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Liver-simulating device surpasses animal-based alternatives

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The HEMIBIO project has developed a Hepatic Microfluidic Bioreactor mimicking the structure of the human liver. The project team is confident that the new device will eventually help remove the need for testing chemicals on animal subjects.

The liver plays a key role in transforming and clearing the chemicals we absorb every day, which makes it one of the main target organs for toxicity tests. But scientists are still looking for the perfect testing method: the latter frequently involve animals, and therefore often fail to reflect the actual impact of tested chemicals on human livers with a 100% reliability.

To overcome this problem, the HEMIBIO consortium has set out to develop a device able to simulate the complex structure of the human liver and to make it available for preclinical toxicity testing as part of the SEURAT-1 initiative.

Now a few months away from the end of the project, the team has successfully developed a liver-on-chip device capable of maintaining metabolically active liver organoids for over a month in vitro, with a 99% accuracy in toxic concentrations measurements.

Prof. Catherine Verfaillie, coordinator of the project and Director of KU Leuven's Stem Cell Institute, highlights some of the key findings of the project and the future plans of the consortium.

How did the HEMIBIO project come about?

HEMIBIO was born from previous collaborations between many of the project partners (Drs Verfaillie, Luttun, van Grunsven, Rogiers, Sancho-Bru, Collas, Nahmias and Van Fleteren) that aimed to create an in vitro model of human liver for the study of toxicity and viral hepatitis, as well as studies related to the creation of parenchymal and non-parenchymal liver cells combined using small microfluidic bioreactors.

As we all had a strong interest in liver tissue engineering, from an applied and basic science point of view, we saw HEMIBIO as an opportunity to extend our collaboration across Europe and make a real impact on the development of alternatives to in vivo toxicity testing. We recruited excellent partners with expertise in liver biology, toxicology, genome engineering, sensor technology and microfabrication, and put together a proposal that was much greater than the sum of its parts.

Why do you feel like animal testing needs to be replaced?

At the time of submission of the HEMIBIO application multiple projects had been funded by the EC which aimed at decreasing the need for animals in toxicity testing.

However the assessment of the toxic effects of chronic exposure not only to drugs and chemicals, but also cosmetics, still required a relatively frequent use of animals. Moreover, aside from ethical considerations, there was also a great need for suitable human cells to be used in toxicity testing, due to the often poor concordance seen between toxicity in animal models and actual effects in humans.

What was your key objective?

Our belief was that, in order to recreate a liver-simulating device that is suitable for long-term toxicity testing, the cellular components of the liver need to be viable for extended periods of time (more than 1 month), with appropriate metabolic and transport function, and a physiology comparable to an in vivo liver.

To achieve this we had to consider the flow through the device, the zonation of the hepatocytes (and some non-parenchymal liver cells), and the impact of non-parenchymal cells on the function and downstream toxicity of the parenchymal component—in particular where one of the toxic endpoints, liver fibrosis and cirrhosis, was concerned.

Liver fibrosis and cirrhosis is caused by an interplay of hepatocyte toxicity and hepatic stellate cells activation which caused a deposition of collagen, along with changes in the liver sinusoidal endothelial cells which lose their specific fenestrated membrane. Such changes can only be studied within a device where the different cell components are present.

What would you say are the main achievements of the project so far?

HEMBIO has led to five major advances. First, we developed a liver-on-chip device capable of maintaining metabolically active liver organoids for over a month in vitro under oxygen gradients mimicking the native microenvironment. This demonstrates the specific advantages of human-on-chip technology, as current methods rely on daily drug exposure and dozens of end-point assays—resulting in limited kinetic information and prognostic value.

A second achievement was the generation of a diverse library of proliferating, metabolically functional, polarized cultures of primary

human hepatocytes—the gold standard for drug toxicity studies. We notably showed that the cells could accurately predict the TC50 [50% toxic concentrations, ed.] profile of 12 compounds with an unprecedented 99% accuracy (compared with 60% of HepG2/C3A cells). We generated five different genotypes from patients from a wide ethnic background, thereby allowing unique studies into inter-patient variability and idiosyncratic toxicity events in large populations.

A third achievement, was, by using cocultures of hepatocyte and stellate cells, the creation of a unique in vitro model to study repeated dose toxicity ultimately leading to liver fibrosis was developed.

The fourth success is the development of genome-engineered pluripotent stem cells—hPSC master cell lines—suitable for FLPe recombinase-mediated cassette exchange (RMCE) in the AAVS1 locus that allow generation of transgenic lines within 3-4 weeks with 100% efficiency and without random integrations. Using RMCE, we successfully incorporated several transgenes useful for lineage identification, cell toxicity studies, and gene over-expression.

Finally, HEMIBIO provided a novel definition of the transcriptional, miRNA and epigenome phenotype of quiescent and activated human hepatic stellate cells; as well as novel culture systems to maintain human stellate cells quiescent in vitro.

When do you think your device could be made available on the market?

The group of Prof. Nahmias at the Hebrew University of Jerusalem, in partnership the Fraunhofer Institute and Upcyte Technologies, demonstrated the ability to expand human hepatocytes and sustain their function in a microfluidic bioreactor for over 28 days under continuous

oxygen measurement. This liver-on-chip technology accurately predicted the TC50 values of acetaminophen, amiodarone, troglitazone, and rotenone, a number of toxins on the gold compound list from Seurat-1, with an R2 of 0.9 in in-vitro in-vivo correlation. The sensitivity of the device enabled the detection of a new mechanism of acetaminophen toxicity as well as new insights into the development of troglitazone-induced damage.

Several provisional patent applications have been submitted for the bioreactor, methods, and sensors. Some of the partners are considering setting up a company over the next couple of months that will provide screening services for the cosmetic and pharmaceutical industries. In addition, The Verfaillie group has now created an iPSC platform for drug-screens, which in the near future will hopefully be automatized, so that large numbers of lines can be generated, differentiated and used in high throughput screens.

Is it realistic to think it could replace in vivo testing in the near future?

We are convinced that the progress made within HEMIBIO will contribute to the possibility of replacing animal testing by in vitro testing, even if a lot of studies that will address in vitro – in vivo correlation will still be needed to completely replace animal studies by in vitro human cell loaded bioreactors.

What are your plans until and after the project ends?

Several partners from the HEMIBIO consortium (and other Seurat-1 clusters) are involved in follow-up local grant schemes (van Grunsven, van de Water, Verfaillie), as well as follow up EC-H2020 schemes (Verfaillie, van de Water), and directly with cosmetics companies

(Nahmias) on studies related to the creation of in vitro liver models for toxicity. These should eventually enable the replacement of animal studies by human in vitro studies using composite liver organoids in microfluidic bioreactors. Hence the progress made within HEMIBIO will be taken forward in these subsequent efforts.

More information: For more information, please visit HEMIBIO project website: www.hemibio.eu/

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