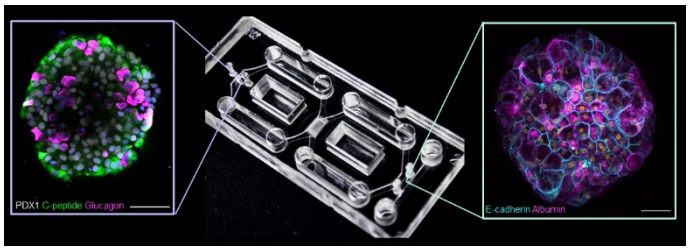
NOR-MPS symposium - microphysiological systems, organoids and organ-on-chip technology



Wednesday February 14th, 2024
Venue: Meet Ullevaal / Ullevål stadion

0900 - 0915 Welcome/introduction
Prof. Stefan Krauss

0915 - 0950 Keynote speaker I
Dr. Jesse Veenvliet
Connecting scales & spaces in stembryos

0950 - 1025 Keynote speaker II
Prof. Anna Herland
Engineering cellular interfaces in Organ-on-Chips

1025 – 1045 Coffee break

1045 – 1220 Presentations of Norwegian Research Groups

PI talks:
1045-1055 Stefan Krauss
1055-1105 Oncosyne
1105-1115 Bill (William) Louch
1115-1125 Frøydis Sved Skottvoll
1125-1135 Håvard Jostein Haugen

PhD/postdoc talks:
1135-1140 Aleksandra Aizenshtadt
1140-1145 Joachim Mossige
1145-1150 Kushtrim Kryeziu
1150-1155 Ingrid Wilhelmsen
1155-1200 Mathias Busek
1200-1205 Stian Kogler

1205-1210 Torben Hildebrand
1210-1215 Chencheng Wang
1215-1220 Nicola Pietro Montaldo
1220-1225 Franziska Jessica Schoeb
1225-1230 Igor Meszka

1230 - 1300 Lunch / installation of posters

1300 - 1345 Poster session

13:45 – 14:45 Presentations of Norwegian Research Groups

PI talks:
1345-1355 Katja Elgstøen
1355-1405 Steven Ray Wilson

PhD/postdoc talks:
1405-1410 Ina Katrine Nitschke Pettersen
1410-1415 Ludivine Delon
1415-1420 Malgorzata Elzbieta Zawadzka
1420-1425 Marissa Maciej-Hulme
1425-1430 Julie Aarmo Johannessen
1430-1435 Kristina Sæterdal Kømurcu
1435-1440 Hege Brincker Fjerdingstad

1440 - 1455 Coffee break

1455 - 15:25 Keynote speaker III
Dr. Anirban Chatterjee:
Merck's ambition to phase out animal work

1525 - 1600 Panel discussion Ethics/Funding/Future Perspectives

Heidi Bentzen - (5 min on ethics)
Ole Johan Borge - confirmed (5 min on funding and innovation)
Anna Herland (5 min on future perspectives)
20 min panel discussion

1600 - 1615 Poster prize and wrap up Stefan Krauss, Viola Lobert, Steven Wilson

Keynote speakers

Jesse Veenvliet, a Max Planck Research Group Leader at MPI-CBG, Dresden, is known for pioneering embryo development modeling in a petri-dish. His research focuses on using "stembryos" to study mammalian embryogenesis' design principles, employing a mix of developmental engineering, omics, imaging, and genetics.

Connecting scales & spaces in stembryos

Embryonic development requires multi-scale coordination of tissue morphogenesis, cellular behaviours, and gene expression to ensure highly reproducible differentiation outcome at the systems level. A deep understanding of the feedbacks that govern such robustness requires the study of the processes that pattern and shape the embryo in live specimens, in toto, across spatial and temporal scales. In mammalian embryogenesis, this remains a major challenge as the embryo develops in utero, precluding easy accessibility. These impediments can be overcome by coaxing pluripotent stem cells to form three-dimensional embryonic organoids (or stembryos) in vitro. Gastruloids are stembryos that break symmetry, elongate and self-organise the major body axes, but lack the stereotypical architecture of the embryo1-5. We have shown that the latter can be unlocked by precisely timed addition of an extracellular matrix, resulting in trunk-like-structures (TLS) that molecularly and morphologically resemble the core part of the embryonic trunk5. In contrast to its in vivo counterpart, these stembryos are easy to access, track, manipulate and scale. I will explain how we leverage these unique properties to identify minimal sets of inputs necessary to pattern and shape the mammalian embryo through controlled modulations of the system. Moreover, I will discuss novel frameworks we have developed that allow us to connect transcriptional, cellular and morpho-states in space and time, and how these help us to uncover the design principles of regulative development during critical windows of mammalian embryo morphogenesis.

Anne Herland, professor and group leader at the Royal Institute of Technology, Stockholm, her research, affiliated with Karolinska Institute, focuses on neural models and Hybrid Bioelectrical Systems, with a particular emphasis on utilizing Organ-on-Chip technology. With 28 peer-reviewed publications and 10 filed patents, Herland is at the forefront of interdisciplinary research bridging engineering and neuroscience.

Engineering cellular interfaces in Organ-on-Chips

Anna Herland[1, 2]

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Engineered human microfluidic Organ-on-Chip models are a promising pre-clinical technology for studies of specific cellular mechanisms and drug responses in physiological environments. In Organ-on-Chips, cells are cultured in interfacing microcompartments with perfusion. In our group, we are developing these systems from three perspectives: first, to increase human physiological relevance through stem cell engineering and a defined three-dimensional microenvironment; second, to incorporate non-disruptive real-time monitoring of cells; and third, to make them user-friendly and cost-effective. Our specific focus is the neurovascular unit (NVU), the restrictive barrier that lines the capillaries in the brain and spinal cord. We have developed and validated protocols to generate vascular and neural cells from human pluripotent stem cells for our NVU-on-Chip models.

To enable higher-throughput fabrication and better control of drug exposure, we developed a new chip material, off-stochiometry-thiolene epoxy (OSTE). OSTE allows a one-step fabrication process to integrate real-time barrier monitoring electrodes in the chip. The chip and perfusion system have been tailored to study barrier penetration of small drugs (Winkler, *ACS Appl. Mater. Interfaces* 2021*)* and biopharmaceuticals, cellular interactions, and inflammatory responses in real-time (Matthiesen *Small* 2021, Matthiesen, *Biosensors* 2022).

Our standard chip format allows cellular interactions through permeable membranes with limited flexibility. To study defined cell interactions in full 3D geometry, we have developed new laser patterning techniques. We recently developed scaffolds with micrometer precision via two-photon printing and could delineate contact and non-contact mediated mechanisms between astrocytes and neurons (Buchmann, Materials Today Bio, 2023). To enable studies of vascular organoid interactions and physiological drug exposure of organoids, we have developed a femtosecond laser patterning method termed cavitation molding. Cavitation molding allows us to pattern 10-20 μm wide lumens in ECM gels in the direct vicinity of an organoid without impairing its viability. These lumens were populated with endothelial cells, forming a defined vascular network around the organoid (Enrico, *Advanced Materials* 2022). We are now designing our neurovascular systems as a combination of top-

down and bottom-up approaches with integrated or downstream sensing. Our specific aim with these systems is to better understand metabolic interactions and disturbances in the neurovascular unit, which we have previously studied in human primary cells (Maoz and Herland, Nature Biotechnology, 2018). We are now investigating NVU interactions in patients with inborn metabolic diseases as well as models of radiation damage.

References

Buchmann, S., Enrico, A., Holzreuter, M.A.,Reid, M., Zeglio, E., Niklaus, F., Stemme, G., and Herland, A. (2023) Probabilistic cell seeding and non-autofluorescent 3D-printed structures as scalable approach for multi-level co-culture modeling. *Materials Today Bio, Volume 21, August 2023, 100706*, doi: 10.1016/j.mtbio.2023.100706

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Matthiesen, I., Nasiri, R., Tamashiro Orrego, A., Winkler, T.E. & Herland, A. (2022) Metabolic Assessment of Human Induced Pluripotent Stem Cells-Derived Astrocytes and Fetal Primary Astrocytes: Lactate and Glucose Turnover. *Biosensors* **2022**, *12*, 839. doi:10.3390/bios12100839

Maoz BM*, Herland A*, FitzGerald EA, Grevesse T, Vidoudez C, Pacheco AR, Sheehy SP, Park T-E, Dauth S, Mannix R, Budnik N, Shores K, Cho A, Nawroth JC, Segrè D, Budnik B, Ingber DE, Parker KK. A linked organ-on-chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells. *Nature Biotechnology*. 2018;36:865-874. doi:10.1038/nbt.4226.

Winkler TE, Herland A, Sorption of Neuropsychopharmaca in Microfluidic Materials for In Vitro Studies, ACS *Appl. Mater. Interfaces* 2021, 13, 38, 45161–45174, doi: https://doi.org/10.1021/acsami.1c07639

Anirban Chatterjee is the head of the Advanced Cell Culture team, part of the Merck Life Science technology pioneering Group, USA. With a PhD and MBA, Anirban acts as a driver of innovation with Merck's ambition to phase out animal work.

Abstracts – short talks

A1

Modelling the metabolic crosstalk between islets and liver on an organ-on-chip (rOoC) platform

Aleksandra Aizenshtadt, Chencheng Wang, Shadab Abadpour, Pedro Duarte Menezes, Ingrid Wilhelmsen, Andrea Dalmao-Fernandez, Justyna Stokowiec, Alexey Golovin, Mads Johnsen, Thomas M. D. Combriat, Hanne Røberg-Larsen, Nikolaj Gadegaard, Hanne Scholz, Mathias Busek, Stefan Krauss

Hybrid Technology Hub Centre of Excellence, Institute of Basic Medical Science, University of Oslo, P.O. Box 1110, 0317 Oslo, Norway.

Type 2 diabetes mellitus (T2DM), obesity, and metabolic dysfunction-associated steatotic liver disease (MASLD) are epidemiologically correlated disorders with a worldwide growing prevalence. While the mechanisms leading to the onset and development of these conditions are not fully understood, predictive tissue representations for studying the coordinated interactions between central organs that regulate energy metabolism, particularly the liver and pancreatic islets, are needed. We have devloped a dual pump-less recirculating Organ-on-Chip (dual-rOoC) platform that combines human pluripotent stem cell (sc)-derived sc-liver and sc-islet organoids. The platform reproduces key aspects of the metabolic cross-talk between both organs, including glucose levels and selected hormones and supports the viability and functionality of both sc-islet and sc-liver organoids while preserving a reduced release of pro-inflammatory cytokines. In a model of metabolic disruption in response to treatment with high lipids and fructose, we show that sc-liver organoids exhibit hallmarks of steatosis and insulin resistance, while sc-islets produce pro-inflammatory cytokines *on-chip*. Finally, the platform reproduces known effects of anti-diabetic drugs *on chip*. Taken together, the platform provides a basis for functional studies of obesity, T2DM, and MASLD *on-chip*, as well as for testing potential therapeutic interventions.

DOI: 10.1002/adhm.202303785

A2

Developing coculture-based drug screening for in vitro diagnostics in pancreatic cancer

Peter W. Eide[1]; Jarle Bruun [1]

[1] *Oncosyne AS, Oslo Cancer Cluster Incubator, Ullernchausseen 64, 0379 Oslo*

Oncosyne is a biotech startup that has developed a technology platform for clinical-grade personalized drug screening. Its first-generation *in vitro* diagnostic test was developed and verified in an observational study, TargetCRC, that initially included 40 patients with primary **colorectal cancer** (**CRC**). In the period 2024-2026, test performance will be validated in a randomized-controlled trial for 2nd line metastatic CRC, DSEE-CRC. The clinical studies are run in collaboration with **Akershus University Hospital**.

Pancreatic ductal adenocarcinoma (**PDAC**) has the **lowest survival rates among major cancers** and accounts for 5% of all cancer deaths worldwide. Available therapies include gemcitabine- and 5FU-based chemotherapy regimens in addition to molecularly targeted therapies. For both classes response rates are less than 25%. There is an urgent medical need for methods that can determine which drugs are effective for individual patients.

The **tumor microenvironment** can modulate the cancer cells' response to drugs and, for PDAC in particular, this modulation appears clinically important. Oncosyne, **Hybrid Technology Hub** and **Sahlgrenska University Hospital** were recently granted 16 MNOK in funding to research & develop a next-generation *in vitro* diagnostic test. To this end, the consortium will (1) adapt Oncosyne's assay for PDAC; (2) evaluate HTH's OoC chip-technology for this use-case; and (3) evaluate the predictive accuracy in a randomized controlled-trial.

Here, we will present Oncosyne's technology platform, ongoing clinical studies on CRC, and plan for developing and demonstrating clinical feasibility for an in vitro diagnostic test leveraging co-culture, OoC, and drug screening for the **betterment of PDAC patients' outcome**.

A3

Stem cell-derived cardiomyocytes: from model system to implantation therapy

William Louch

Institute for Experimental Research, University of Oslo & Oslo University Hospital

The adult human heart has very limited regenerative capacity, and thus cellular loss during disease is highly detrimental. However, implantation of stem cell-derived muscle cells (cardiomyocytes) represents an exciting opportunity for repopulating the diseased heart. In recent years, the development of cardiomyocytes from human induced pluripotent stem cells (hiPSCs) has opened the door to creating such cells. Unfortunately, hiPSC-derived cardiomyocytes (hiPSC-CMs) produced to date exhibit poor maturation, inadequate contractility and unstable electrical stability leading to poor in vivo function upon transplantation. My group aims to improve hiPSC-CM differentiation, by fine-tuning the mechanical cues of the cells' environment, and manipulating the proteome to enhance the maturation of cellular substructure. We also employ hiPSC-CMs as a model system, to understand the cellular mechanisms that generate the heartbeat, and investigate how this process is disrupted by human mutations. Here, we are particularly focused on truncating mutations in titin which lead to an inherited heart failure condition. Titin is a giant elastic protein which our data shows is crucial to coordinating contraction across the cell. Our work is aimed at correcting cellular contractility in hiPSC-CMs derived from these patients, by altering the expression of specific titin isoforms.

A4

Towards Multi-Modality Organ-on-Chip Platforms; The Role of Silicon Micro-Nanofabrication

Frøydis Sved Skottvoll[a] , Enrique Escobedo-Cousin[a], Michal Mielnik[a]

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The development of Organ-on-Chip (OoC) technology has demonstrated significant promise for resembling human physiology, showcasing its advantages through various microfluidic designs, biological- and fabrication materials. In the scientific community, there is a growing consensus that the future advancement of OoC technology relies on the creation of robust platforms that offer increased throughput, enhanced customization, and more sophisticated integrated modalities for sensing and actuation. These evolving requirements are well-matched with the capabilities of silicon-based electronics and miniaturized micro-electromechanical systems (MEMS), areas where SINTEF MiNaLab has established expertise. This talk will delve into the potential of silicon-based MEMS within OoC technology, providing an overview of possible applications for actuation, sensing, sample preparation, and the hybrid integration of silicon-based and polymer-based materials.

A5

A New Technique for Creating High-Density Blood Vessel Networks in Organoids

Maik Schot[1], Timo Koch[2], Liebert Parreiras Nogueira[3], Andries van der Meer[1], Andreas Carlson[2], Håvard Haugen[4], Jeroen Leijten[1]

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[2] Department of MathematicsUniversity of Oslo, Norway
[3]Oral Research Laboratory, [4]Department of Biomaterials, Faculty of Dentistry, University of Oslo, Olso, Norway

INTRODUCTION: The vascular tree is crucial for the survival and function of large living tissues. Despite breakthroughs in 3D bioprinting to endow engineered tissues with large blood vessels, there is currently no approach to engineer high-density capillary networks into living tissues in a scalable manner. Here, photo annealing of living microtissue (PALM) is presented as a scalable strategy to engineer capillary-rich tissues. Specifically, in-air microfluidics produces living microtissues composed of cell-laden microgels in ultrahigh throughput, which can be photo-annealed into a monolithic living matter. Annealed microtissues inherently create an open, interconnected pore network within the resulting living matter. Interestingly, utilising soft microgels enables microgel deformation, which leads to the uniform formation of capillary-sized pores. Importantly, the ultrahigh throughput nature underlying the microtissue formation uniquely facilitates scalable production of living tissues of clinically relevant sizes (>1 cm3) with an integrated high-density capillary network. In short, PALM generates monolithic, microporous, modular tissues that meet the previously unsolved need for large engineered tissues containing high-density vascular networks, which is anticipated to advance the fields of engineered organs, regenerative medicine, and drug screening.

CONCLUSIONS: Our results reveal the potential of (photo)annealing of living microtissues to engineer living, monolithic yet microporous tissues containing an interconnected, high-density capillary-like network that serves as a blueprint for vascularisation and perfusion. Despite the intricate design of high-density capillary networks within the engineered tissues, these perfusable networks were created within minutes in a simple one-step approach, overcoming the compromise between resolution and speed inherent to traditional biofabrication approaches such as 3D bioprinting. This novel bottom-up tissue engineering approach effectively uncouples diffusive lengths from construct size by having its maximal diffusive length dictated by the size of the individual microtissues, enabling the creation of viable, large, engineered tissues. The capillary network facilitates near instantaneous and homogeneous delivery of key molecules such as nutrients and oxygen within engineered tissues, circumventing undesired diffusion gradients associated with traditionally engineered tissues. We envision that this novel biofabrication method may find widespread applications ranging from the scalable production of engineered donor organs and advanced drug testing models to lab-grown meat.

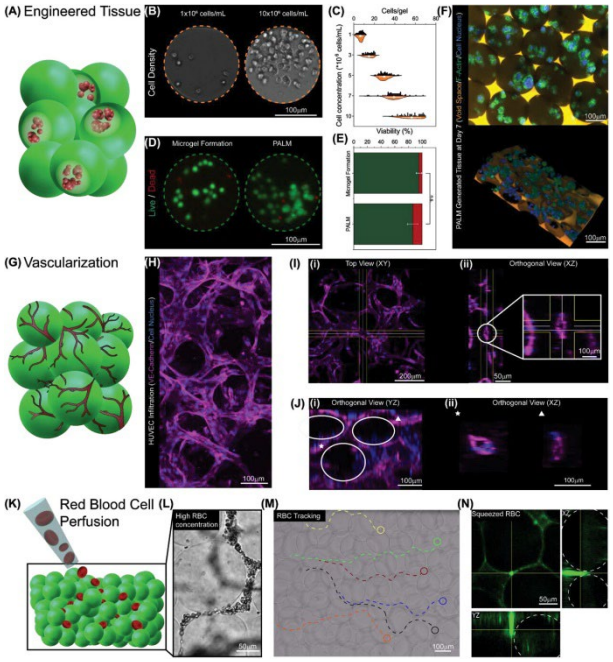

Fig. 1. Engineered and vascularised tissues generated through PALM. a) Schematic depiction of microgels containing cells annealed into a monolithic tissue through PALM. b) Microphotograph of microgels containing distinct concentrations of hepatocytes. c) Quantification of the amount of encapsulated cells per microgel dependent on the cell concentration in the precursor solution (n > 35). d) Fluorescence microscopy image of cell viability after encapsulation (i.e., physical cross-link) and PALM (i.e., covalent cross-link). e) Quantification of cell viability post encapsulation and post-PALM f) Fluorescence confocal microscopy image and 3D reconstruction of an engineered tissue generated through PALM using living building blocks after seven days of culture. Cells and interstitial space are fluorescently labeled with Phalloidin/DAPI and Tetramethylrhodamine-Dextran (TRITC–Dextran), respectively. g) Schematic depiction of PALM-generated construct's ability to vascularize the capillary pore space. h) Confocal maximum intensity projection of VE-Cadherin/DAPI-labeled HUVECs growing into the void space within packed microgels..

ACKNOWLEDGEMENTS: Dutch Research Council (Vidi, Grant No. 17522) and the European Research Council (Starting Grant, No. 759425).

A6

Pump-less recirculating organ-on-chip (rooc) platform to model the metabolic crosstalk between pluripotent stem cell-derived islets and liver organoids.

Aleksandra Aizenshtadt[1*], Chencheng Wang[1,3], Mathias Busek[1,2], Shadab Abadpour[1,3], Stefan Gruenzner[4], Alexey Golovin[2], Justyna Stokowiec[2], Hanne Scholz[1,3] and Stefan Krauss[1,2].

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The worldwide pandemic of obesity is associated with a substantial increase in the prevalence of metabolic-associated fatty liver disease and type 2 diabetes mellitus. Both conditions are metabolically interlinked and often coexist in patients, calling for reliable human *in vitro* models mimicking the interactions between pancreatic islets and the liver.

We recently developed a novel scalable pump-less recirculation OoC platform (rOoC) that generates a directional, gravity-driven flow and allows to link islets and liver organoids "*on chip"* without the need of external tubing[1]. The dual rOoC iteration used in this study includes a membrane between two recirculation loops allowing directed transport of proteins from the islets to the liver compartment, while supporting the exchange of glucose and other small molecules between the circuits. It is therefore well suited to model and interrogate the inter-organ(oid) crosstalk.

The platform reproduces key aspects of the metabolic cross-talk between both organs, including glucose levels and selected hormones, and supports the viability and functionality of both sc-islet and sc-liver organoids while preserving a reduced release of pro-inflammatory cytokines. In a model of metabolic disruption in response to treatment with high lipids and fructose, sc-liver organoids exhibit hallmarks of steatosis and insulin resistance, while sc-islets produce pro-inflammatory cytokines *on-chip*. Finally, the platform reproduces known effects of anti-diabetic drugs on-chip. Taken together, the platform provides a basis for functional studies of obesity, T2DM, and MASLD *on-chip*, as well as for testing potential therapeutic interventions.

1. M. Busek, A. Aizenshtadt *et al.*, *Lab. Chip* (2022), doi:10.1039/D2LC00919F.

A7

Flows of life

Joachim Mossige

RITMO Centre for Interdisciplinary Studies in Rhythm, Time and Motion, University of Oslo

How does mammalian life evolve, from a fertilized egg to a fully developed organism? This intriguing question has baffled us for centuries, and despite of intensive research efforts over the years, it is still far from understood. Especially, we lack a good description of the critical gastrulation stage, where a spherical ball of cells starts to elongate due to collective cell migration, curiously behaving as a flowing liquid. In the ITOM-project, we set out to describe these flows and how they shape early embryonic development. To establish a controlled experimental protocol, we use stem cell derived embryo models (gastruloids), which we study in the lab using a special home-built light-sheet microscope.

A7_2

Heterogeneity of tumor organoids derived from patients with colorectal cancer liver metastases

Kushtrim Kryeziu[1]; Solveig K. Klokkerud[1]; Max Z. Totland[1]; Jonas Langerud[1,2]; Christian H. Bergsland[1,2]; Barbara Niederdorfer[1]; Seyed H. Moosavi[1,2]; Kristoffer Lassen[3]; Henrik M Reims[4]; Trygve Syversveen[5]; Arild Nesbakken[1,2,6]; Sheraz Yaqub[2,3]; Tormod Guren[7]; Anita Sveen[1,2]; Ragnhild A. Lothe[1,2]

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Tumor organoids have emerged as powerful personalized medicine tools for predicting therapy response and nominating treatments in functional oncology trials. Recent studies have developed large living biobanks of patient-derived organoids to bridge the gap between basic and translational research. We analyzed hundreds of organoids from multifocal colorectal cancer liver metastases for heterogeneity in morphology, protein markers, genomic profiles, and drug sensitivity patterns within and between patients.

Tumor organoids recapitulated the corresponding tumor tissue samples at multiple levels such as morphology, expression of cancer cell-intrinsic proteins, transcriptomes and gene mutations. The diagnostic CRC biomarkers homeobox protein CDX2 and cytokeratin 20 were significantly associated with the morphology of the organoids. CDX2 expression was highest in tumor organoids with a “cystic” phenotype, while cytokeratin 20 expression was highest in “solid” organoids. The cystic organoids with well-differentiated histopathology were frequently wild-type for *KRAS*, *NRAS*, and *BRAF*, and were significantly more sensitive to anti-EGFR treatment. Cystic organoids showed upregulated WNT signaling, while solid organoids had TGF-beta pathway upregulation and were relatively resistant to conventional chemotherapies like 5-fluorouracil.

Together, these findings demonstrate that patient tumor organoids can recapitulate tissue heterogeneity and molecular phenotypes relevant to targeted therapy selection. The development of living organoid biobanks coupled with genomic and drug testing enables more effective functionally-guided treatment decision making.

A8

Investigating the Role of Vitamin A and Lipid Droplets on the Activation of Human Stem Cell-Derived Hepatic Stellate Cells

Ingrid Wilhelmsen,[1,2], Thomas Combriat[2], Andrea Dalmao Fernandez[1], Justyna Stokowiec[2], Chencheng Wang[2], Petter Angell Olsen[1,2], Aleksandra Aizenshtadt [2**,] Stefan Krauss [1,2**]

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***shared senior authorship*

Metabolic dysfunction-associated fatty liver disease (MAFLD) is a worldwide health concern that eventually leads to fibrotic scarring in which hepatic stellate cells (HSCs) play a crucial role. The HCSs display a quiescent phenotype in the homeostatic liver, which is characterized by the storage of vitamin A (VA) in intracellular lipid droplets (LDs). During MAFLD development, HSCs activate and transdifferentiate to a myofibroblast-like phenotype. Throughout this process, the HSCs lose their VA-containing LDs. However, the connection between VA and LDs and their impact on HSC activation is poorly understood. Additionally, *in vitro* HSC activation is notoriously challenging to model, as human HSCs are difficult to obtain and spontaneously activate upon *in vitro* cultivation. In this study, we use human stem cell-derived HSCs (scHSCs) as an *in vitro* model to study the effect of VA and lipid starvation on HSC activation induced by TGF-β. The cells were analysed by a plethora of methods, including analysis of VA and LDs by holotomographic live-cell imaging and Raman spectroscopy and metabolism by radioactive substrate oxidation assays, mRNA sequencing, and protein secretion assays. We found that while starvation of VA and lipids did not cause a pro-fibrotic phenotype on its own, the starvation in combination with TGF-β increased the activation-related transcriptomic profile of the scHSCs, indicating that the loss of VA and LDs contributes to the activation process. However, analysis of activation-related cytokines and proteins suggested that TGF-β alone caused the most pro-fibrotic effects. In conclusion, the results indicate that VA and LD loss is necessary, but not sufficient, for the activation of HSCs. We also highlight that the timing of the VA and LD loss may be important for the activation process of the HSCs, as protein secretion of pro-fibrotic compounds was decreased in VA- and lipid-starved conditions. Lastly, we demonstrate the suitability of scHSCs as an *in vitro* research model of HSC activation.

A9

Pump-less recirculating Organ-on-Chip (rOoC) platform – a versatile platform for biomedical research”

Mathias Busek

Hybrid Technology Hub - Centre of Excellence, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway.

Most contemporary Organ-on-a-Chip (OoC) systems rely on pumps for fluid control, which can be prone to malfunctions and air bubble formation, leading to reduced stability and reproducibility over time. In response to this, pump-less OoC platforms are gaining popularity due to their user-friendliness and potential for creating more predictive, complex human-like organ models for substance testing and disease modeling.

Recently, a pump-less recirculation OoC (rOoC) platform that leverages directional gravity-driven flow has been developed at HTH in Oslo. This platform is adaptable to different flow conditions and facilitates the examination of endothelial lining, blood vessel sprouting, immune cell circulation, and integration of 3D cell models like organoids. Moreover, we've developed a computational model to predict shear stress and mass transport within the rOoC, allowing customization for diverse research applications.

In this study, I will showcase different rOoC layouts adapted for different biological applications, introduce methods of reproducible cell/organoid loading and give an outlook of new developments like online monitoring and lab automation with the rOoC.

In summary, our rOoC platform offers a reproducible and scalable solution for research related to disease modeling, organ interactions, and immune studies. It is user-friendly and equipped with features like controlled flow and online monitoring.

A10

Automated filtration and filter-flush system and analysis for organoid and organ-on-chip samples

S. Kogler[ab], G.M. Pedersen[a], F. M. Ramirez[a], A. Aizenshtadt[b], Mathias Busek[b], S. J. K. Krauss[b], S. R. H. Wilsona[b], and H. Røberg-Larsena[b]

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In this study, we explore the application of an automated filtration and filter-flush system (AFFL) for the determination of the diabetic drug tolbutamide and its metabolite 4-hydroxy tolbutamide in medium derived from organoids and Organ-on-Chip (OoC) systems.
The AFFL system, known for its versatility and self-cleaning capabilities, is employed for LC-MS analysis, overcoming challenges associated with the complex composition of the medium.
The method is validated following FDA and ICH guidelines, demonstrating low limits of detection and quantification. The research compares drug metabolism results between static organoid exposure experiments and OoC systems, highlighting the potential advantages of advanced in vitro models.

A11

Contrast-enhanced micro-CT imaging for cell spheroids and small organs

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INTRODUCTION: Confocal microscopy is a widely employed technique for visualizing cell spheroids, organoids, and small organs, providing high-resolution imaging with a broad spectrum of stains. However, it has limitations in penetration depth, detecting only a few micrometers beneath the sample surface [1], and requires sample clearing for light transmission. Conversely, micro-CT imaging is not restricted by sample transparency or penetration depth but faces challenges in resolution and low soft tissue contrast. Here, we show contrast-enhanced high-resolution micro-CT imaging as a promising technique to assess the ultrastructure of spheroids, organoids or small organs using phosphotungstic acid (PTA) and Lugol's iodine as a contrast enhancement agents.

METHODS: Osteoblastic cell spheroids, murine adrenal glands, ovaries and testicles were fixed in 4% PFA and contrast-enhanced with 0.3% PTA in water for 24h or 1.5 Lugol's iodine for 48h. The samples were washed in distilled water three times and mounted for µ-CT imaging in water for cell spheroids, or embedded in paraffin. Scans were performed with the SkyScan 2211 (Bruker, Belgium) at pixel sizes ranging from 0.4 to 4.5 µm and rotation steps of 0.19° to 0.47° over 360° depending on sample size. The reconstructions were performed with the software NRecon (Bruker, Belgium) and analyzed using Dragonfly (Object Research Systems (ORS) Inc., Canada).

RESULTS: Entire sample volumes could be visualized, and total volumes could be calculated. Samples with volumes < 0.8 mm^3 showed the presence of cells. In volumes with lower cell density, the morphology of individual cells could be observed (Fig. 1). Also, native tissues exhibited great details in ultrastructure (Fig. 2).

DISCUSSION & CONCLUSIONS: The described method revealed the spheroids' and organs' ultrastructure within the entire sample using PTA for spheroids and Lugol's iodine for organs as a contrast enhancement agent for µ-CT imaging. With larger sample size, compromised resolution due to the geometric magnification was the consequence. However, visualizing smaller volumes of a larger sample can increase the resolution. This contrast-enhanced micro-CT technique provides a valuable supplementary method for analyzing the ultrastructure of spheroids, organoids, or small organs that exceed the inspectable volume limits of confocal microscopy.

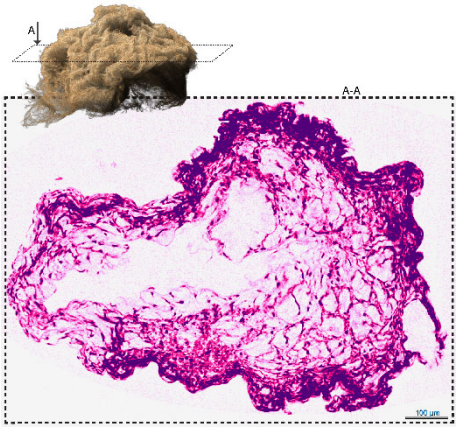

Fig. 1. Cross-section of osteoblastic cell spheroid from contrast-enhanced micro-CT showing individual cells.

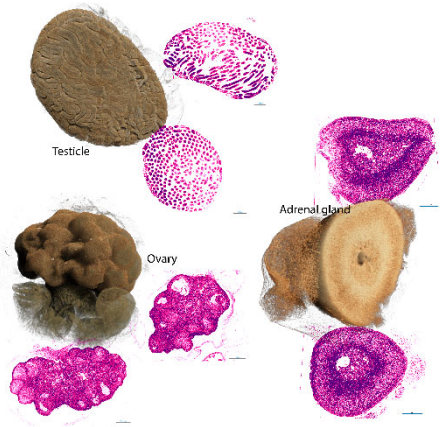

Fig. 2.3-D reconstructions of the murine testicle, ovary and adrenal gland from contrast-enhanced micro-CT visualizing organ-specific ultrastructure.

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ACKNOWLEDGEMENTS: We acknowledge financial support from the Baltic Research Programme project No. EEA-RESEARCH-85 EGGSHELL under the EEA Grant of Iceland, Liechtenstein and Norway No. EEZ/BPP/VIAA/2021/1.

NOR-MPS Symposium (Abstract)

Chencheng Wang
February 14th 2024
Meet Ullevaal, Oslo

Stem cell derived-islet organoids for studying metabolism disorders *in vitro*

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Metabolism disorders such as diabetes and non-alcoholic fatty liver disease research have faced persistent challenges due to the absence of genuine disease models that should ideally be unlimited and capable of replicating the abnormalities associated with the development, structure, and function in pathological conditions. Significant efforts are ongoing to develop stem cell-derived islets (SC-islets) for disease modeling, drug screening, and transplantation therapy. However, it remains unclear to what extent SC-islets generated from current protocols recapitulate the physiology of human primary islets that can be used for studying metabolism disorders *in vitro*.

Here, we present an optimized SC-islets differentiation protocol based on fine-tuning the WNT signaling pathway during differentiation. Our protocol led to SC-islets that showed a significantly increased population of Chromogranin A positive endocrine and C-peptide positive (Cp+) cells compared to benchmark protocols. The SC-islets contain >60% of Cp+ cells, which have a small population of glucagon-insulin (>15%), somatostatin-insulin (>10%) bi-hormonal cells, and glucagon-insulin-somatostatin positive tri-hormonal cells (>10%). The bi-hormonal and tri-hormonal cells decreased during prolonged culturing *in vitro*. The SC-islets could maintain glucose-stimulated insulin secretion ability for 4 *weeks in vitro* with an average insulin secretion index of 1.45, reflecting a flexible time window for subsequent utilization. As expected, insulin secretion increased while glucagon secretion decreased from these SC-islets in response to an elevated glucose concentration, reflecting their physiological potential in regulating glucose homeostasis similar to human primary islets. The oxygen consumption rate during glucose stimulation increased in SC-islets, indicating they may have matured aerobic oxidation ability similar to primary islets. Furthermore, fatty acids amplified insulin secretion from SC-islets, like in human primary islets, but amino acids failed to trigger significant insulin secretion in SC-islets. The cell identity dynamic and nutrient stimulation profile of SC-islets reported in this study provided parameters to consider while choosing SC-islets for modeling metabolism disorders *in vitro.*

In summary, we present an improved differentiation protocol for generating SC-islets. The insulin secretion ability of SC-islets can be maintained for 4 weeks in a chemical-defined medium. These SC-islets could be a potential mini-organ source to fine-recapitulate the major physiological function of primary human islets for studying metabolism disorders *in vitro*.

A13

Base Excision Repair in Aging-Related Disease Pathologies

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Genomic instability is one of the hallmarks of ageing and a risk factor in the development of neurodegenerative diseases (NDD) such as Parkinson's disease (PD). Different DNA repair mechanisms targeting various types of DNA lesions counteract genomic instability. One of these mechanisms is the Base Excision Repair (BER) pathway, dedicated to the removal of single-base lesions in nuclear and mitochondrial DNA. DNA glycosylases detect and remove the base lesion and generate apurinic apyrimidinic (AP) sites and single strand breaks (SSBs). Subsequently, a DNA polymerase catalyzes DNA synthesis, filling in the gap created by the removal of the damaged base. Following this, a DNA ligase seals the newly synthesized DNA strand, ensuring the restoration of the DNA sequence and completion of the repair process. Here we show that, in C. elegans PD model, the lack of the initial damage-recognition step, carried out by the NTH-1 DNA glycosylase, is neuroprotective. The dopaminergic neurons of animals expressing a-synuclein and with a deficiency of NTH-1, remain morphologically fitter than the isogenic NTH-1 proficient strain and, notably, with improved neuronal functionality.
Furthermore, NTH-1 deficiency increases the mitochondrial transcription rate, activating mitohormesis, the source of neuroprotection in our model. This effect disappears when re-supplementing NTH-1 from an extrachromosomal array. In addition, expressing the human homologue of NTH-1, NTHL1, in the dopaminergic circuit, also ablates neuroprotection. As we transition these insights into human models, we observe heightened levels of oxidative phosphorylation (OXPHOS) proteins, alterations in mitochondrial size as well as metabolic rates in NTHL1 knockout cell lines. These observed alterations in cellular dynamics, particularly within the mitochondria, underscore the potential impact of manipulating DNA-glycosylases in mitigating the effects of neurodegeneration. Our next step aims to utilizing patient-derived human induced pluripotent stem cells (hIPSCs) carrying SNCA gene triplication to engineer NTHL1 knockout via CRISPR-Cas9. This approach will enable us to investigate the functional consequences of NTHL1 loss on mitochondria and neuroprotection within differentiated dopaminergic neurons and brain organoids.

A14

AI meets biology: Predicting stem cell differentiation success with deep learning

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The efficiency of stem cell differentiation varies greatly, even when using cell clones. This is a problem for many applications of stem cell derived cells, including organ-on-a-chip technology as well as cell therapy. To select successful differentiation experiments early on, we imaged induced pluripotent stem cells during differentiation. We use these images and corresponding biological labels to train deep neural networks to predict the cells' differentiation efficiency.

A15

Gastruloids – to the organs (and beyond?)

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Gastruloids are stem cell derived embryo models that can model a variety of embryonic developmental stages and processes. As such gastruloids are increasingly being used to study the "black box" of embryo development, tissue morphogenesis, and evolutionary biology. In our project, we are focused on the development of gastruloids – 3D embryo models that resemble the post-gastrulation embryo – from human ES cells. Our aim is to develop the robust protocol for anteriorized gastruloid formation that can be further exploited for heart and foregut development, and subsequently for the induction of liver and pancreas organogenesis. Previous work done in our team has shown that published protocols for human gastruloid formation, which rely mainly on the induction of the WNT pathway[1], are not optimal. We have tested a variety of conditions to improve gastruloid protocols and have managed to develop a comparable robust protocol for human gastruloids based on differential morphogenic induction followed by controlled aggregation.

1. https://doi.org/10.1038/s41586-020-2383-9

A16

Global metabolomics aims at capturing as many metabolite signatures of a sample as possible.

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From hundreds to thousands of metabolites are typically seen using this approach, making global metabolomics a powerful tool in hypothesis generation.
Using liquid chromatography- high resolution mass spectrometry we have developed both a robust global metabolomics and global lipidomics method, suitable for clinical applications.
The method has proven well suited for liquid samples, but can in theory be applied on all sample types, including tissue.
To evaluate the methods usefulness for organoid analysis, we have performed a pilot study on global metabolomics in brain organoids. Strengths and weaknesses, opportunities and limitations, in the use of global metabolite analysis for characterization of organoids will be discussed.

A17

Mass spectrometry and proteomics for studying organoids and organ-on-chip

Steven Ray Wilson and Hanne Røberg-Larsen

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The Bioanalytical chemistry (BACH) group at the Department of Chemistry (UiO) focus on applying separation science and mass spectrometry to studying organoids and organ-on-chip systems. Examples of the work are the studies of drug metabolism, metabolomics, and lipidomics. In this talk, proteomics-related approaches are focused upon, including global proteomics studies of liver organoids and more targeted approaches, i.e. the measurements of glucose-regulatory hormones in islet organoids. Finally, our ongoing approach of studying the effects of persistent organic pollutants is discussed.

A18

Establishing PDX-derived organoid models from pseudomyxoma peritonei

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Pseudomyxoma peritonei (PMP) is a rare, slow-growing, abdominal cancer characterized by mucinous tumor spread to the surfaces of the peritoneal cavity. The standard treatment is cytoreductive surgery (CRS) followed by hyperthermic intraperitoneal chemotherapy (HIPEC), which cures half of the patient. However, for patients that are not cured by CRS-HIPEC, there is no efficacious treatment, and our research focuses on development of novel treatment options.

Patient-derived tumor organoids are generated by culturing tumor tissue in the form of 3D structures in a gel-based growth medium. Organoid cultures are more complex than conventional 2D models, but less complex than animal models, although potentially more efficient in terms of time and costs. Organoid cultures from individual patients may be utilized to develop patient specific treatment.

PMP is characterized by a low number of slow-growing tumor cells producing large amounts of mucin, making it challenging to culture ex vivo, compared to other cancers. We have established several patient-derived xenograft (PDX) mouse models from PMP patients that can provide a regularly available source of PMP tissue. To develop the technology for PMP, organoid cultures have been established from four of the PDX-models. The models have been characterized with respect to mucin and cytokeratin expression using fluorescent confocal microscopy and immunohistochemistry. Results from these analyses show that the organoids have the same characteristics as the tissue of origin, specifically the original patient tumor and tumor from the PDX-model.

A19

Pump-less and Recirculating Placental Syncytium-on-Chip induced only by Shear Stress for *in vitro* Drug Testing

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The placenta is a key embryonic membrane that allows the embryo to attach to the uterine wall and acts as a barrier between the maternal and fetal circulatory systems. Here, we have used a recently published pumpless recirculation organ-on-chip (rOoC) platform with an integrated porous membrane (MirOoC) to recreate aspects of the human placental barrier.[1] The platform provides the opportunity to recapitulate nutrient exchange and test its barrier function on known and unknown hazardous compounds. The barrier function of the human placenta is performed by a layer of multinucleated giant cells called the syncytiotrophoblasts and is the main site of exchange for drugs, metabolites, nutrients and waste products in the interaction between the maternal and embryonic/fetal circulation. Due to the blood flow, a wall shear stress (WSS) acts on the syncytiotrophoblasts and has previously been described as an important factor in the regulation of many cellular events within the syncytiotrophoblast.[2] However, most placenta-on-chip models developed to date have relied on chemically-induced differentiation of trophoblast BeWo cell line to establish a syncytium-on-chip.[3,4] Employing commonly used BeWo cells, we compared the effect of chemical (forskolin treatment in Transwell) and mechanical (WSS) cues on their properties. We show that BeWo cells undergo syncytialisation as efficiently under WSS exposure as observed under forskolin treatment. This suggests that our microfluidic placenta-on-chip system has the potential to be a better *in vitro* model of the placental biology as it is devoid of chemical components which could hamper further drug testing or direct tissue/tissue interaction studies. We demonstrate that a specific range of WSS yields a functional syncytium with hormone secretion, cell fusion, polarization, barrier properties, and key transporter expression, such as transferrin receptor and glucose transporter-1 proteins, which are crucial for nutrient exchange across the cell membrane.[5] Moreover, we tested changes in the levels of FMS-like tyrosine kinase-1 and Placental Growth Factor involved in the regulation of the endocrine balance of the placenta which verify the development of a more physiologically relevant placental syncytium-on-chip compared to the currently used chemically-induced differentiation protocols.[6] Finally, to highlight the usefulness of our model, we verified barrier function in response to particles and pro-inflammatory drug exposure. We also found that our WSS-induced model keeps physiological endocrine balance in longer term (11 days) culture on-chip unlike in static forskolin-treatment. Our results show that BeWo cells cultured under optimized WSS represent a useful model to recapitulate healthy-placental syncytium-on-chip for future drug testing as well as tissue-tissue interaction studies (*e.g.* adult or embryo tissue models).

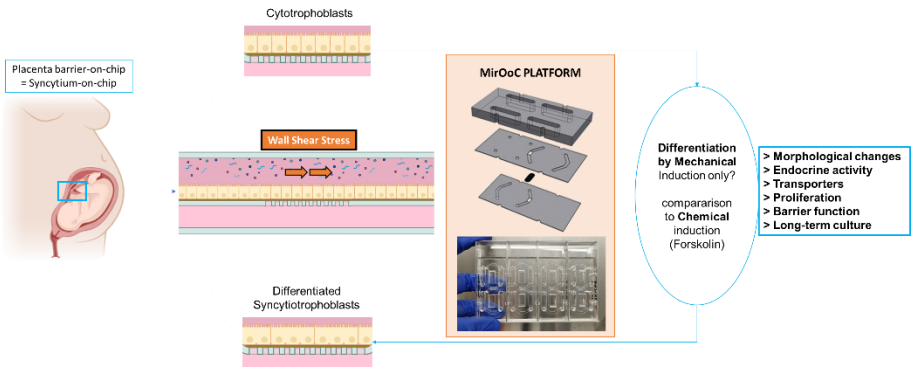

Figure 1: *Generating physiologically relevant placental syncytium-on-chip with only Wall Shear Stress on a Pump-less and Recirculating device (MirOoC) for Drug Testing.*

Acknowledgments
Many thanks to Alexei Golovin for his technical support in the fabrication of microfluidic devices and rotating/tilting platforms. Thanks to Dr. Olga Bibikova for Dynamic Light Scattering characterization of the particles.

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A20

Mass Spectrometry Imaging of gastruloids and organoids

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Mass spectrometry Imaging (MSI) will be employed for studying liver organoids and gastruloids. These investigations will allow for better understanding of their biology leading to creating more mature organoids that could replace animals models.

The nanospray desorption electrospray ionization (nanoDESI) platform is assembled and tested in-house.

Commercially available instrumentation is also employed. Matrix assisted laser desorption ionization (MALDI) and desorption electrospray ionization (DESI) imaging was performed using respectively a timsTOF fleX from Bruker and Waters DESI-XS instruments. Collected images provide comprehensive insights into potential finding using MSI. These pilot projects gave also good basis for optimization of all steps in the process, from sample preparation to image acquisition.

Steatotic and normal liver organoids, and gastruloids formed suing various protocols, will be analyzed with DESI and MALDI MSI with the goal of mapping metabolite distribution. Additionally the MSI will be applied to organoids exposed to per- and polyfluoroalkyl substances (PFAS) as a first step in mapping organic pollutions in various cells using organoids and gastruloids as models.

A21

GlycoMap: Integrated glycomics of tumour organoids for cancer precision medicine

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Tumour heterogeneity remains the primary cause for inter-patient variation in treatment response. One of the largest sources of tumour heterogeneity arises from post-translational glycosylation of proteins and disturbances of glycosylation are an established hallmark of cancer. Glycomics (the comprehensive study of the glycome) offers the potential to link glycan associated genome/transcriptome data with relevant glycan structures. The recent development of tumour organoids from patient biopsies has enabled the study of patient-representative biological processes *in vitro*, offering a unique platform for individual patient drug screening and basic science analyses. The GlycoMap project aims to develop an integrated glycomics workflow to produce glycan data from tumour organoids that reflects the global glycosylation status of a patient. Using organoid GlycoMaps, we plan to subgroup patients based on glycan status and probe treatment sensitivities of patients, as well as better characterize the cancer glycome.

A22

Patient-derived tumoroids in renal cell carcinoma

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University of Oslo

Renal cell carcinoma (RCC) is the most common form of kidney cancer and is known for its heterogeneity and resistance to chemotherapy. However, the emergence of immune checkpoint inhibitors has revolutionized treatment outcomes for many patients. Despite this progress, predicting disease progression and identifying patients who will respond to immunotherapy remains a challenge - especially in a cancer as fundamentally heterogenous as RCC. By analyzing protein levels of key players in the autophagy pathway in different RCC subtypes, we have observed distinct clusters that highlight the heterogeneity of autophagy mechanisms involved. We believe understanding these mechanisms is crucial for elucidating treatment response in RCC.

In this study, our objective is to establish renal cell carcinoma tumoroids/organoids using tumor and normal tissue samples, obtained through collaboration with urologists at Aker hospital. To further investigate the role of autophagy in RCC progression and therapy resistance, we will study the molecular mechanisms underlying autophagy-mediated resistance in metastatic RCC and hopefully identify biomarkers that can predict response to immunotherapy. We will perform molecular profiling of the patients to enable us to identify specific molecular signatures associated with autophagy-mediated resistance, and perform an autophagy modulating drug screen in combination with current treatments to help elucidate relevant targets. By focusing on the heterogeneity of the patient subgroups, we aim to uncover the underlying mechanisms that contribute to differential treatment outcomes in RCC patients and potentially identify biomarkers to stratify patients beyond what we believe is possible with traditional histology based diagnostics.

A23

Mass Spectrometry Reveals that Oxysterols are Secreted from Non-Alcoholic Fatty Liver Disease Induced Organoids

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Using liquid chromatography – mass spectrometry, we show that liver organoids produce and secrete oxysterols. Oxysterols are a group of bioactive cholesterol metabolites, which has been connected to metabolic dysfunction-associated steatotic liver disease (MASLD, previously known as non-alcoholic fatty liver disease (NAFLD). We find that steatotic organoids secrete elevated levels of 26-hydroxycholesterol compared to untreated liver organoids. Other oxysterols, such as 7α,26–dihydroxycholesterol and 7α,25-dihydroxycholesterol, are only detected in steatotic samples.

By this, we demonstrate the advantage of combining organoids and mass spectrometry for disease modeling.

K. S. Kømurcu, I. Wilhelmsen, J. L. Thorne, S. Krauss, S. R. Wilson, A. Aizenshtadt, H. Røberg-Larsen, J. Steroid Biochem. Mol. Biol.2023, 232, 106355.

A24

Presentation of the Core Facility for Human Pluripotent Stem Cells

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Norwegian Core Facility for Human Pluripotent Stem Cells

The Core Facility for Human Pluripotent Stem Cells offers services related to research on human ES and iPS cells. We provide reprogramming of iPS cell lines from patients and healthy donors, for example for in vitro disease modeling. We have already-established human ES cell lines and a large bank of human iPS cell lines established through ongoing REK-approved projects. We also offer a range of fee-based services including iPS cell characterization, instruction in iPS cell-related techniques, and use of cell culture facilities and instruments.

The Center is also spearheading the development of a national “super-donor” allogeneic iPS cell bank for clinical applications, in collaboration with the Norwegian Bone Marrow Donor Registry and the Oslo University Hospital Ex Vivo Laboratory for GMP clinical cell production. This project aims to establish a national bank of clinical grade haplotyped hiPS cell lines that can be used to derive differentiated cells for cell replacement therapies in patients.