Bioengineered human livers mimic natural development

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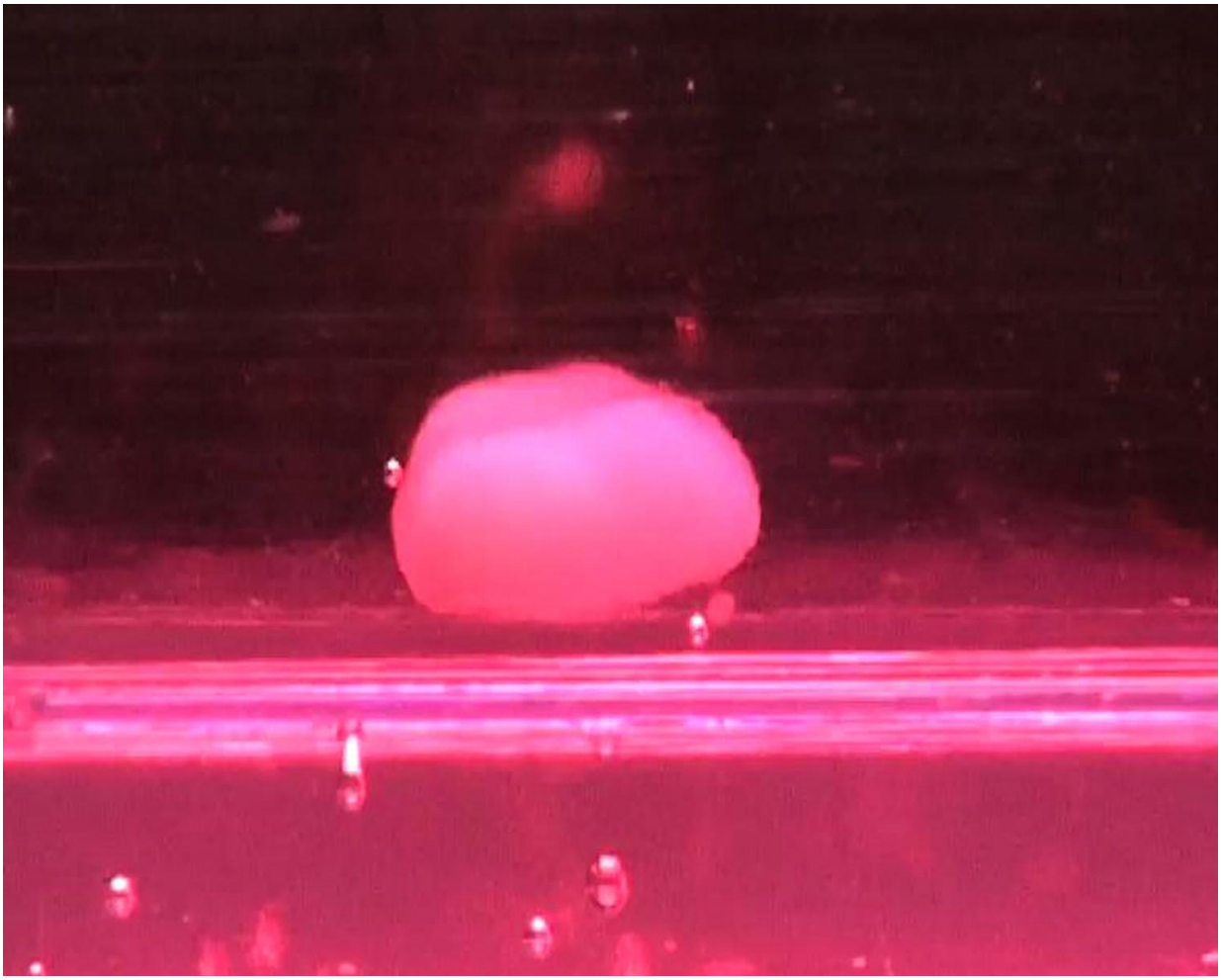


Bright-field microscopic image shows a three-day-old human liver organoid grown by scientists who report research results in Nature. The tiny liver -- suspended in solution inside an under-lit petri dish well -- is about 10mm wide. Generated from human pluripotent stem cells (hPSCs), the miniature organs are being developed for their potential to study and treat liver disease. Credit:

An international team of researchers bioengineering human liver tissues uncovered previously unknown networks of genetic-molecular crosstalk that control the organ's developmental processes - greatly advancing efforts to generate healthy and usable human liver tissue from human pluripotent stem cells.

The scientists report online in *Nature* on June 14 that their bioengineered human liver tissues still need additional rounds of molecular fine tuning before they can be tested in clinical trials.

The research was led by Takanori Takebe, MD, a physician/investigator at Cincinnati Children's Hospital Medical Center (Division of Gastroenterology, Hepatology & Nutrition) in the United States, and Barbara Treutlein, PhD, Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany.

The only current treatment for end-stage liver disease is a liver transplant, and the number of livers available from deceased donors is limited. Because of this, a major goal in regenerative medicine is to attain self-organizing human tissues - in which cells experience a series of coordinated molecular events precisely timed and spaced to form functioning three dimensional liver buds, the authors write.

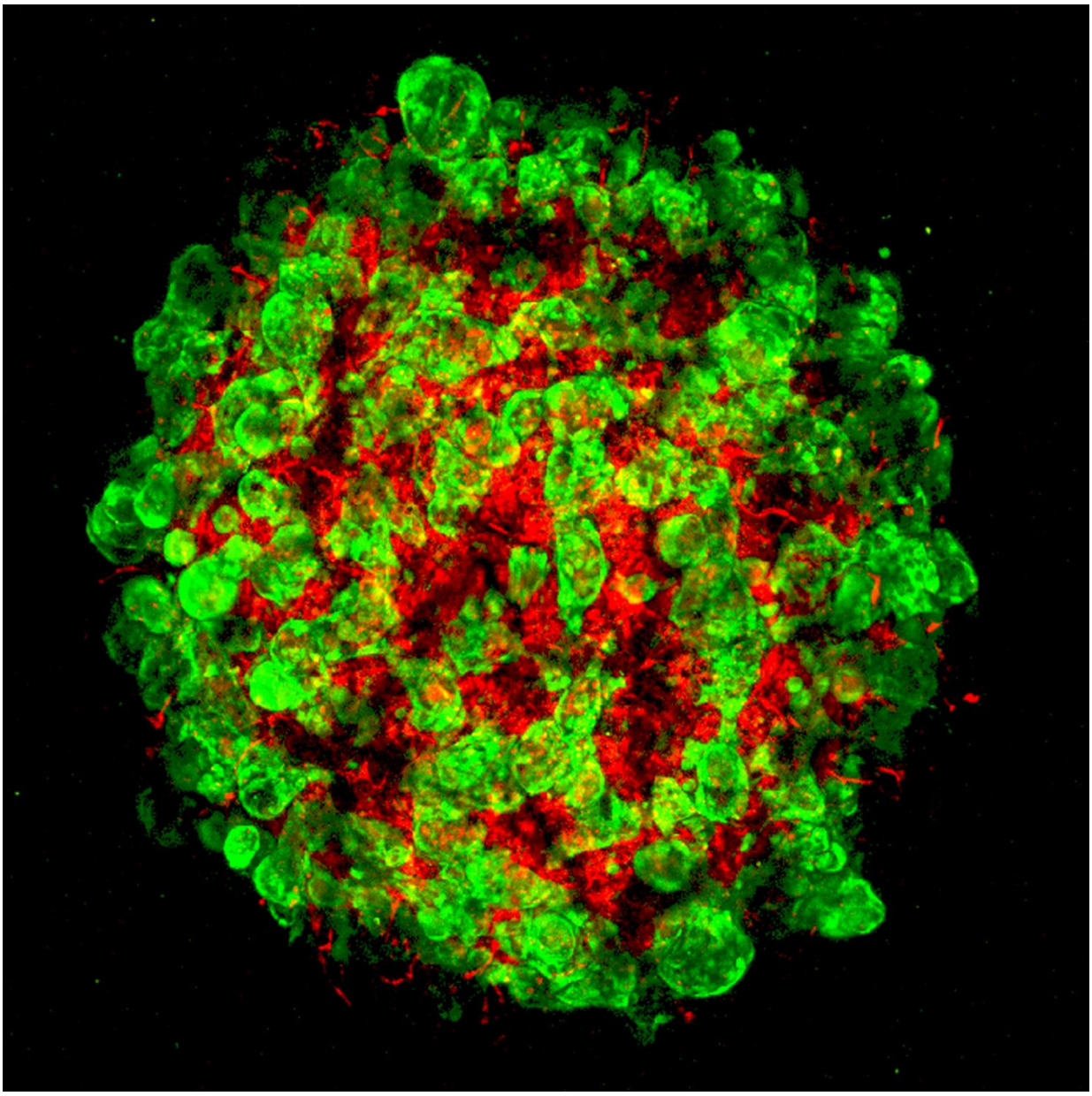
Nailing down the precise details and context of developmental molecular-cellular crosstalk in the endoderm of an embryo - where livers form - is critical to this technology's therapeutic potential.

"The ability to bioengineer transplantable livers and liver tissues would be a great benefit to people suffering from liver diseases who need

innovative treatments to save their lives," said Takebe at Cincinnati Children's. "Our data give us a new, detailed understanding of the intercellular communication between developing liver cells, and shows we can produce human liver buds that come remarkably close to recapitulating fetal cells from natural human development."

Genetic Blueprint

In the current study, the authors used single-cell RNA sequencing (RNA-Seq) to monitor how individual cells change when they are combined in a three-dimensional (3D) microenvironment. This is where vascular cells, connective tissue cells and hepatic cells engage in a complex communication.



Colorful confocal microscopic image shows detailed development of a human liver organoid that was tissue-engineered by scientists with human pluripotent stem cells (hPSCs). Green sections of the image show forming hepatic tissues and red sections show developing blood vessels. Reporting their research results in *Nature*, scientists are developing the miniature organs for their potential to study and treat liver disease. Credit: Cincinnati Children's/Max Planck

The main advantage of using single-cell RNA-Seq technology is it provides a blueprint of gene activity in each and every cell type. The researchers zeroed in on developing a complete blueprint of active transcription factors (genes that tell other genes what to do) and the signaling molecules and receptors in each of the different cells before and after they come together to form liver tissue.

Authors report they observed a dramatic change in the genetic-molecular conversations and how the cells behave when they all develop together in a 3D microenvironment.

Single-cell RNA-Seq analysis also helped researchers benchmark the engineered 3D liver tissues generated from stem cells against naturally occurring human fetal and adult liver cells. Researchers observed that the lab-grown liver buds have molecular and genetic signature profiles that very closely resemble those found in naturally developing human liver cells.

In particular they highlight molecular crosstalk between a signaling protein that cells produce to stimulate formation of blood vessels (VEGF) and a protein and receptor that communicates with VEGF to help trigger formation of a blood supply to the developing liver (KDR). The current study shows the communication between VEGF and KDR is critical to instructing the development and maturation of liver tissues.

Researchers indicate they observed this crosstalk during development of mouse liver cells, natural human liver cells and in their bioengineered livers.

"Our data reveals, in exquisite resolution, that the conversation between cells of different types changes the cells in a way that likely mimics what is going on during human development," said Treutlein at Max Planck. "There is still a lot left to learn about how to best generate a functioning

human liver tissue in a dish, nevertheless, this a big step in that direction."

Natural vs. Bioengineered

The authors noticed the gene expression landscape in the generated liver buds - such as precisely where and when genes express themselves - did not completely match natural human liver cells. The remaining gaps between natural and bioengineered tissues may come from different developmental cues caused by the unique microenvironment of cells developing in a petri dish versus that of cells developing in a person or animal.

The new cellular and molecular data uncovered in the current study will be "exploited in the future to further improve liver bud organoids" and "precisely recapitulate differentiation of all cell types" in fetal human development, the authors write.

More information: J. Gray Camp et al, Multilineage communication regulates human liver bud development from pluripotency, *Nature* (2017). DOI: 10.1038/nature22796

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