Ouch!

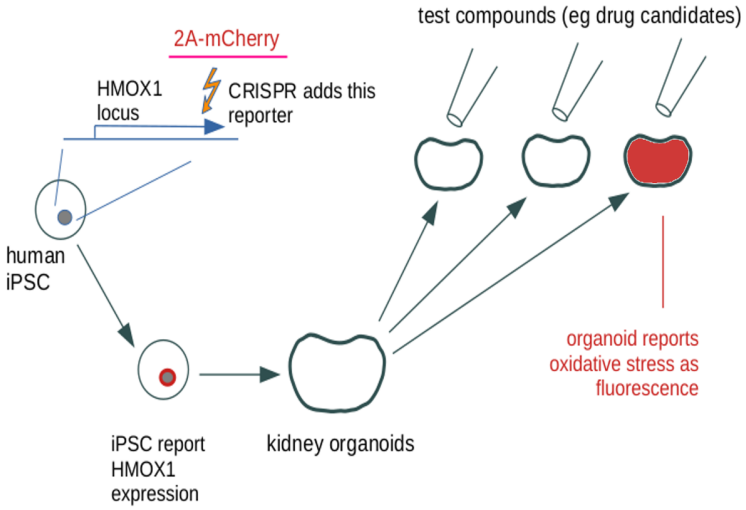
To be useful as a new drug, a candidate molecule must, at the very least, meet these two conditions: (i) it must be effective at controlling the target disease, and (ii) it must not be toxic (or at least, it must have little enough toxicity that its overall effect is very positive). Testing for toxicity is not as reliable as anyone would like. There are several reasons for this, but one of them is that some human organs are significantly different, in important details, from the corresponding organs in experimental animals. Heart and kidney are two prominent examples, and unexpected effects on these organs cause many drugs to be withdrawn during clinical trials.

Companies that develop drugs have therefore been interested for a long time in using human cells in their early testing, so that a potential problem is spotted before too much has been spent on the drug candidate in question. Human heart cells and human kidney cells are not human hearts and kidneys, and everyone acknowledges that things may damage an entire organ without having any noticeable effect on a simple dish of cells. Anything that has a bad effect on how dissimilar cells of a tissue communicate with one another would be an example of something that could not be detected in a culture of only one of those cell types. So, for this reason, there is a move to follow simple tests on simple cultures with later tests that involve more realistic organ substitutes, at least for those drug candidates that show no trouble on the simple tests.

Organoids - multi-tissue three-dimensional entities made from stem cells, that have micro-structures similar to real organs - are a relatively recent invention that show obvious promise for the application. The problem is that the more complex the organoid, the harder it is to make measurements from it that are simple enough to be done across thousands of samples by a machine, which is what the drug development process really needs. My lab made the first, primitive renal organoids back in 2010 and we have been making steady improvements to them ever since and for this reason, probably, a major European drug development company approached us with the problem. We, the lab and the drug company, agreed to work informally together, without money flowing but with help for assays etc., and that any results from our collaboration would be published openly so that any lab or company could benefit. This is not unusual - such companies know that safety testing systems need to be public and widely used anyway, if they are to be accepted by regulatory bodies.

Having listened to the needs of the company, our idea was to try to engineer cells so that, if they

were subject to toxic stress, they would produce a fluorescent signal. In other words, they would say "Ouch!" in a manner very easily detected by a machine. So the first thing to do was to produce renal organoids, subject them to a range of drugs known perfectly well to be toxic to kidneys, and see if they turned on any genes strongly in response to most or all of them.



This figure, the graphical abstract from our iScience paper (see Links), shows the general idea of the system. Image reproduced under the terms of its copyright licence: CC-BY-4.0

Clearly, any organoids we made would have to be made from human pluripotent cells rather than our usual mouse cells. We therefore used a good ready-made protocol from Melissa Little's lab, who published one of the first two human kidney organoid protocols in 2015 (Jo Bonventre's lab publishing the other). Having made the organoids, we subjected them to known toxicants (and some non-toxic controls), and used a technique called RNAseq to assess their gene expression. To cut somewhat long story short, one gene stood out. Called HMOX1, the gene codes for a protein that protects cells against oxidative stress.

The next step, done mainly by Melanie Lawrence and Shuwan Liu, was to use the CRISPR technique to add a red fluorescent protein 'tag' to the stem cells' own HMOX1 gene. Having engineered the cells, Melanie and Shuwan subjected them to various concentrations of hydrogen peroxide to subject them to varying levels of oxidative stress. Those not subject to stress showed very little fluorescence, while those subjected to more and more hydrogen peroxide glowed more and more brightly up to the point that it was so toxic it was killing them.

With the basic idea proved in the stem cells - they said "Ouch!" in the language of fluorescence -

the next step was to make kidney organoids from them, subject them to a range of toxic and harmless drugs, and see if they could 'tell' us what they had experienced. Our drug company colleagues offered to send us a panel of compounds, and we agreed that these should be 'blind-coded'. That is, they would know what was in tube 1, tube 2 etc., but we would not. We would apply the compounds at different dilutions to our organoids, and see if and how 'loudly' they would say "Ouch". We would then send them an encrypted copy of our results. When they had this, they would send us the decoding of what sample was what, and we would send them the decryption key to see our results. That way, at no point could anyone connect specific compounds to specific results until we both had copies of the results and the compound decoding. It is not, of course, that we did not trust one another. The problem is that if people know what they are expecting to see, they have a habit of seeing it. Or they get so worried about being biased that they make the opposite error. Using a rigorous blinding procedure like the one described above gives everyone confidence that human wishes and fears cannot have contaminated the results.

And the results of the experiments, done mainly by Melanie Lawrence and Mona Elhendawi, were really encouraging. It turned out that the cells responded to toxicant molecules, with the rapidity of the response depending on the compound used. If the fluorescence was examined at both 24 and 72h, then if either one of these showed an 'ouch response, this correctly identified a known toxicant in the panel.

Have we solved the problem of renal toxicity testing? No, of course not - we have made a step towards a better system, that's all. Our system will need testing against a much larger panel of compounds before real confidence can be placed in it. Also, we used just one line of human induced pluripotent stem cells for these experiments, from an American male as it happens. To be useful in pre-clinical drug testing, this kind of system needs to be built using cells from a range of genders and to represent many different peoples of the world.

Developing test systems is not the focus of this lab. We have now published the study in iScience (see Links) and, if anyone else want to take the idea on to a commercial idea, we would be happy to help them on their way, but not to play too large a part ourselves. We have other priorities for our organoids, and still hope one day to turn them into functioning kidneys.

Links:

The iScience paper: https://www.cell.com/iscience/fulltext/S2589-0042(22)00154-7

Jamie Davies, Edinburgh, February 2022