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RESULTS PACK ON ORGANOIDS

Organoids: mini organs in a dish for disease research and new cures

EDITORIAL

ORGANOIDS: MINI ORGANS IN A DISH FOR DISEASE RESEARCH AND NEW CURES

Producing miniature versions of organs in a dish may sound like science-fiction, but thanks to advances in stem cell technology and bioengineering scientists are now able to artificially grow a mass of cells into organoids with similar properties to organs. Organoids represent cells grown in specific three-dimensional (3D) environments, creating mini, simplified organs that retain some physiological function. They are derived from one or a few cells, from a tissue, embryonic stem cells or induced pluripotent stem cells, which can self-organise in 3D cultures.

Following the observation that cells do not behave in 2D cultures as they do in vivo, *3D cultures of organoids have emerged as promising model systems for studying tissue development and generating new therapies. With recent technological breakthroughs, 3D culture models have come to represent a more physiologically relevant approximation of the* in vivo *environment. Hence, researchers in the fields of the physical sciences and engineering are seeking to improve the 3D culture conditions to grow mini organs in a dish.*

Organoids are increasing in complexity as researchers begin probing more deeply the mechanisms behind stem cell lineage and differentiation. Models have now been grown for many organs, including brain, liver, kidney, breast, retina, and organs of the gastrointestinal tract, among others.

Mini organs to model disease

This CORDIS Results Pack presents the first results from EU/ERC-funded research in the organoid field. It sheds light on five projects and their scientific advances in organoid technology and how they can be used as in vivo*-like models.*

The Mini Brains project generated brain organoids to study the mechanisms of various neurological disorders and discover novel drugs to treat them. TOXANOID proved that their technology can outperform current in vitro *systems and replace a significant portion of animal-based toxicology studies. The team successfully developed organoid systems for several organs including small intestine, colon and liver.*

Technologies for 3D *in vitro* organoids

ColonCan developed novel ex vivo *3D organoid cultures that replicate genetic events in colorectal cancer (CRC), the second most common cause of cancer-related deaths, and used them to test novel therapeutics. COMIET created a new cell culture platform for epithelial tissues to advance the* in vitro *modelling of diseases, preclinical screening for drug efficacy and toxicity, as well as the understanding of organ development. The CLOC project produced liver organoids* in vitro *using hepatocytes cultured on 3D scaffolds as novel models for studying liver development and disease, and potential treatment of inherited cholestatic disorders.*

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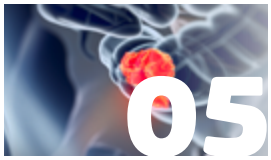
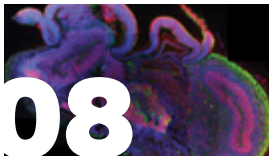
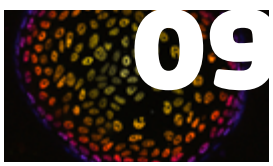






Organoids to treat liver diseases

Cell transplantation is a promising new approach for organ regeneration in disease or after injury. To support the differentiation of hepatocytes from stem cells, European researchers developed *ex vivo* organoids which precisely mimic liver architecture.

Inherited cholestatic disorders (ICDs) are characterised by defects in bile synthesis and secretion, leading to progressive liver disease. Many ICD patients do not respond to medical treatment and need a liver transplantation. With 10 % of patients dying while waiting on the transplant list due to shortages of liver donors, there is an imminent need for alternative therapies.

Bioengineered tissues are emerging as a promising solution for reducing the need for donor organs. However, the complexity of liver organisation makes the generation of a functional liver challenging. To address this issue, researchers in the ERC-funded CLOC (Cultured Liver Organoids for Investigation and Treatment of Inherited Cholestatic Diseases) project produced liver organoids *in vitro* using hepatocytes cultured on three-dimensional (3D) scaffolds.

CLOC brought under its umbrella multidisciplinary experts including surgeons, engineers and cell biologists who worked together to guarantee the success of the project. "We wanted to develop novel treatments for ICDs but also models for studying liver development," explains Prof. Paul Gissen from University College London, principal investigator of the CLOC project.

Stem cells for liver organoids

The scarce availability of viable primary hepatocytes and their poor *in vivo* engraftment, severely limit the success of the cell transplantation approach. On the other hand, the pluripotency of stem cells renders them an ideal source for tissue bioengineering. In particular, human induced pluripotent stem cells (hiPSCs) derived from reprogrammed skin fibroblasts by overexpression of particular factors have become a significant tool of regenerative medicine. Production of patient-specific hiPSCs can be scaled up, generating fully immuno-compatible differentiated cells for clinical application.

CLOC researchers decided to seed hiPSCs onto a 3D extracellular matrix (ECM) scaffold. "We generated liver organoids from a decellularised scaffold obtained from the mouse liver. We then repopulated the scaffold with differentiating hepatocytes that were derived from hiPSCs," continues Prof. Gissen.

The resulting organoids preserved the micro-architecture, blood vessel network and ECM of liver. They were subsequently placed in a bioreactor where they were cultured for several weeks under a constant flow of oxygen. Compared to two-dimensional cultures, results showed that seeding hiPSCs on a 3D scaffold promoted faster cell maturation, a prerequisite for successful *in vivo* transplantation in an adult organism.

Advantages of CLOC organoids

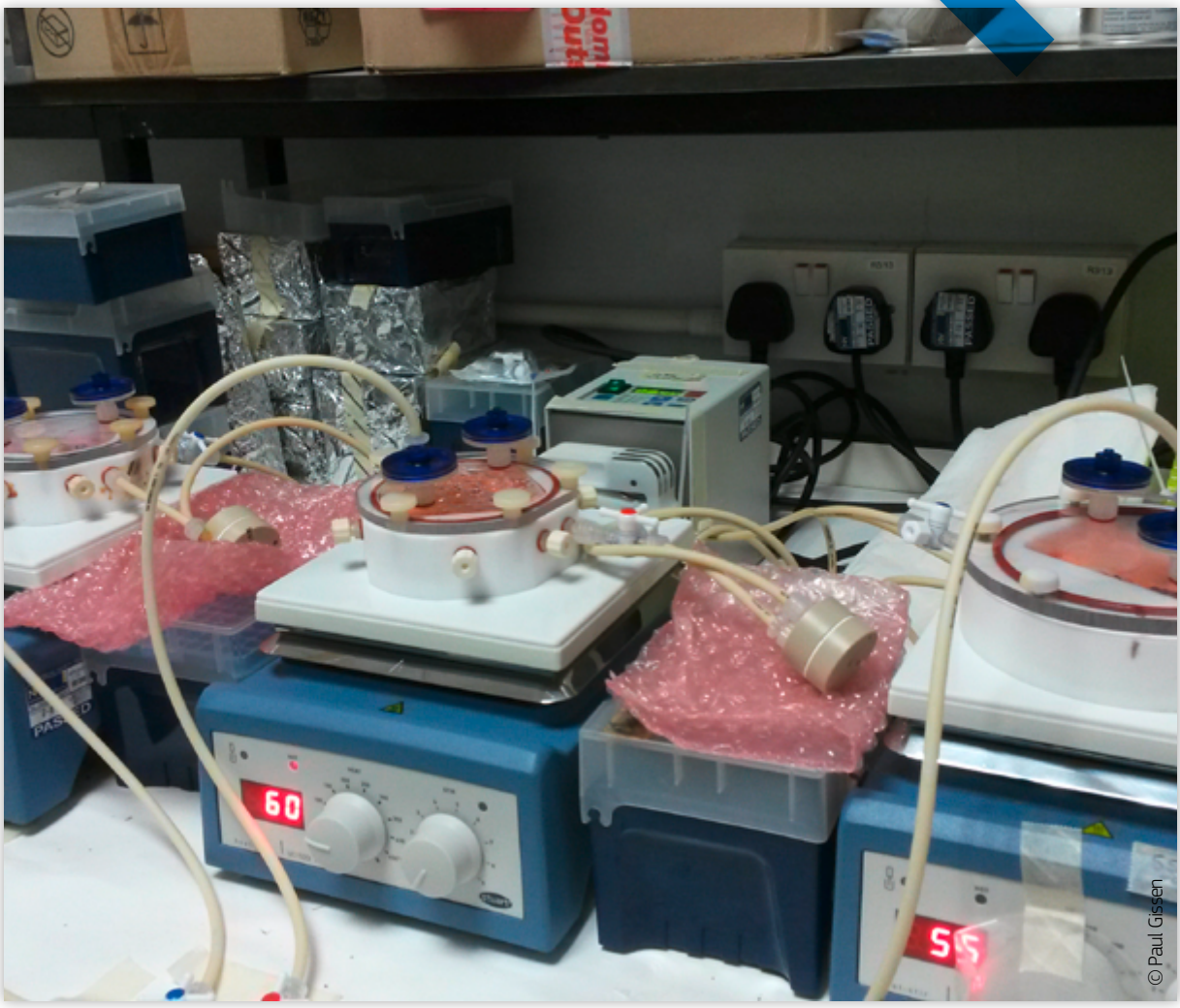
According to Prof. Gissen, "the 3D environment provides mechanical stimuli to the cells, and alongside tissue-specific signalling, enhances liver cell function and proliferation. In addition, key ECM components assist hiPSC differentiation into hepatocytes." Importantly, mouse liver is a readily available source of decellularised scaffolds and requires few cells to repopulate.

We wanted to develop novel treatments for ICDs but also models for studying liver development.

By using hiPSCs obtained from ICD patients, CLOC researchers were able to generate *in vitro* models of ICDs for drug screening purposes. As Prof. Gissen outlines, "the next step is to test the generated organoids in mice with liver defects and also to generate organoids from different materials such as plastic."

Undoubtedly, the safety of transplanting hepatocytes from hiPSCs warrants further investigation. Although hepatocyte transplantation has been used to treat various metabolic disorders, direct infusion of cells will unlikely reverse polarised bile flow and correct ICDs. Liver organoids offer hope in this respect since in addition to supporting the terminal differentiation of stem cells prior to cell transplantation, they could also be used for whole organ regeneration.

Project	**CLOC – Cultured Liver Organoids for Investigation and Treatment of Inherited Cholestatic Diseases**
Hosted by	University College London in the United Kingdom
Funded under	FP7-IDEAS-ERC



© Paul Gissen

Organoids in the fight against cancer

Colorectal cancer (CRC) is the second most common cause of cancer-related deaths. European researchers developed novel *ex vivo* three-dimensional organoid cultures that replicate genetic events in CRC, as tools to test novel therapeutics.

CRC emerges in the epithelium of the gastrointestinal tract, most often as a result of driver mutations in the adenomatous polyposis coli (APC) tumour suppressor gene. APC mutations increase signalling in intestinal stem cells and enhance expression of target oncogenes such as MYC (c-Myc). It is apparent that additional events are necessary for CRC development such as mutations in the KRAS and TP53 genes, which are involved in regulating key cell processes, such as the cell cycle. However, the impact of these changes on the development of CRC and response to therapy is not well understood.

To address this issue and further study CRC, ERC grantee Owen Sansom, leader of the EU-funded ColonCan (Targeting downstream effectors of Wnt signalling in colorectal cancer) project, developed a number of preclinical models that faithfully recapitulate the course of the human disease, including metastasis. "Our aim was to identify and test potential novel targets and therapies for CRC," explains Prof. Sansom. A key objective was to assess the impact of cooperating mutations and decipher the signalling mechanisms by which these genetic changes contribute to the phenotype of APC-deficient cells.

CRC preclinical models

Researchers utilised state of the art technologies including ribosomal and metabolic profiling to study CRC cells from their preclinical models. They discovered that KRAS mutations alter signal transduction within APC-deficient CRC cells. The project results suggested an overall increase in global protein production and alterations in nutrient stress response pathways and cellular metabolism in cells harbouring both mutations. Furthermore, they discovered that the growth factor TGF-β suppresses tumourigenesis in intestinal cells.

Our aim was to identify and test potential novel targets and therapies for CRC.

The scientific team also generated new mouse models of metastatic CRC using genetic engineering. A comparison of transcriptomic data generated from these models with data from human primary CRC indicated that the models recapitulate the cancer subtype with the worst overall survival. "These new models can be used to test therapeutic agents, for modelling stratified clinical trials, and are an excellent platform for testing immunotherapy combinations," continues Prof. Sansom.

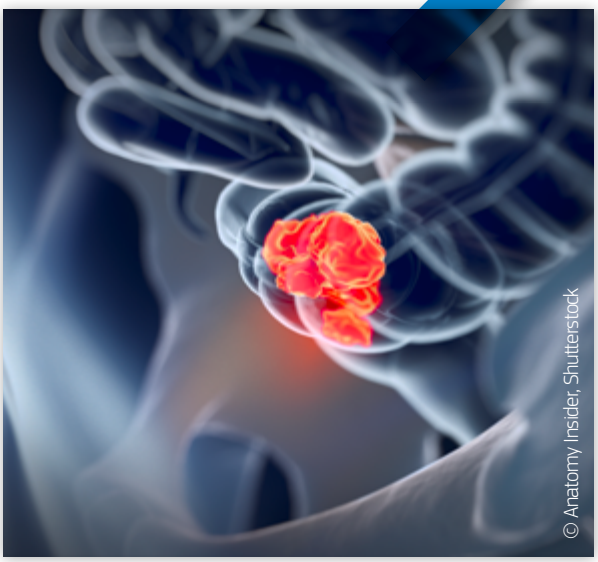
In addition, considerable effort went towards the development of organoid cultures as *ex vivo* CRC models or for orthotopic transplantation into mice. This new technology essentially mimics the properties and the three-dimensional structure of the tumour of origin as it emerges from tumour-initiating cells.

Novel treatments against CRC

All preclinical models were employed to test current therapies for CRC. Intriguingly, scientists observed drug resistance to many targeted therapies, suggesting that alternative approaches are urgently needed. Prof. Sansom emphasises the clinical significance of the ColonCan study "through the identification of new opportunities for treating CRC." Indeed, targeting nutrient stress and/or metabolism has emerged as a specific approach to kill CRC cells with mutations in both APC and KRAS genes. Considering that approximately 40 % of CRC patients carry both of these mutations, these findings open up new treatment avenues for late stage CRC.

Taken together, ColonCan's newly generated model systems (mice and organoids) precisely describe the human situation and will serve as powerful tools for testing new therapeutic strategies. "The ultimate aim is to use these models to drive trials of novel treatments," envisages Prof. Sansom. To accelerate clinical studies, researchers are working to establish a European preclinical platform for cross-validation of models and therapeutic testing in CRC.

Project	**ColonCan – Targeting downstream effectors of Wnt signalling in colorectal cancer**
Hosted by	Beatson Institute for Cancer Research in the United Kingdom
Funded under	FP7-IDEAS-ERC



Tissue engineering to mitigate the negative impact of digestive diseases and disorders

Epithelial tissues cover all exposed body surfaces, line most of our organs and form barriers that protect the body against physical, chemical and microbial harm. An EU initiative, funded through the European Research Council, is providing a new cell culture platform for epithelial tissues that can advance the *in vitro* modelling of diseases, preclinical screening for drug efficacy and toxicity, and the understanding of organ development.

Functional *in vitro* models of epithelial tissues are key elements in basic biological research, disease modelling, drug discovery and regenerative or personalised medicine. Clinical applications of tissue engineering are hampered by the risk of bacterial infection due to the lack of functional epithelial engineered tissues. In the case of the small intestinal epithelium, functional *in vitro* models are needed to accurately predict the absorbance of drugs delivered orally.

Trouble-free engineering strategies for microfabrication

"The ultimate goal of COMIET is to engineer intestinal epithelial tissue models that mimic physiological characteristics found within *in vivo* human intestinal tissue," says Prof. Elena Martinez, leader of the ERC project. To achieve this, an experimental

approach will combine microfabrication techniques, tissue engineering components and the self-organising characteristics of intestinal organoids.

To date, the project team has set up a simple strategy to microfabricate 3D villi-like structures on very soft materials. The 3D model shows functional parameters that are closer to physiological tissue than conventional flat monolayer culture systems. This will improve predictability of drug absorption, for instance. The findings have been submitted for publication and form the basis of a PhD thesis.

According to Prof. Martinez, researchers have developed a strategy to "open up" intestinal organoids that are 3D closed structures. The aim is to form monolayers that cover flat substrates and the project's fabricated 3D scaffolds. The results were advanced at the Organoids EMBO I EMBL Symposium 2016 and will soon be published. Project partners also demonstrated that the 3D villi architecture has a direct impact on bacteria adhesion and invasiveness potential.

In vitro human intestinal models that faithfully replicate *in vivo* behaviour

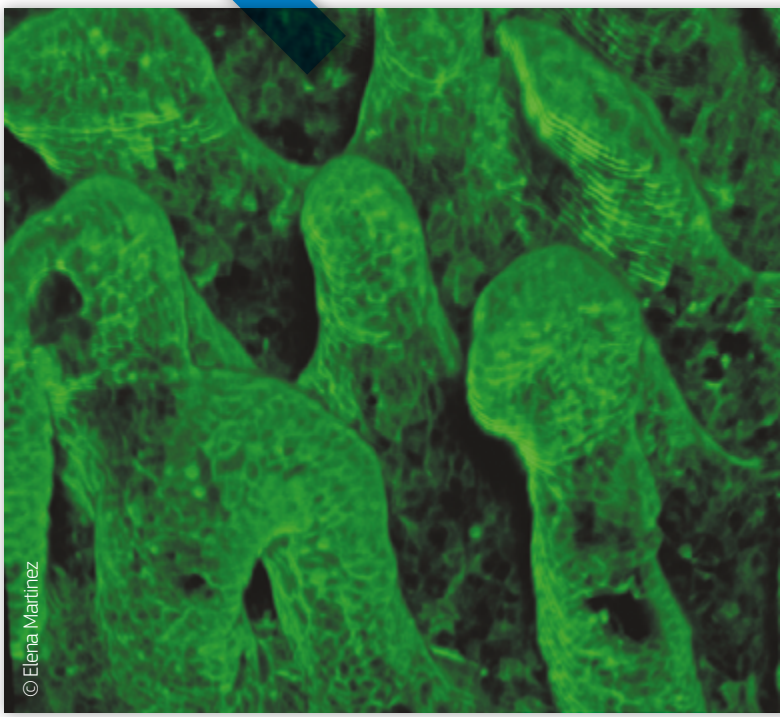
Prof. Martinez explains that the most significant output of COMIET (Engineering Complex Intestinal Epithelial Tissue Models) will be to demonstrate that engineering strategies can be successfully used to provide intestinal epithelial stem cells with physical and biochemical cues that guide their compartmentalisation, barrier formation and renewal as *in vivo*. "If this concept is successfully demonstrated, a similar approach could also be used to mimic other epithelial tissues with complex geometries such as kidney, skin or lungs," she says.

In the field of basic research, end users will benefit from a system that recapitulates the main physiological characteristics of the *in vivo* tissue. As a result, it can be used in developmental studies, but also as an *in vitro* model of human diseases.

The aim is for COMIET to open up new research avenues in human intestinal diseases.

Those involved in drug screening, drug absorption and toxicology assays also stand to benefit from a system that improves predictability of current assays. "The aim is for COMIET to open up new research avenues in human intestinal diseases," concludes Prof. Martinez. "Patients will ultimately gain from the project outcomes, as the system can be used for personalised medicine strategies."

Project	**COMIET – Engineering Complex Intestinal Epithelial Tissue Models**
Hosted by	Institute for Bioengineering of Catalonia in Spain
Funded under	H2020-ERC


© Elena Martinez

Cerebral organoids: an innovative treatment for neurological disorders

Being able to generate human tissue *in vitro* in a dish is expected to revolutionise biomedical research. European researchers generated brain organoids to study the mechanisms of various neurological disorders and discover novel drugs to treat them.

Pharmaceutical research usually employs animal models and conventional cell culture methods to study disease. However, fundamental differences in developmental and physiological aspects between humans and the commonly used animal models constitute a major bottleneck. For brain disorders, such as neurodegenerative and developmental diseases, especially, existing methods fail to recapitulate the complexity of the human brain, causing major pharmaceutical companies to severely downsize their respective research.

To address this issue, scientists from the ERC-funded Mini Brains (Cerebral organoids: human mini brains in a dish open up new possibilities for drug development in neurodegenerative and developmental diseases) project developed a stem cell-derived three-dimensional organoid culture system, termed cerebral organoids. "The idea was to use these cerebral organoids as a highly cost-effective tool in the discovery and development of therapies for neurodegenerative and developmental diseases," explains project leader Dr Jürgen Knoblich.

Cerebral organoid technology

Researchers cultured human embryonic stem cell lines and induced pluripotent stem cells under specific growth conditions to promote the differentiation into several brain tissues. More specifically, they generated the *in vitro* primitive cell layer called neuroectoderm, where the nervous system derives from, and maintained it in a specific scaffold to support complex tissue growth.

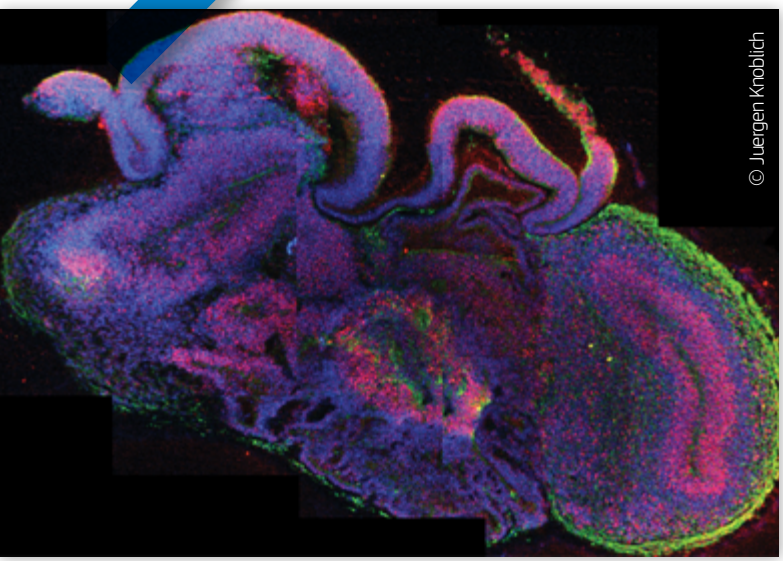
After approximately 20 days of culture in a bioreactor, neuroepithelial tissue surrounding a fluid-filled cavity was formed, reminiscent of a cerebral ventricle. "Ten days later, defined brain regions, including a cerebral cortex, retina, meninges as well as choroid plexus, developed," continues Dr Knoblich. Although they could survive indefinitely in the bioreactor, the lack of circulation prohibited brains from growing further.

The clinical potential of cerebral organoids is tremendous; for the first time, we can develop organoids derived from patients' blood or skin cells. This will provide new insights into the mechanisms that lead to neurological disorders.

Clinical potential of cerebral organoids

Scientists were able to grow organoids affected by microcephaly, a human genetic disorder in which brain size is significantly reduced. Intriguingly, they observed that while the neuroepithelial tissue was smaller compared to normal organoids, microcephaly organoids were characterised by increased neuronal outgrowth. This led Dr Knoblich and his team to suggest that, in patients with microcephaly, neural differentiation during brain development happens prematurely at the expense of stem and progenitor cells, affecting brain size.

In another part of the project, researchers used organoids to study complex interactions, such as cell migration and axon growth, between different developing brain regions. Emphasis was given to the inhibitory GABAergic interneurons which play a central role in brain activity regulation and are associated with epilepsy, schizophrenia and autism. Naturally, these interneurons



emerge in a ventral part of the human brain and migrate over a long distance to the dorsal regions. If this migration, which is guided by various chemical signals such as CXCR4, fails, then epileptic seizures can occur.

Furthermore, researchers combined bioengineering to improve the architecture of organoids. They used microfilaments to generate floating scaffolds that maintained their self-organising properties but displayed enhanced structure.

Using patient-specific cerebral organoids for research and drug screening offers an alternative to animal experiments, reducing costs and animal use. It also has the potential to decrease the cost of drug development, reduce the brain disease burden and increase the rate of approved drugs for brain disorders. "The clinical potential of cerebral organoids is tremendous; for the first time, we can develop organoids derived from patients' blood or skin cells. This will provide new insights into the mechanisms that lead to neurological disorders," envisages Dr Knoblich. With a new ERC-funded project, Mini Brain, scientists hope to take neurological research a step further.

Project	**Mini Brains – Cerebral organoids: human mini brains in a dish open up new possibilities for drug development in neurodegenerative and developmental diseases**
Hosted by	Institute of Molecular Biotechnology GmbH in Austria
Funded under	H2020-ERC

Mini organs in a dish

Toxicology and pharmacology assays are currently carried out in cell lines or experimental animals. Scientists from the TOXANOID project developed mini organs in a dish, which outperform existing *in vitro* systems.

Organoids are three-dimensional structures made of adult cells that mimic the tissue of origin in basic organisation and function. They emerge from tissue-specific stem cells, a small subpopulation of cells that resides within each organ and can regenerate it throughout life.

Current *in vitro* drug screening systems employ tumour-derived cell lines, primary cells or animals but none of them is ideal. To address this issue, scientists from the EU-funded TOXANOID (Pharmacological safety testing in human adult stem cell-derived organoids) project proposed to develop organoids for small intestine and liver that could be used for drug screening purposes.

This is the first ERC Proof of Concept grant for researcher Hans Clevers, stemming from his project STEMCELLMARK, which had identified a molecular marker for tissue stem cells in multiple organs. "Based on our previous findings we were able to grow and expand epithelial organoids from single stem cells expressing the Lgr5 marker," explains Dr Helmuth Gehart, key team member in the project.

Harnessing the benefits of organoids

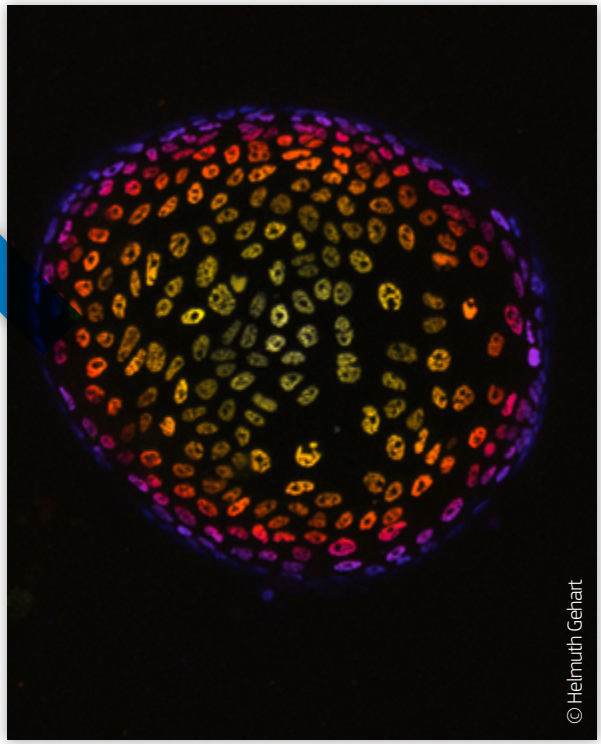
Organoids have a number of advantages compared to existing *in vitro* systems. Similar to cell lines, they are highly expandable and

thus represent an essentially unlimited resource. Importantly, unlike cell lines, they reflect the healthy tissue and contain the enzymes required to metabolise pharmacological compounds. In addition, they overcome the scarcity, batch to batch variability and *in vitro* culture challenges associated with primary cells. Furthermore, organoids help overcome the ethical issues raised with animal testing, and their human origin avoids false positive or negative results due to species differences.

Collectively, these attributes render organoids suitable for standardised assays such as those required to test the safety and efficacy of pharmaceutical compounds. "Organoids resemble primary cells in form and function and mimic therefore the organ of origin. Naturally, there is a wide spectrum of applications for these miniature organs, ranging from disease modelling, drug development and regenerative medicine to safety testing of new treatments," continues Dr Gehart.

The team successfully developed organoid systems for a number of organs including small intestine, colon and liver. Despite differences in culture conditions, they all share high proliferation rates and the ability to form the various epithelial cells of the parent organ.

During the study, scientists demonstrated the feasibility of using organoid-based assays to study drug toxicity in intestine and liver. Organoids outperformed cell lines, such as HepG2 cells, and behaved comparably to primary cells, demonstrating the capacity to metabolise drugs in a simple and cost-effective *in vitro* assay.



© Helmuth Gehart

Organoids resemble primary cells in form and function and mimic therefore the organ of origin.

Researchers had to overcome certain challenges associated with the three-dimensional nature of organoids. In this context, they generated a novel assay that was tested using known toxic and non-toxic compounds and delivered highly reproducible results.

The future of organoids

Overall, the TOXANOID study proved that their technology can outperform current *in vitro* systems and replace a significant portion of animal-based toxicology studies. In addition, organoids help understand how tissues take up and degrade pharmaceutically active compounds.

"Fuelled by TOXANOID results, we are currently working on another ERC project, ORGANOID, which plans to take our cultured mini-guts a step further," outlines Dr Gehart. This project aims to determine the effects of the microbiome and immune system on the gut by dissecting their interactions in healthy and diseased tissue.

Finally, researchers plan to focus on drugs that have been misidentified by conventional toxicity tests in the past and to promote their technology for drug development. Towards this goal, they are working closely with industrial partners aiming to expand organoid applications for rare genetic diseases such as cystic fibrosis and personalised medicine.

Project	**TOXANOID – Pharmacological safety testing in human adult stem cell-derived organoids**
Hosted by	Royal Netherlands Academy of Arts and Sciences in the Netherlands
Funded under	H2020-ERC

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