'Micro' livers could aid drug screening

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MIT researchers have devised a novel way to create tiny colonies of living human liver cells that model the full-sized organ. The work could allow better screening of new drugs that are potentially harmful to the liver and reduce the costs associated with their development.

Liver toxicity is one of the main reasons pharmaceutical companies pull drugs off the market. These dangerous drugs slip through approval processes due in part to the shortcomings of liver toxicity tests. Existing tests rely on liver cells from rats, which do not always respond to toxins the way human cells do. Or they rely on dying human cells that survive for only a few days in the lab.

The new technology arranges human liver cells into tiny colonies only 500 micrometers (millionths of a meter) in diameter that act much like a real liver and survive for up to six weeks.

Sangeeta Bhatia, associate professor in the Harvard-MIT Division of Health Sciences and Technology (HST) and MIT's Department of Electrical Engineering and Computer Science, and HST postdoctoral associate Salman Khetani describe their model liver tissue and its behavior in the November 18 online issue of *Nature Biotechnology*.

To build these model livers, Khetani uses micropatterning technology—the same technology used to place tiny copper wires on computer chips—to precisely arrange human liver cells and other supporting cells on a plate. Khetani adapted this method from Bhatia's early work as an HST graduate student building micropatterned co-

cultures of rat liver cells and supporting cells.

Such precisely arranged cells results in what Bhatia calls a “high-fidelity tissue model” because it so closely mimics the behavior of a human liver. For example, each model “organ” secretes the blood protein albumin, synthesizes urea, and produces the enzymes necessary to break down drugs and toxins.

To predict how close their model tissue is to real liver tissue, which has over 500 different functions, they also evaluated its gene expression profiles, measures of the levels of gene activation in the tissues. They found that these profiles are very similar to those of fresh liver cells, “giving us confidence that other [liver] functions are preserved,” said Khetani.

For drug testing purposes, this affinity to the human liver allows each colony to provide a window into the human liver’s response to a drug without having to expose human patients to the drug in a clinical trial, said Bhatia.

Further, because the engineered tissue lives for so long, it has the potential to make new types of toxicity tests possible. For instance, it opens the door to testing the effects of long-term drug use akin to taking one pill a day over multiple weeks. It also will allow more extensive testing of drug-drug interactions.

In addition to being a good biological model, the engineered tissue is designed to be seamlessly integrated into an industrial pharmaceutical science setting.

To mass-produce plates of the miniature liver models, Khetani relies on a technique called soft lithography. This technique fashions a reusable micropatterned rubber stencil from a silicon master. Each stencil

contains an array of 24 wells, and each well contains a matrix of 37 tiny holes. Khetani “peels and sticks” the stencil onto plates and places the liver cells into the holes, patterning over 888 miniature model livers across the microwells in a matter of minutes.

In tests of drugs with a range of well-known toxicity levels, assays (chemical detection tests) on the miniature liver models showed the expected levels of toxicity. “Our platform was able to predict the relative toxicity of these drugs as seen in the clinic,” said Khetani. For instance, troglitazone, a drug withdrawn from the market by the FDA due to liver toxicity, showed toxicity levels much higher than its FDA-approved analogues, Rosiglitazone and Pioglitazone.

The model uses a fraction of the costly human liver cells used in other test platforms and can be assembled using frozen cells. Moreover, the expanded toxicity testing capabilities have the potential to allow drug developers to identify toxicity earlier in the development process, thereby avoiding the expense of investing in formulas that are bound to fail.

A startup company called Hepregen has licensed the technology and is working to introduce it into the pharmaceutical marketplace.

“My hope is that this new model will make drugs safer, cheaper, and better labeled,” said Bhatia.

Source: Massachusetts Institute of Technology