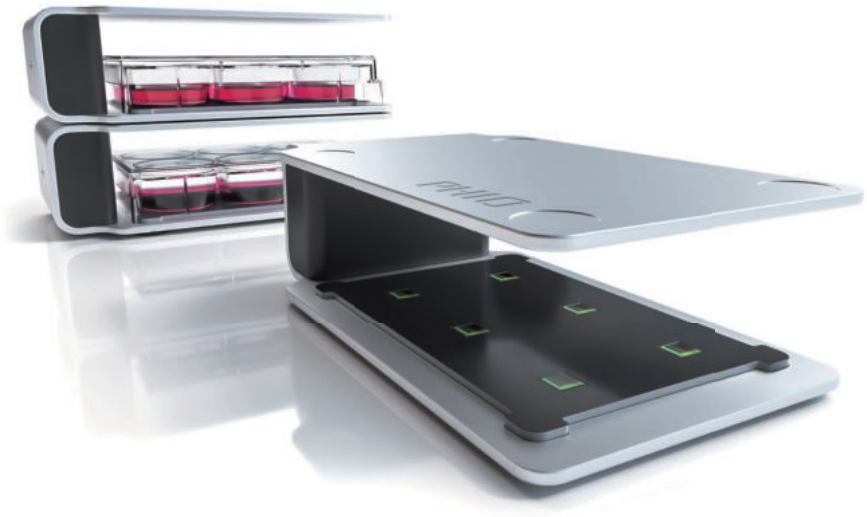
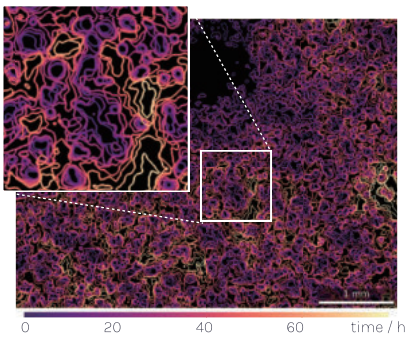
Cellwatcher M

Multiply your assay output by six.

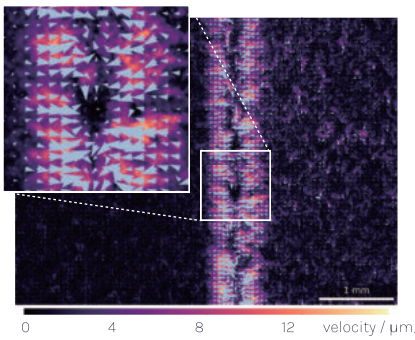


Easily track cell growth, viability, motility, phenotype and drug response, without leaving your desk. Get continuous data, processed objectively and automatically, without any additional manual work steps. Benefit from higher quality of assays monitored inside the incubator and find your publication-ready results online.

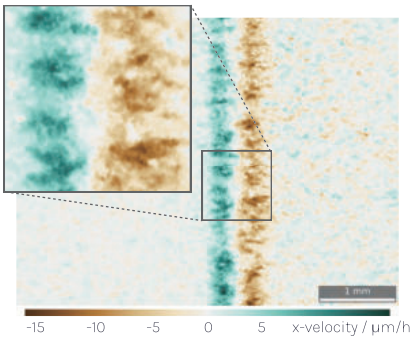
Acquire multiparametric data & six conditions at a time.



Cluster growth of stem cells



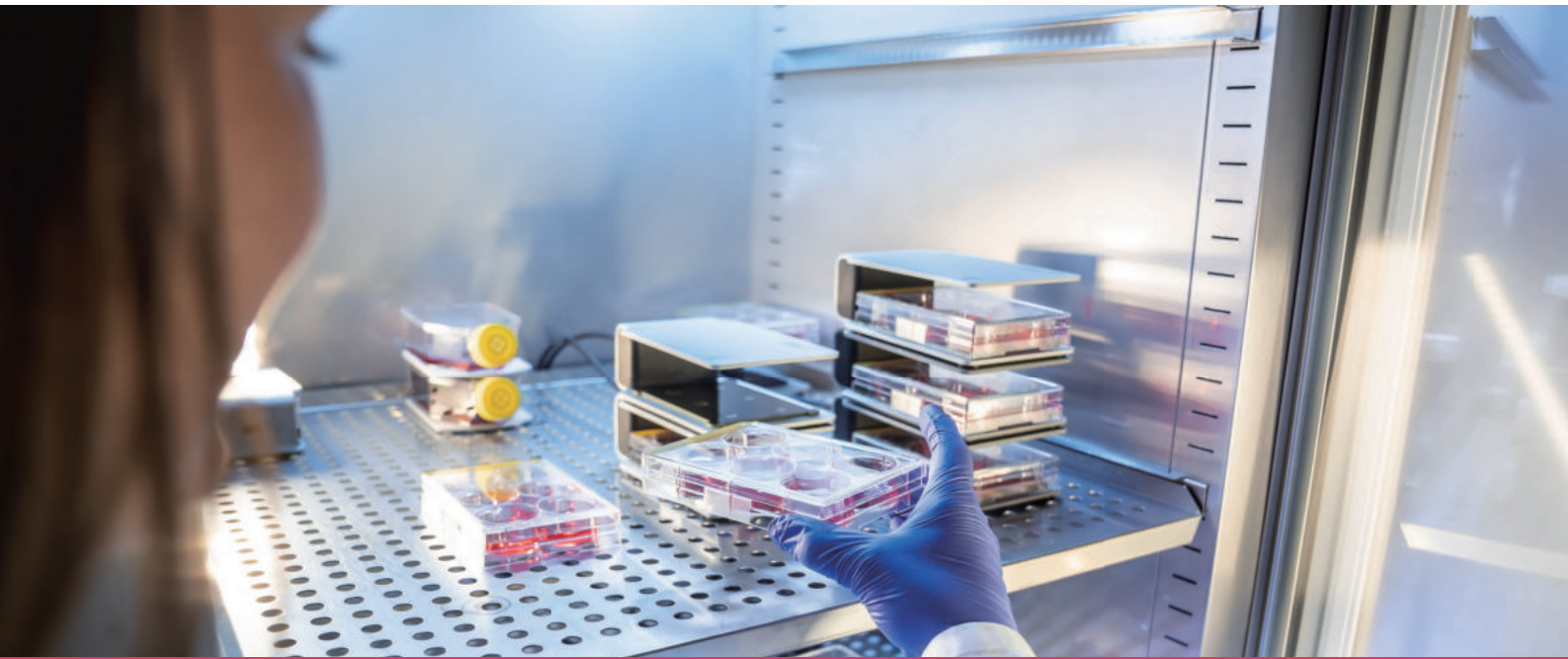
Spatially resolved cell motility of a scratch



Cell motility heatmap of a scratch

Objective AI analysis, done in real-time, ensures reproducible results without the expertise, effort, and errors of manual image analysis. Simply monitor your cells' behavior across multiple conditions simultaneously and get results instantly. Top-notch visualizations and multiparametric data down to the single cell level - Cellwatcher M and PHIOme level up your analysis of e.g. wound healing, drug screens, cluster formation, and more. Access all of your data through your browser via the PHIOme cloud: anywhere, anytime.

Live cell monitoring inside the incubator. Easy.



Countstar
Smart Cell Analysis

Our Galactic Four to Monitor Your Cell Culture Processes

Countstar® Altair

Countstar® Rigel

Countstar® Mira HT

Countstar® Mira Plus

Cell Titer – Viability – Productivity – Phenotyping
Image Based – Precise – Cost-Effective

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ALT BIOTECH

sphere FLUIDICS

work small, think big.

Cell Selection,

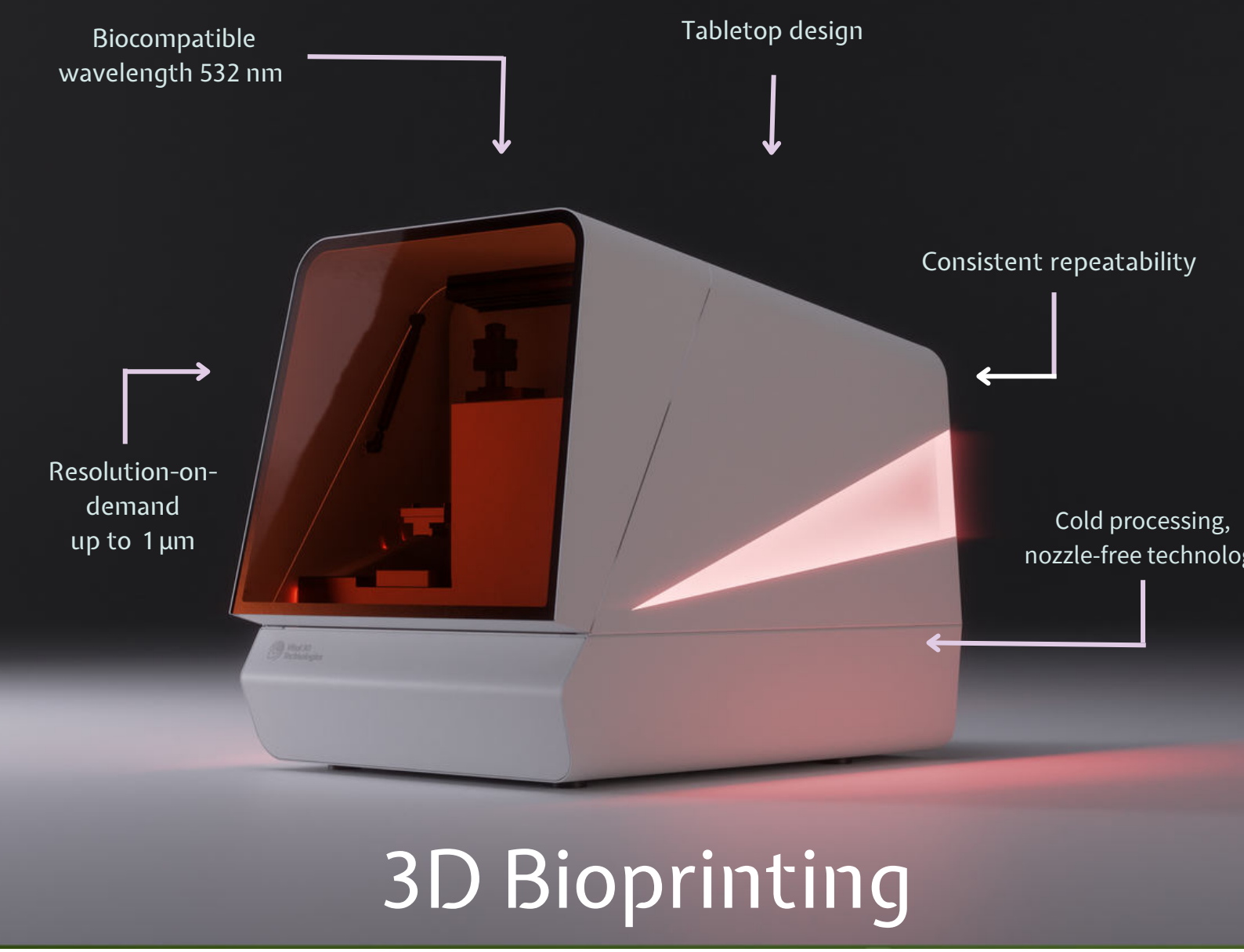
Sorted.

Simplify and accelerate your cell line development with Sphere Fluidics' automated platforms

- ✓ Completely integrated system
- ✓ Select "high producing clones" from a large transfection pool
- ✓ Cuts project lengths from months - weeks
- ✓ Dramatic reduction in reagent costs
- ✓ Provides evidence of monoclonality
- ✓ Cell friendly processing
- ✓ Traceable and certified AOF reagents and consumables
- ✓ Ease of use - low training requirements
- ✓ Small footprint

spherefluidics.com

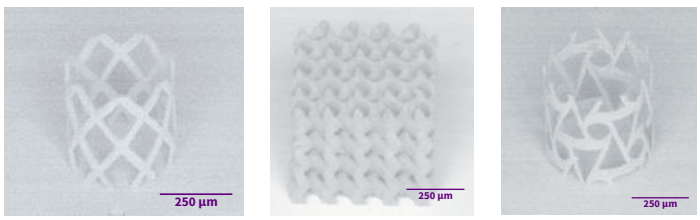
Vital 3D Technologies



3D Bioprinting Innovations

Vital3D Technologies is developing innovative 3D bio-printing tools dedicated to the production of fully functional organs.

Company technology stands out due to its extraordinary printing efficiency thanks to resolution-on-demand capability. Our first commercially available device - Vital Light 3D - is the fastest bio-printer in the market with printable feature size down to ~1 µm.



vital3D.eu **/vital3d**

WORLD PRECISION INSTRUMENTS

Accelerate Your Life Science Research with Our New EVOM™ Auto High-Throughput Screening System

The Gold Standard for Automated TEER Measurement of Both 24 and 96 HTS Multiwell Plates

EVOM™ Auto with both 24 and 96 HTS multiwell plate capability is a high throughput screening (HTS) platform offering fast, non-invasive sample scanning by comparing electrical resistance measurements (transepithelial/transendothelial electrical resistance: TEER). TEER measurement experiments are simple to set up and less time consuming than more complex molecular studies. TEER measurement can be used as the primary scanning method to identify any physiological changes that can then be further evaluated by other methods. EVOM™ Auto can capture TEER measurements in 24 and 96 HTS multiwell plates from Corning, Millipore, or MatTek. The sample preparation time in these HTS plates is efficient, allowing for fast, multi-channel pipetting options.

APPLICATIONS

- Epithelial & Endothelial Barrier Studies
- Confluence
- Lung Viral Infection
- Toxicity
- Antibody-Antigen Binding
- Blood Brain Barrier (BBB)
- Cancer Tissue Studies
- Intestine, Kidney and Liver Tissues

Additionally, the EVOM™ Auto electrode disinfection capability during measurement minimizes sample cross-contamination. Its wireless device control offers the convenience of operating the instrument from a distance, and the small footprint of EVOM™ Auto enables you to use it inside a sterile cell culture hood or an incubator. The EVOM™ Auto provides a fast and efficient platform for early drug discovery, by narrowing down drug targets and drug concentrations through automated, non-invasive sample scanning.

EVOM™ Auto Expedites Life Science Research by Accelerating:

- Creation of New and Effective 2-D and 3-D *In Vitro* Disease Models to Understand Disease Initiation and Progression
- Identification of Physiological Changes in Disease Models
- Quality Control of 2-D and 3-D *In Vitro* Tissues for Novel Drug Identification
- Target Screening, Identification and Verification
- Formulation Optimization & Compare Effectiveness
- Evaluate Cytotoxicity
- Novel Assay Development

WORLD PRECISION INSTRUMENTS

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Determining

The Feasibility of Transporting Retinal Organoids and Scaffolds Using the Cellbox.

Cellbox Solutions

The Gamm Lab utilizes stem cell technologies to investigate the cellular and molecular events during retinal differentiation and generate cells to develop advanced disease models. By understanding the behavior of these cell types, they hope to optimize strategies to delay or reverse the effects of blinding disorders such as retinitis pigmentosa and age-related macular degeneration.

Gamm Lab, University of Wisconsin-Madison.

> "Truth-in-advertising for battery life, and cell viability claims are sufficient for cross-campus transport. The Cellbox can be used to ship organoids without significant changes to the cell viability or performance.

THE PROJECT

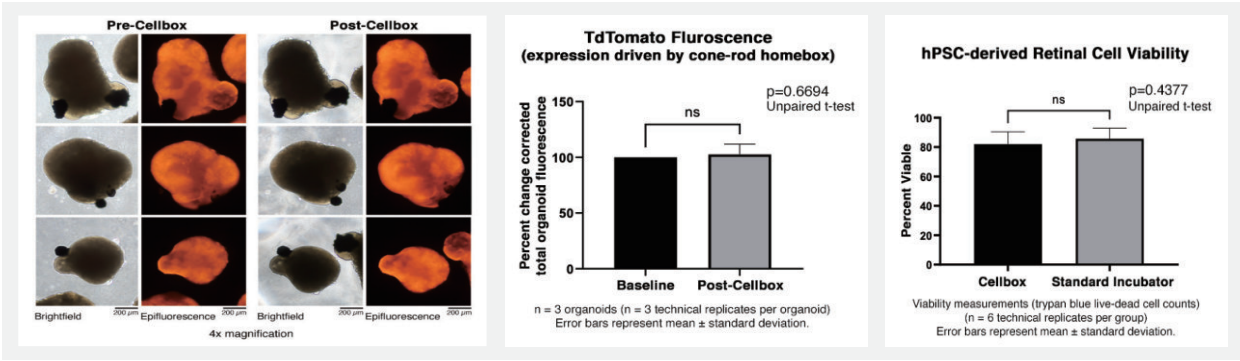
Transporting organoids and biomaterial scaffolds can be very stressful to delicate retinal neurons and typically requires the use of special shipment media. Cryopreservation or transport at ambient temperature is often not possible due to the fragile nature of the cells and their extracellular matrix. For this reason, the Gamm Lab looked for alternatives to shipping delicate cell products and found the Cellbox.

The purpose of this trial was to evaluate the Cellbox as a viable means of transporting the fragile retinal organoids and biomaterial scaffolds. This was done in two experiments - the initial testing of the empty device; checking the cell viability and usage feasibility pre- and post-transport.

Using a controlled heating device that also provides CO_2 allows for a safe transport of these cells under standard incubation conditions. Cellbox offers a solution to transport samples in a controlled environment with temperature and CO_2 regulated during product maturation and maintenance phases.

The Cellbox device was first tested to confirm the advertised running times. Once this was validated, organoids were packaged according to Cellbox recommendations and transported across campus to another building for imaging.

Total organoid TdTomato fluorescence (an indicator of photoreceptor survival) and cell viability tests were performed before and after the transport, and the comparison yielded no significant differences in any of the samples after Cellbox transport.



Agrostemmoside E: a potent tool for transfection assays

Transfection is the process to bring nucleic acids into eukaryotic cells. However, on the way to the cell, protection mechanisms kick in, which degrade the foreign genetic material. Hence, chemical modifications of the nucleotides and protective nanoparticles were developed. In this way, it is understood that the nanoparticle is endocytosed once it reaches the cellular membrane. Thereby, the genetic cargo reaches intracellular compartments such as early endosomes, which are acidified to become lysosomes. This process leads to further degradation and it is established that *only one percent* of the genetic material is able to escape the endosome into the cytosol, making this step **the major current obstacle** for efficient delivery of the genetic cargo.

Plant secondary metabolites, such as triterpene glycosides (saponins) have an amphiphilic structure, which allow them to interact with cellular membranes and trigger endosomal escape. In our group we could establish the continuous isolation of Agrostemmoside E (AG1856) in high purity. This saponin offers a beneficial therapeutic window and reliable, cell line dependent results. Application of this molecule to cellular assays can reduce genetic material needed for any transfection assay, since more genetic material escapes the degradation process.

Spheroid culture of murine primary chondrocytes and ATDC5 chondrocyte cell line for musculoskeletal research

Authors: Melanie Timmen, Simone Niehues, Amelie Franke, Elnaz Enderami, Richard Stange

Institute of Musculoskeletal Medicine, Department of Regenerative Musculoskeletal Medicine, University of Muenster, Muenster, Germany

Background: Cartilage tissue consists of chondrocyte cells and surrounding extracellular matrix. Chondrocytes pass through different stages from progenitor cell (origin form mesenchymal stem cells) to proliferating cells that secrete proteins forming the extracellular matrix to hypertrophic chondrocytes that form bloated cell bodies. The extracellular matrix contains different type of collagens mainly, but also a wide variety of proteoglycans, growth factors and cytokines dependent on the impact of the tissue. Cartilage appears mostly avascular and can be calcified e.g. within the growth plate or during bone healing. Growth of chondrocytes *in vitro* seems to be difficult, because of different behavior of cells in plastic associated 2D cultures or as 3D, which is often performed in micromasses or hanging drop cultures. We focused on investigation of chondrocyte growth from different sources in small spheroids and characterize the behavior of the cells. Our aim is to reconstitute cellular interaction of chondrocytes with other cell types like endothelial cells and osteoblasts.

Methods: Isolation of primary chondrocytes: The cartilaginous knee joint (hind limb) of 3-5days old mice were sequentially digested using 3mg/ml Collagenase IV. Cells were seeded in non-adherent U-bottom wells and cultured for up to 32 days in basal medium (DMEM low glucose, 10% FBS, 0,2mM L-Ascorbat-2-Phosphate, 100 U/ml Penicillin/Streptomycin (P/S)) or differentiation medium (DM) (basal medium with 100nM dexamethasone, 1mM pyruvate, 40µg/ml proline, 10ng/ml TGFbeta1, ITS-Premix. ATDC5 cells were cultured in DMEM F12, 5% FBS, 1mM pyruvate, 100U/ml P/S, and ITS-Premix. Spheroid of both cell types were seeded with 2.500 and up to 30.000 cells per well, medium was changed every 48h and cells were cultured at 37°C under normoxia (5% CO_2). For analysis, spheroids were fixed using 4% paraformaldehyde and embedded in paraffin. Slices (5µm) were stained for proteoglycans using Alcian blue staining. Immunhistocytology with antibodies against collagens as well as quantitative real time PCR to evaluate mRNA expression of marker genes for chondrocyte differentiation was performed.

Results: Primary chondrocytes build up nice spheroids with every condition used although some loose cells did not join the spheroid. Neither the two different media nor the different cell numbers/spheroid negatively affected the formation of spheres. We observed no necrotic areas within the spheroid tissue especially within the bigger spheroid or after long cultivation time (32 days). Alcian blue staining revealed the formation of extracellular matrix from day 7 on. From day 14 on cellular morphology pointed to the appearance of hypertrophic chondrocytes that was stable until day 32. However, at the outer edge of the spheroid alcian blue negative areas could be found. Collagen Type 2 positive staining was found at day 7 at the outer edge of the spheroid getting thicker with time. Using chondrocyte differentiation medium expression of marker genes like Col2a1 (collagen type II) and colX (collagen type X, hypertrophic chondrocytes) as well as Sox 9 were stimulated. ATDC5 spheroids formed faster and no loose cells were observed. Spheroid size increased during cultivation pointing to further proliferation. Alcian blue staining also revealed the formation of matrix starting at day 14, so the differentiation of chondrocytes seems to be somewhat slower compared to primary cells. Even after 21 days of culture or in bigger spheroids, no necrotic regions have been noticed. At day 21, cellular morphology indicated the onset of hypertrophic chondrocyte development. However, due of the high cell density (30,000 ATDC5 cells), alcian blue negative areas could be detected.

Conclusion: Both, primary chondrocytes and ATDC5 cell line, form nice cartilaginous spheroids with appropriate extracellular matrix, differentiation of cells from pre- to hypertrophic cell morphology and expression of marker genes. Both reveal to be a good basis for development of co-cultures with other cell types like endothelial cells and osteoprogenitors.

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Development of an organoid-based cell transformation assay for intestinal carcinogenicity screening of chemicals

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Abstract The high incidence of colorectal cancer (CRC) is closely associated with the exposure to environmental carcinogens. In order to assess intestinal carcinogenic potential of chemicals, we established an adult stem cell-derived intestinal organoid model from mouse intestine (mASC-IOs) and develop a cell transformation assay (CTA). Typical chemicals were selected to assess the intestinal carcinogenesis using mASC-IOs-based CTA. The experimental design was optimized including dose range, frequency and duration of administration, indicators and period of experiment and so on. Sixteen environmental chemicals (Cd, iPb, Cr-VI, iAs-III, Zn, Cu, PFOS, BPA, PCB-126, MEHP, AOM, DMH, BaP, MNNG, Aspirin, Metformin) were selected to test the sensitivity, specificity, and accuracy of this assay. mASC-IOs maintained the biological characteristics of their origins in terms of self-organization, proliferation, differentiation, organoid morphology, genetic stability following limited splitting. To conduct mASC-IO-based CTA, mASC-IOs were treated with chemicals for 72 h after seeding, and the treatment was repeated 4 times. The carcinogenic latency of chemicals was examined for at least 10 weeks, determined by single-cell colony-formation rate in colony formation assay and anchorage-independent growth assay, and confirmed by tumorigenicity assay in nude mice. The results of carcinogenicity from 16 chemicals were highly corresponding to the epidemiological results (10/13), *in vivo* mouse studies (12/16), *in vitro* studies (11/14) and CTA results conducted following OECD guideline (6/11). Increasing proliferation of mASC-IOs was an early key event in chemical-induced oncogenic transformation. Frequency of colony formation in single-cell colony formation assay and anchorage-independent growth assay was significantly correlated with tumor formation rate in nude mice, which could be used to analyze the dose-dependent carcinogenic potential. We also found that the degree of tumor malignant in nude mice was determined by the differentiation level of mASC-IOs in the group of chemicals with carcinogenicity. In addition, the expression level of the most commonly mutated genes in human CRC, including APC, TP53, KRAS, and SMAD4 were highly correlated with the activity of chemical-induced oncogenic transformation. Taken together, we have successfully developed a mASC-IO-based CTA for intestinal carcinogenicity screening of chemicals, which plays an important role in promotion of the development and application of organ-specific screening systems.

This work was supported by NSFC 82204080 and NKPs 2017YFC1600200.

Keywords: Colorectal cancer, Intestinal organoids, Cell transformation, Environmental chemicals, Carcinogenicity screening

PeptiGels™: A technological platform for the design of fully defined synthetic 3D hydrogel scaffolds for cell engineering applications.

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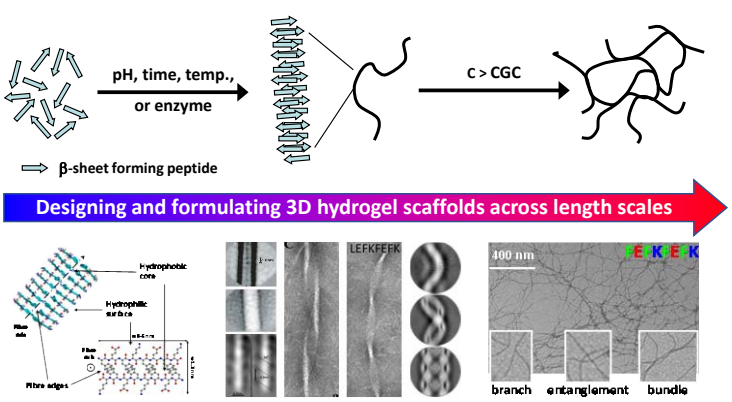
The use of non-covalent self-assembly has become a prominent strategy for the construction of increasingly functional biomaterials for a range of applications. A variety of molecular building blocks can be used for this purpose, one such block that has attracted considerable attention in the last 20 years is *de-novo* designed peptides. Our group work focusses on the development of a technological platform for the design of novel biofunctional 3D hydrogels scaffolds exploiting the self-assembly of so-called β-sheet forming peptides. These scaffolds can be easily functionalised using specific biological. Through the fundamental understanding of the self-assembly and gelation processes of these peptides across length scales we have been able to design hydrogels with tailored properties.



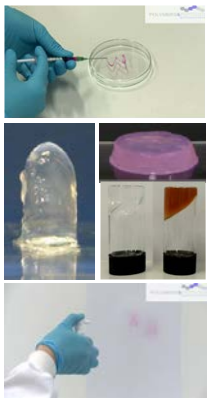
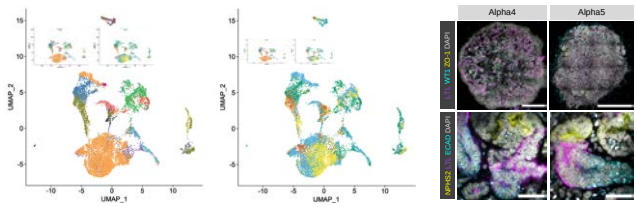


Figure: (Top) Schematic representation of the self-assembly process of β-sheet forming peptides. (Bottom) Characterisation across length scales for molecular assembly to network cross-linking. (Right) Examples of injectable, sprayable and functional hydrogel scaffolds

These 3D hydrogel scaffolds have been used in a range of contexts including cell culture, drug delivery tissue engineering, bioprinting and organoid engineering:



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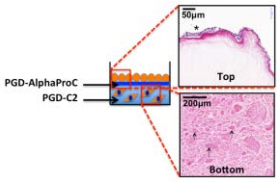

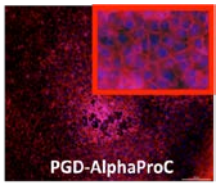

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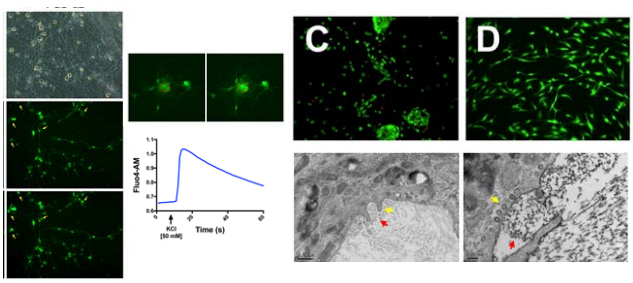

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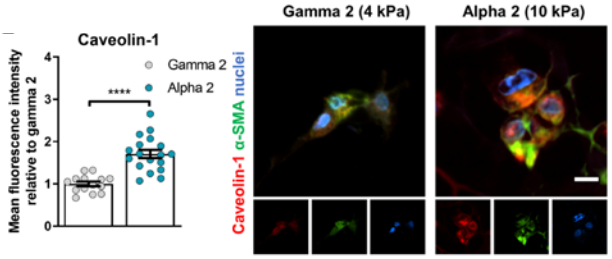

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Creation of skin cell spheres and improved methods for measuring cell viability in the 3D model

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The most common *in vitro* model of skin for testing pharmaceuticals and cosmetic products is the 2D cell monolayer. However, it has a significant drawback as it oversimplifies the interactions within the model (1). In contrast, 3D *in vitro* models, including spheres and equivalents, provide a more accurate representation of cell-cell interactions. Several methods for producing spheres, such as the hanging drop method (HD) and limiting cell adhesion (LCA), are widely known (2-3). Unfortunately, they usually require additional equipment or do not provide sufficient repeatability (4). Moreover, many existing protocols for cell viability assays are tailored to 2D models, hence the optimization step before conducting experiments on the 3D *in vitro* models is needed. Therefore, our study aims to optimize methods of sphere creation and to adjust the existing viability assays for their usage in these advanced models.

This study was conducted on the primary human skin cells and commercial cell lines. The HD and the LCA methods were compared in the context of sphere formation. To determine which surfactant would better limit the cell adhesion Pluronic F127 (Poloxamer 407), Pluronic F68 (Poloxamer 188) and Kolliphor EL (PEG35 castor oil) were applied as coating agents. Cell viability was analyzed by the dehydrogenase (MTT) and esterase (FDA) activity assays.

The LCA method provided more repeatable results of the sphere formation than the HD method. Above all, spheres differed in morphology depending on the used cell type. Different melanoma cell lines showed varied capacity to form compact cell aggregates, which could be correlated to their adhesion properties visible in the 2D culture. A low cytotoxic influence of the two coating agents Pluronic F127 and F68 on skin cells was confirmed, whereas the application of Kolliphor EL led to cell membrane damage. The surfactant's influence on the cell adhesion depended on its concentration. The influence of the extracellular matrix proteins on the process of cell arrangement into aggregates is minor. Although the FDA assay can be directly applied to the sphere model, the MTT protocol needs modifications to improve the dissolvent of formazan from cell spheres.

Our findings indicate that the basic 3D cell culture protocols can be further optimized to facilitate the preparation of these models and their usage.

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Keywords: 3D *in vitro* models, skin cells, spheres, limiting cell adhesion, surfactants.

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