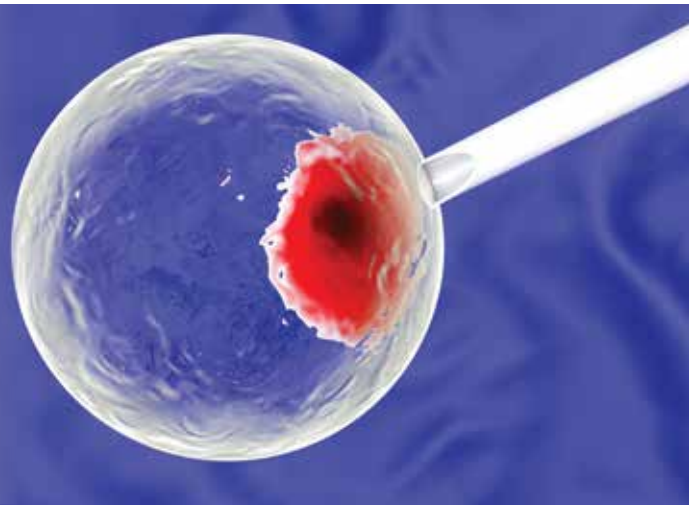
Patient in a Dish: Toxicologists Embrace Stem Cells



Toxicity assays based on embryonic and adult stem cells could soon replace unreliable cellular and animal testing, helping toxicologists detect which drugs might be dangerous long before they reach the market.

By Alan Dove

Pharmaceutical researchers live with a recurring nightmare. After spending years and billions of dollars developing a new drug, carefully shepherding it through preclinical and clinical trials, and finally receiving government approval to deliver it to patients, the compound suddenly starts hurting some of the people it was supposed to help. The promise of a new therapy collapses instantly into a morass of lost research investments and litigation.

"If you look back over the last 10 to 12 years, this is happening continuously, every year there are several drugs that go all the way [to market] and then have to be withdrawn or have their use significantly curtailed," says Stephen Minger, chief scientist for life sciences at **GE Healthcare** in Cardiff, United Kingdom.

The problem stems from the limitations of toxicology assays. In the standard drug approval process, researchers test a drug's toxicity in animals and relatively small numbers of people. Animal models don't represent human physiology accurately, and clinical trials seldom include a large enough population to identify rare idiosyncratic effects.

The ideal solution would be to grow realistic human tissues and organs in the lab, mimicking the physiology of a large population of patients without the cost and ethical baggage of massive clinical trials. Armed with the latest technologies for culturing embryonic and adult-derived human stem cells, toxicologists and cell biologists are now starting to do exactly that.

HEART LINES

Traditionally, toxicologists have tested compounds in immortalized cell lines and primary tissue cultures. Immortalized lines are effectively lab-adapted tumors that bear only a passing resemblance to cells in the body. Primary cultures represent a tissue's normal physiology more realistically, but don't live long enough for extended experiments.

Stem cells combine the best traits of both types of cultures, as they can be grown continuously and induced to differentiate into realistic tissues of all types. About five years ago, Minger and his colleagues began to exploit those characteristics to develop new assays for drug toxicity. "We've taken human embryonic stem cells and converted them into large populations of adult human cardiomyocytes that in our hands and the hands of our customers are extremely predictive," says Minger, adding that "at least retrospectively, we can show toxicity in compounds that have had to be withdrawn by the [U.S.] Food and Drug Administration that never had a hint of toxicity [in traditional assays]." Several pharmaceutical companies are now using GE's cardiomyocyte assay, called Cytiva, to test drugs in development.

Other companies are also developing stem cell-based assays for cardiac toxicity. **Cellular Dynamics** in Madison, Wisconsin, for example, offers a competing line of cardiomyocytes, called iCell, derived from induced pluripotent stem (iPS) cells. Unlike embryonic stem cells, iPS cells come from adult volunteers. A combination of growth factors reprograms the adult cells into an embryonic-like state, from which they can be directed to differentiate into all major tissue types.

For researchers who want to develop their own stem cell assays, reagent choices abound. General lab supply companies now offer culture media for stem cells, and specialty firms such as **Cellectis** in Paris, France and **Stem Cell Technologies** in Vancouver, British Columbia also cater continued>

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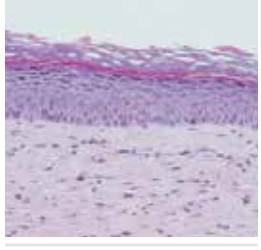

A model of human skin derived from induced pluripotent stem cells by researchers in the SCR&Tox program.

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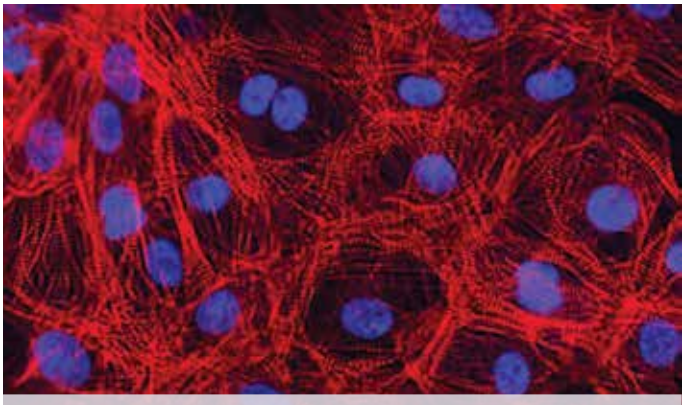

Embryonic Stem Cell-Derived Cardiomyocytes

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to the field. Whichever path they choose, assay developers must address several problems to produce useful toxicology tools. While many of the protocols to differentiate stem cells into particular tissues seem straightforward, the resulting cells are often in an immature state and mixed with partially differentiated progenitor cells.

Assays for pharmaceutical research also have to be consistent, which is a major challenge in the stem cell field. "A lot of the reagents that we use for the production of [stem] cells … are notoriously variable," says Minger. To address that, his team broke down the process of cardiomyocyte differentiation into discrete steps, then isolated and controlled as many variables as possible. The result is a production line that consistently produces cultures where at least 50% of the cells are mature cardiomyocytes.

DRUGS AND DEVELOPMENT

Generating fully mature adult cells will likely remain a major challenge, given the inherent biology of stem cells. "Stem cells … are really designed to do a good job of getting the fetus set up and structuring the body for its initial stab at life," says Robert Chapin, senior research fellow at **Pfizer** in Groton, Connecticut. However, the immaturity of stem cell-derived tissues is more a feature than a bug for Chapin and his colleagues, who study developmental toxicity.

Indeed, stem cell systems should be nearly ideal for determining whether a compound will be toxic to a developing fetus, especially if researchers can recapitulate the biology of multiple tissues in a single culture. "One of the [goals] is getting multiple cell types derived from stem cells together [in] 3-D cultures, because these cells don't operate in isolation," says Donald Stedman, senior principal scientist at Pfizer.

Pfizer now incorporates its own murine stem cell-based assay into its drug development pipeline to test for developmental toxicity. Regulatory agencies rely heavily on mouse and rabbit preclinical data to predict drugs' developmental effects in humans, rather than insist on clinical trials in pregnant women. A drug that is toxic to mouse embryos won't get approved for use in pregnancy, so companies want to weed out compounds likely to fail in mice.

To validate the assay, Chapin's team tested over 90 compounds that had been studied previously in animals, and found nearly perfect agreement between the stem cell assay and real developmental toxicity results. "We use a stem cell assay to help us identify those compounds that are clearly toxic and clearly don't need to go forward, and if we're lucky and we run an unknown compound through the assay and it comes out very clean, then it's easy to give that one a nice bill of health," says Chapin.

Such extreme results are the exception rather than the rule. More often, the investigators find that new compounds exhibit some toxicity at some doses. "In practice we wind up making judgment calls and talking about probabilities," Chapin explains.

The short track record and probabilistic results of stem cell assays have slowed their adoption. "I would say there's been quite a bit of resistance within the pharma community to [stem cell-based assays], because they're uncertain what [the technology] is telling them," says GE's Minger. For example, he adds, "if you see a compound that's toxic on our [Cytiva] cells that's not toxic on all the other standard tests, what do you believe?"

Early proponents of stem cell assays are confident those concerns will erode as more data come in. Researchers may also be swayed by the unique capabilities of stem cell assays, such as more direct tests of human developmental toxicity. "I'm hoping that from the developmental toxicity side there will be assays developed that are predictive using human embryonic stem cells, and we'll be able to decide whether those have any more or less value than the animal [systems] that we use," says Stedman.

TEAM EFFORTS

Using stem cells to replace animal testing is also a popular idea in Europe. In 2011, the European Commission and a consortium of cosmetics manufacturers jointly funded the Safety Evaluation Ultimately Replacing Animal Testing (SEURAT) program, which encompasses several large projects aimed at developing alternative safety tests for cosmetics and other chemicals. Meanwhile, European authorities began restricting animal testing, culminating in a full Europe-wide ban on the sale of animal-tested cosmetics in March of 2013.

Within the SEURAT cluster, the Stem Cells for Relevant, Efficient, and Normalized Toxicology (**SCR&Tox**) project focuses entirely on developing stem cell-based assays for toxicity in five types of tissues: heart, liver, muscle, skin, and nerves. Though cosmetics companies fund SEURAT, pharmaceutical researchers will likely find many of SCR&Tox's assays useful as well. "The objective of the SEURAT cluster

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is to replace in vivo repeated dose toxicity testing," says Vania Rosas, project manager for SCR&Tox.

To do that, SCR&Tox researchers have been working on differentiating iPS cells into the five mature tissue types the project studies. Rosas says they've confronted many of the same problems other investigators have had, especially the tendency of stem cells to retain immature characteristics even after differentiation. Nonetheless, SCR&Tox-developed assays will soon find their way into industry laboratories for testing and validation, and Rosas hopes regulatory agencies will begin accepting stem cell-based toxicity data for cosmetics within a few years.

Another large European research project, **StemBANCC**, is also working to improve the use of stem cells in toxicology assays. While SCR&Tox researchers are developing general-purpose toxicology assays based on iPS cells derived from healthy volunteers, StemBANCC scientists are focusing on iPS cells derived from 500 patients with various diseases. These disease-specific cells will form the basis for a new generation of drug development assays, including toxicological tests.

"The generation of induced pluripotent cells is just a technical goal. More important [is] to then differentiate them into a cell type that is relevant for a disease … and then try to understand the disease in a dish," says Martin Graf, head of the stem cell platform at **Hoffmann-La Roche** in Basel, Switzerland and one of the leaders of the StemBANCC project.

Besides cell samples, StemBANCC will also collect detailed clinical data from the patients. "One of the big things about this project is the depth of the clinical phenotyping that we're going to be doing on these patients, I think the real value of the cell lines subsequently is having that phenotyping information," says Zameel Cader, academic director of StemBANCC and a professor of clinical neurosciences at **Nuffield College** in Oxford, United Kingdom. Besides a diagnosis and history, each StemBANCC line will come with extensive data from diagnostic tests characterizing the patient's disease progression, treatments, and drug reactions.

Ultimately, the project seeks to give pharmaceutical researchers realistic laboratory models of individual patients, yielding more reliable predictions of a new drug's efficacy and toxicity long before it reaches the clinic. "I think in vitro toxicology using patient-derived material is really the first step towards trying to address the fall-off in drugs during their development," says Cader.

THIS IS YOUR BRAIN ON A CHIP

Other researchers are taking the patient-in-a-dish concept a step further by trying to grow multiple tissue types together in organ-like 3-D cultures. Luc Stoppini, professor of tissue engineering at the **University of Applied Sciences of Western Switzerland** in Delemont, Switzerland, began such a project after an initial disappointment with conventional stem cell culture. "People were claiming that they had [stem cell-derived] neurons, because they were expressing beta-3 tubulin which is one of the markers of neurons, but when I looked at them they were like fibroblasts, not really differentiating with axons, neurites, and synapses," says Stoppini.

As a neurobiologist, Stoppini wanted a more realistic neuronal system. Allowing the stem cell-derived "neurons" to grow in a 3-D culture system caused them to develop more neuronal shapes, and the cells also began transmitting electrical signals. The trick was to let the 3-D cultures grow much longer than traditional flat cultures; the neurons often continue developing for several months. "The message here is that we really need time to get human neuron function," says Stoppini. He adds that this slow development may make it hard to scale some assays to the high throughput needs of pharmaceutical companies.

Nonetheless, the payoff for getting such a system working could be huge. In particular, Stoppini says the long-term cultures can develop some of the non-neuronal cell types that are crucial for normal nervous system functions, such as astrocytes and oligodendrocytes. His team can now derive these miniature brains from both embryonic and iPs cells, raising the possibility of mimicking specific neural diseases with patient-derived cells.

The investigators have also taken the process a step further, growing 3-D brain cultures on microfluidic chips dotted with electrical sensors. Microcapillaries bring fresh nutrients to the culture while the sensors record electrical activity.

Stoppini is also connecting the brain chip to other stem cell-derived microfluidic organ cultures. Future pharmaceutical researchers might be able to feed an experimental drug into a patient-derived intestine, which would then deliver its metabolites through a vascular system to a miniature liver, finally affecting the activity of an in vitro brain. "At the end of the day we expect to have what we call the petri dish of the 21st century, that's to have a culture embedded in a biochip where all the biosensors … are integrated," says Stoppini.

The latest iteration of the technology also includes a Wi-Fi transmitter, so researchers can monitor the system without even opening the incubator. Stoppini adds that, "it's an extraordinary period that's opened new avenues of research by combining the biological tools [with electronics] … so we can really have some glimpse of how these things are working."

Alan Dove is a science writer and editor based in Massachusetts.

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