Utrecht-based HUB wants to become the world's leading organoid centre

MCO-170 series

HUB (HUBRECHT ORGANOID TECHNOLOGY), UTRECHT, THE NETHERLANDS

Since its founding in 2013, the number of HUB (Hubrecht Organoid Technology) employees has almost doubled every year, to more than 40 today.

CEO Robert Vries believes this exponential growth will continue for a while: 'We are currently receiving more project requests for primarily diagnostics and screening than we can take on. And with the major steps currently being taken in the field of personalised medicine, this number will certainly increase.'

More than 10 years after the cultivation of the first organoid – at the Hubrecht Institute by Toshiro Sato in the group headed by Hans Clevers – the path to commercial applications is open. So says Robert Vries, who has been working since 2013 to make HUB (Hubrecht Organoid Technology) the world's leading organoid centre. At the core of this ambitious goal is the exclusive licence for the patents for making organoids out of adult stem cells, called ASC organoids. These patents, which stem from the research done at the Hubrecht Institute – a KNAW (Royal Dutch Academy of Sciences) institute – are the property of the KNAW. 'With this licence, for which we pay the KNAW an annual fee, we can commercially exploit the technology in the form of licences and/or research and development work for primarily pharmaceutical companies. The benefit of this expanded patent portfolio, which includes over 50 patents in 14 patent classes and encompasses all aspects of (making) the ASC organoids, is that companies that want to work with such organoids cannot circumvent us,' says Robert Vries.

Almost all organs

The big advantage of organoid technology is that you can allow the human cells to grow in the mini-organs without them changing; the cultures remain extremely stable both from the genotype as well as the phenotype aspect. ASC Organoid Technology uses epithelial cells. This renders this method suitable for making 3D mini-structures that contain epithelial cells, such as the liver, intestine, lung, thyroid, stomach and kidney. The method is not suitable for tissue lacking epithelial cells, such as muscle tissue (including the heart), blood vessels and neurons. To make such structures, better options are available using pluripotent stem cells (IPS). However, this approach cannot compare to the ASC method with respect to the structures with epithelial cells and is only suitable for cultivating healthy cells.

The focus of organoid research was initially also on cultivating healthy cells, with the ultimate goal of regenerative medicine and cell therapy. In the meantime, the attention has switched to preclinical drug screening and diagnostics and, by extension, personalised medicine. 'In any case, the road to cell therapy is very long, even longer (and also more uncertain) than the road to launching medicines on the market based on small molecules. However, we noticed that we could grow not only healthy cells but diseased cells as well, such as cancer cells. That opened the door to applications that can be developed a lot faster, for example disease

modelling by growing organoids directly from diseased patient tissue. Furthermore, direct cloning of multiple individual cells from primary tumours enabled molecular and functional analysis of tumour heterogeneity. And we can also work with CRISP-mediated genome modification. Human organoids appear to be very receptive to this, which offers an enormous range of possibilities in the area of modelling of malignant transformation and mutagenesis after defect DNA repair.'

Immediately profitable

The basis for the founding of HUB in 2013 was implementing organoid technology for use as a test when developing new medicines or as a diagnostic tool. It was decided to set the organisation up as a non-profit company, in the form of a foundation. 'We saw an enormous amount of different applications. This is not so easy to accommodate in one for-profit company: investors often want focus, so either regenerative medicines or diagnostics, and often for a specific disease. And that's exactly what we didn't want. We also wanted to properly set up the platform and entrench it in the Netherlands through the structure with the KNAW patents. This meant that we had to be profitable from the beginning; first earn money and then spend it. That was a challenge because – certainly in 2013 – we had to earn that money with a very early version of the technology,' says Robert Vries.
HUB, which was founded by the Hubrecht Institute and the UMC Utrecht, was started with two former post-docs at the Hubrecht: Robert Vries, who focused on business development, and current Scientific Director Sylvia Boj, who oversaw the lab activities. In the beginning, several technicians at the Hubrecht Institute also worked with us, who were hired on for specific projects. In the meantime, in addition to the around 25 people working in the various laboratories, the organisation is increasingly hiring business developers, regulatory specialists and lawyers, who have to keep the currently more than 40 licensing agreements with companies all over the world on the right track.

Research technician Josje Heuvelmans in a section of HUB's treasure chamber, a biobank that currently has almost a thousand organoids stored at -80 °C in freezers provided by PHC Europe.

HUB (Hubrecht Organoid Technology) uses CO_2 incubators by PHC Europe for cultivating organoids. Pictured is lab assistant Ramazan Senlice next to one of the currently seventeen incubators.

Cystic fibrosis

Organoids can be applied in many areas, from in vitro preclinical models for screening the effectiveness of potential medicines to patient-specific optimisation of drug therapies (personalised medicine). All of this starts with the development of good models. Just as with animal models in preclinical research, the behaviour of an organoid in a test also has to have a certain clinical relevance.

One of the first illnesses for which this has been extensively tackled is cystic fibrosis (CF). CF is a hereditary disease that causes serious damage to the lungs and the digestive system, for example. Due to a defect in the CFTR gene of the 70,000 to 100,000 CF patients worldwide, organ secretions become thick and sticky, so that the affected organs no longer function properly.

'A challenge in the treatment of CF lies in the diversity of the genetic defect. More than 2,000 different mutations have been identified in CF patients, with twelve mutations occurring in half of the population and the rest being distributed over the other half. Therefore, a medicine can be less effective for one patient than for another. While in the past you could only determine this experimentally, we can now predict the effectiveness. For this, we use a test developed at the UMCU/WKZ (Utrecht University Medical Centre / Wilhelmina Children's Hospital) by Jeffrey Beekman, the in vitro FIS assay, with FIS standing for forskoloin-induced-swelling. An important investment we made for this is the design of a CF biobank with more than 400 organoid cultures of CF patients that represent more than 100 mutations. With this biobank, companies can predict the effectiveness of new substances on the entire population or specific mutations within this population. Various studies on other diseases show

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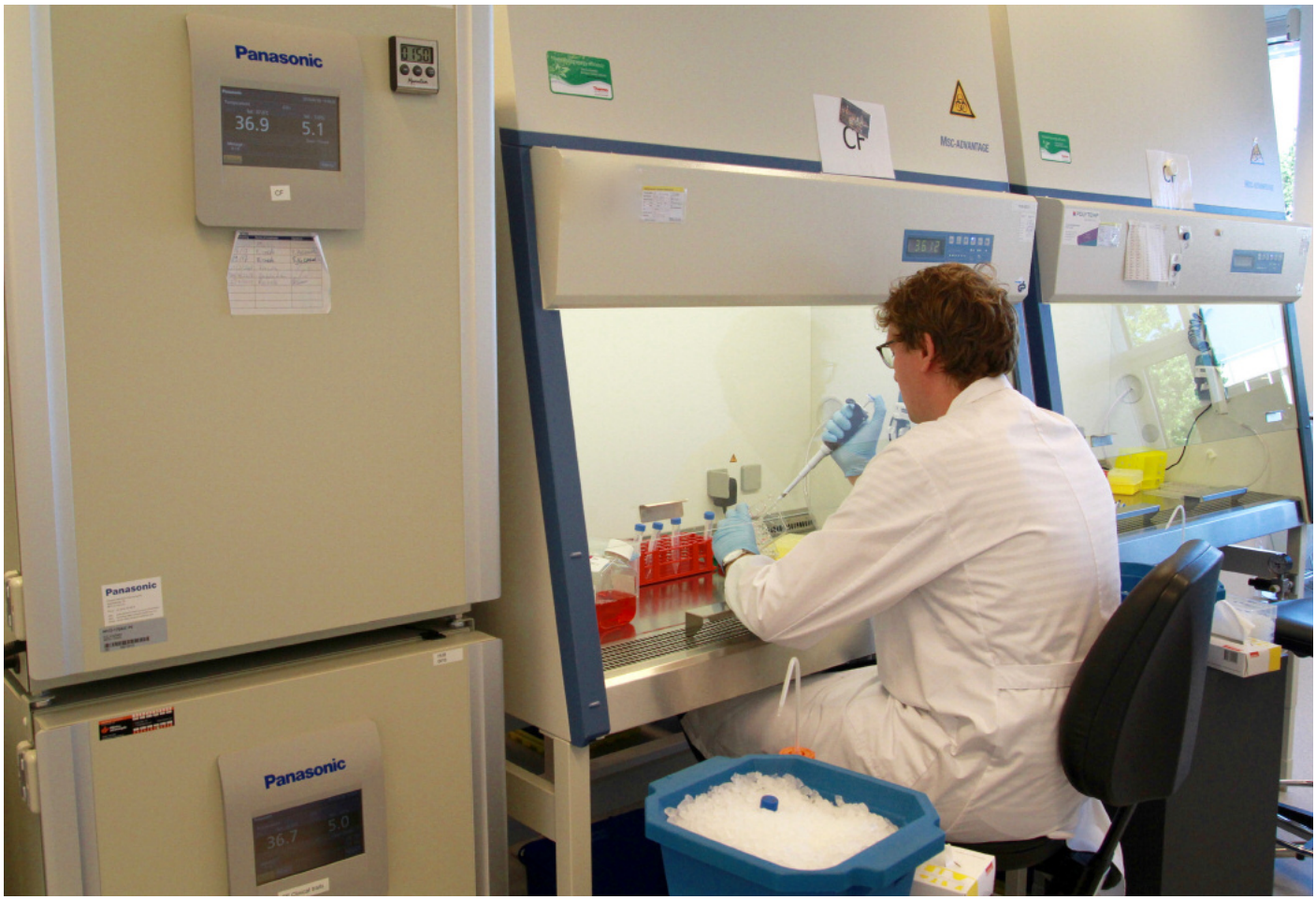

The laboratories for tissue cultivation are organisationally divided across three departments: oncology, CF and other illnesses, and screening.

EUR 15,000 for an organoid

You need about five months and EUR 15,000 in order to cultivate sufficient organoid material for a biobank. This not only applies to organoids with which you conduct additional experiments (and whereby you cultivate new cells from an original organoid in the 'master cell' bank for the 'working cell' banks that serve as the source for experiments), but also to those organoids that are cultivated for patient studies. The latter are now responsible for the bulk of the biobank's growth, with currently almost 1,000 different organoids. The costs for creating these organoids are relatively low when compared to the costs of the in part still experimental CF medicines. If you consider that you can improve the effectiveness of treatment (thus only prescribe medicines that work), then in addition to the gain in patient benefit, these patient-specific organoids will even bring about savings. For the original organoid, too, things are not as bad as they seem: after all, with those EUR 15,000 you are investing in an organoid that you can use for years for all sorts of research. This might then end up costing just a few euros per experiment.
Robert Vries is convinced that these costs will decrease: 'If we can scale up the production of organoids to the numbers we see in oncology, this technology will also become a lot more inexpensive.'

Robert Vries, CEO of HUB (Hubrecht Organoid Technology), predicts that his company will become increasingly busy with the ultimate breakthrough of personalised medicine.

that this is an especially effective method. These studies show a correlation of over 80% between what happens in the patient and what happens in the organoid. If you create a new medicine based on these samples, the chance that you already know you're heading in the right direction is a lot bigger than if you rely on classic preclinical testing. One of the reasons medicines are so expensive is because the majority doesn't make it to the finish line: more than 90% fail. If you already know what is going to happen before you start with clinical trials, you can go through the process a lot more efficiently,' says Robert Vries.
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Personalised medicine
A step further is to predict which medicine works (best) with the aid of an organoid cultivated from stem cells from the diseased tissue of a CF patient. 'You might think that it's just a small step from drug screening to organoid cultures, but in practice there's a lot more to it. When it comes to diagnostics for predicting what types of medicines patients need, there's an entire protocol in place along with the associated technology. Academically speaking, there is a kind of proof-of-concept for this, but you do need to prove that the approach is actually suitable for clinical application. We therefore also need to conduct clinical trials for the organoids themselves, which is what we're currently doing. We haven't had any negative tests to date, so we've had a successful run!'
Patients have also discovered the potential of organoids and in their enthusiasm tend to oversimplify things, which Robert Vries can well imagine, certainly for serious illnesses for which no medicines are available yet, such as CF. 'Patients themselves call us to ask us to create an organoid of their bodily material and send it to a pharmaceutical company that is developing medicines for their illness, so that the company can make these specifically for him or her. That's not possible, of course, but it may happen in the future. And, in fact, it's already happening for groups of patients with specific genetic defects. Eloxx Pharmaceuticals, for example, is developing a

medicine against a stop codon – in part thanks to the use of organoids – which seems that it will also be effective for a certain form of CF. In this case, patients have literally said: I have that mutation, do something!'

Busy at the lab

The initial positive results for CF have quickly garnered interest in other disease profiles, in particular oncology. 'While CF is a "clear" illness, with low incidence, things are a lot more complex and comprehensive for cancer. But the medical need is also high in this field, which provides a great deal of motivation in our work. However, we are too small to tackle all illnesses. What's more, we dedicate one-third of our time to the technological development of new scientific methods, which we optimise and scale up for application in the pharmaceutical industry.'
The robust growth of our company, which grew in parallel to the worldwide market for 3D constructs (almost zero in 2013; now 700 million euros) is also reflected in the laboratories, which are currently spread among two adjacent buildings due to a lack of space. The plan is to relocate to a new property at the beginning of 2022, but until this happens we expect to have to lease additional space. The laboratory work is currently organisationally divided across three departments: oncology, CF and other illnesses, and screening. From a physical standpoint, these departments are rather interlinked. For example, molecular biology and histology are housed in one building, where a quarantine lab and an ML2 lab for virology can also be found.

Seventeen incubators

Tissue cultivation takes place in the 'main building', which also houses the offices. Here, there is a clear separation between the various diseases in the current 17 CO_2 incubators: (patient) cells that are associated with a specific illness are grown in each incubator. The caution when separating such equipment per department and/or disease is also reflected in the selection process of the incubator supplier: PHC Europe, which also supplied several -80 °C freezers. 'We simply need to have the right equipment. This certainly applies to the incubators, which are essential to our work. In addition to specific conditions regarding stability and notification (alarms), if an incubator experienced a failure or its temperature deviated slightly, it's important for us to be able to find this information in the logged data. After all, these are valuable materials, often stemming from seriously ill patients. There's no compromising in this regard,' says Robert Vries.

About sample safety:
"After all, these are valuable materials, often stemming from seriously ill patients. There's no compromising in this regard"

Providing reliable capacity in incubation and ULT freezing

Incubators are a key piece of equipment at the HUB. The Unit has 17 CO_2 Incubators.

The following models:

MCO-170AICUVH-PE

With a capacity of 165 litres and weighing 80 kg, the MCO-170AICUVH-PE occupies a of 620mm x 750mm x 905mm.

MDF-DU700VH

The -80°C ULT Freezers from PHC is a VIP ECO ULT Freezer (model name MDF-DU700VH-PE). This range of VIP ECO Freezers reduces environmental impact and saves money by achieving an optimum footprint, using natural refrigerants, and is designed for minimal energy consumption.

INFORMATION

PHC Europe	**www.phchd.com/eu/biomedical**
HUB	**hub4organoids.eu**

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