Published on *Up Close* (http://www.upclose.unimelb.edu.au)

#306: Organs on a chip: How 3D models of living tissue are changing biomedical research

VOICEOVER

This is Up Close, the research talk show from the University of Melbourne, Australia.

DYANI LEWIS
I'm Dyani Lewis. Thanks for joining us. Our understanding of biological systems including our own body is largely based on laboratory studies. By looking at cells grown in Petri dishes or conducting experiments on animals we can pick apart how biology works. But how well do our lab techniques actually represent real life? In many cases, when candidate drugs make it to clinical trials we discover that promising outcomes in animals don't always translate into the same in humans.
But what if we could advance our medical knowledge without the use of animal models or tissue culture experiments? Is there a better way, a more accurate way of investigating human physiology in the lab? Such is the exciting promise of organs-on-a-chip.
To learn more about the exciting field of organ-on-chip technologies we're joined on Up Close today by Professor Donald Ingber, Founding Director of the Wyss Institute for Biologically Inspired Engineering at Harvard University. Don is also the Judah Folkman Professor of Vascular Biology at Harvard Medical School and Boston Children's Hospital and Professor of Bioengineering at the Harvard School of Engineering and Applied Sciences. He's in Melbourne to deliver the 2014 Graeme Clark Oration.
Welcome to Up Close, Don.

DONALD INGBER
Thank you.

DYANI LEWIS
Don, could I start by asking why create an organ-on-a-chip when we already have things like tissue culture where you grow the tissues in Petri dishes and we also have

very good model organisms like worms and fruit flies and mice?

DONALD INGBER
Well first off most cells in dishes don't express the functionality that you see in our bodies and that's been known for years and people have been striving to make better cultures but they still don't exhibit the functionality that you'd want to be able to test drugs. You also don't have flow and you don't have the vascular system that produces different tissues which is very important if you're trying to measure, for example, drug delivery and the per cent of a drug that gets to an organ and clearance from the body. And you don't have organ-organ interactions which are absolutely critical in our bodies for physiology.
The simple systems like flies and worms, they're tremendous for analysing molecular mechanism but, you know, a fly is not a human. I think in general the finding is that more often than not results from animal tests do not predict what happens in human clinical trials. I often say to my students if someone told you to design your next experiment by the failures you have that you would just laugh, but that is in fact the way the pharmaceutical industry works and they're kind of captive because the regulatory agencies require animal tests because there's nothing better.
Animals, sometimes they're good but oft times they're not and the pharmaceutical companies know there's a problem, the regulatory agencies there are problems. So everyone has been striving to come up with better alternatives.

DYANI LEWIS
So the organ-on-a-chip that you've been involved in developing, can you explain to us what it actually looks like?

DONALD INGBER
Yes, so the first one we developed is a lung-on-a-chip if we take that as an example. It's the size of a computer memory stick. It's optically clear, flexible silicone rubber. We use microchip manufacturing to make a very small hollow channel or series of channels inside it less than a millimetre in diameter. And if you imagine you're in this channel, think of it like you're driving through a car tunnel.
For the lung-on-a-chip we have a suspended membrane horizontally midway across it and we have engineered holes in that membrane so it's porous. We coat it with molecules that are called extracellular matrix which is what you could think of like the egg carton that cells sit on, it's what holds cells together in the body in tissues. We take human cells from the air sac of the lung, the alveolus and we culture them on top of that membrane and we flow air over it and then we take human lung capillary blood vessel cells and we put them on the bottom side of the same membrane and we flow medium by it.
That creates the tissue-tissue interface that actually makes an organ. An organ is two or more tissues that come together to give you new functions. So it's not cells in a dish or tissues, it's organs on in this case a chip.
The other thing we do is we have side chambers that are hollow, full height and we apply cyclic suction and that, because it's all flexible rubber, it makes the middle membrane with cells attached stretch and relax, stretch and relax to the same degree and at the same rate that our cells do when we breathe in and out in our lung

and giving the flow, the air cell interface and the breathing motions you get functionalities that no one's ever seen before in a dish.

DYANI LEWIS
So that pliability, being able to stretch and bend the tissue or the layer of cells that are in there, that's quite important I take it and something that certainly wouldn't be possible in the normal Petri dish scenario.

DONALD INGBER
There are certain dishes that are flexible that you can stretch and you actually can have potent effects on cell function but the combination of having the ability to create tissue-tissue interfaces which are physiological, in this case the capillary cells and the air sac cells, plus the stretching, plus the flow all of that together creates the physiological environment that induces these cells to exhibit functions that look very much like in the body. So if we do this with the gut-on-a-chip, very similar to the lung-on-a-chip but instead of breathing motions we give peristaltic like cyclic distortion at that rate and we give trickling flow like the gurgles you hear going through your intestine.
And when we take cells that have been used for years by pharmaceutical scientists and they think they're very poorly differentiated or specialised and not very functional, we put them on this and once we give them the flow and the peristaltic like deformation they spontaneously organise into villi which are the finger-like interdigitations or protrusions that increase surface area of absorption in your intestine, plus they put out mucous, plus they form all the different cells types of the small intestine. And because of that we can now culture microbiome which are the microbes that live in our gut, the normal healthy microbes that are now being found to be important for diseases, for response to drugs, for absorption of nutrients. None of that has ever been seen on a regular culture dish.

DYANI LEWIS
It's really quite incredible, especially the spontaneous creation of the villi because I guess traditionally we would have thought that give some cells some oxygen, give some cells some nutrients and that there is life in a dish, but this is bringing in the biophysical aspects of life.

DONALD INGBER
Yes, so I've worked for 35 years, kind of a voice in the woods in the beginning because people thought I was nuts, saying that mechanical forces, tension, compression, shear are as important as genes in chemicals and hormones. That has turned out to be the case but it took a long time for people to realise it because there's no way that physics is not involved. It can't just be chemistry and this is true at the molecular level, at the cell, tissue, organ and whole body level. You know, if you lie in bed for too long or if you're an astronaut your bones start resorbing. If you don't do exercise your muscles [unclear].
The cells fuel the physical environment. This is true in all organs, all tissues and so that is a key part of what we do and yes, if we don't have the breathing motions in the lung we don't see functionality but we also don't see toxicities from, for example,

when we test aerosol or airborne based particles like smog. We give those to the lung-on-a-chip we see an injury response but only with breathing motions.
When we developed a disease model we developed a model of fluid on the lungs, pulmonary oedema and we used a drug that is a cancer drug that's used clinically but its dose limiting toxicity is fluid on the lungs where fluid goes from the bloodstream into the air space. We find when we injected it intravenously in our device, meaning through the vessel channel, we get fluid going into the air space at the same dose and the same time course as in humans but largely only with breathing motions. Without it you see a small but minimal effect.

DYANI LEWIS
So it sounds like the lung-on-a-chip, it's really able to mimic quite well what would be happening in a real human lung. How do you go about validating your results? I mean your results are different to what you see in tissue culture, but how do you say yes this is what we see in real life?

DONALD INGBER
The benchmark is always recapitulating physiology of the human or in vivo and so we have to use animal models to validate some of this ourselves but there's also a lot of knowledge in human tissues and organs. For example, we've recently developed a small airway chip, as opposed to the air sac we now have a small airway, and they're given the right conditions on these chips the cells essentially form a multilayered epithelium, a cell lining just like in the small airway but more importantly they put out cilia and they put out mucous and they move.
It's like mucous cleanses particles that come out of air, we see the particular clearance and we can measure and quantitate the velocity, the response to infection and looking at the hormones, the factors that are secreted when there's an inflammatory response and we could show that it's very similar to what you see in human. In fact the ciliary response, the mucociliary clearance is almost identical to what's been reported in human lung. So we always go back in vivo.
The hard part is when we come up with something that no one's ever seen before. So in the chip we could actually find, as I said, you need breathing motions for certain things. You can't do an animal study without breathing motions in general so we had to go and develop animal models where we can artificially basically put it on like a heart lung bypass, is the simplest way to envision it, and we could actually have a ventilator and have the blood flowing through the heart and the lungs and explore if you stop the ventilation what happens. And we got the exact same response as we got in our chip without breathing.
So that was actually a prediction and so we learned something from these chips. They provide a window on organ level physiology in terms of molecular scale resolution. So most people think drug companies just screen for chemicals till they find a drug. It's not true at all. They really have to understand molecular mechanism. They worry about toxicities and mechanism of toxicity. These chips can enable one to study that in human cells in an organ level context.

DYANI LEWIS
I'm Dyani Lewis and you're listening to Up Close. We're discussing organs-on-a-chip

with bioengineer Don Ingber.
Don, in tissue culture if you want to have a look and see how your cells are going you can usually just put them under a microscope and have a look but with the complex 3D structures that you get forming in these organs-on-chips how do you monitor what's going on?

DONALD INGBER
We can do exactly what you can do in a culture dish as well as what you could do with animal testing. Because we use optically clear materials, sort of like a living three dimensional section through the organ, it's not building a physical organ, we can focus a microscope right at the cell level. We could do high resolution imaging, we could do all the tricks that biologists do with fluorescent molecules. We could look at calcium imaging. We really have a window on physiology with molecular scale resolution.
There are other people who are trying to approach replacing animal testing by doing tissue engineering in a dish, like make a three dimensional centimetre cube of a liver let's say. The problem is you can't see what's going on in the middle of it. This is a real value of the feedback we get from pharmaceutical partners, cosmetics companies is that you could actually in real time envisualise what's happening.

DYANI LEWIS
The lung-on-a-chip that you described though, it still does only contain those two different types of cells, the cells that line the alveoli or the lung sacs and then the cells that would line the vascular tissue.

DONALD INGBER
Well that's where we start. OK so this is a term called synthetic biology. It's often used to build up artificial gene circuits in cells. But this is synthetic biology at the organ level so we start with the simplest model, the two tissues that make that part of the organ but then if we look for function and we don't replicate it we say something's missing and we [unclear] back. For example, we did an infection with bacteria, a bacterial infection mimicking pneumonia in the lung-on-a-chip by putting living bacteria in the air space. And you don't get inflammation without the immune cells so we had to add white blood cells so we took white blood cells and injected them into the blood side.
If there were no bacteria they'd just float around like our white blood cells but if there were bacteria the lining cells get activated, which is what happens in our vessels, and they get sticky and the white blood cells stick and we can watch them in real time under the microscope stick, move through the cells, go through the tissue, come out the other side and engulf the bacteria. So we watched the entire human inflammatory response in real time at high resolution in this little device. It's all because it's clear and leaves microfabrication to orient the tissues in a way so that they're exposed for analysis.

DYANI LEWIS
That's exactly the question I was going to ask actually about the immune system because in many of our organs, I guess all of our organs they aren't isolated from the

rest of our body so I guess eventually you need to bring in other components of the body even though it is an organ-on-a-chip.

DONALD INGBER
So a few years ago we presented the idea that if you make these organs, and we're now working on like 15 different organs, they have a capillary or a blood vessel channel, in theory you could link the blood vessel channels to each other and flow them and you would have a human body-on-chips. So we actually are funded by DARPA which is the Defense Advanced Research Projects Agency in the United States to build an automated instrument with the goal of having essentially a DVD-like player that you'd have different DVDs but on each DVD there'd be different organs. You could have 10 lungs if you want to have replicates or you could do lung, liver, kidney, heart or heart, liver, kidney, bone marrow. We actually are collaborating with Sony DADC which is a company that makes DVDs to help us mass produce the actual chips at this point.
So we have started to do that and we've started to link different organs and we could actually show physiological coupling between different organs. So that's the long range idea.
In terms of the immune cells and the white blood cells, we just published a paper where we developed bone marrow-on-a-chip, and [in] this we used a little different technique where we used tissue engineering in an animal to form artificial bone and then the bone marrow forms. We do it again with microchip manufacturing. We make a little clear silicone rubber holder that has a cylindrical hole and we put materials that engineers have discovered over the years to induce bone to form and we wait eight weeks and there's white bone and then we remove it from the animal, we punch holes in it with a needle and then we have another device that has microfluidics, meaning hollow chambers with flow going by. It has a cylindrical shape that is exactly the same as the bone. The bone takes the shape of its container when it forms, kind of amazing, and you pop it in and you could keep it alive with the flowing medium.
We create entirely functional marrow. You could literally lethally irradiate another animal and replace its entire blood system by taking the cells out. That may be a source of blood cells for all the other organs.

DYANI LEWIS
Are there some organs that you just can't fabricate like can you fabricate a brain-on-a-chip or something like that?

DONALD INGBER
So yes, again it's synthetic biology so we have to add back and so we're starting with a blood-brain barrier which is very important for drug delivery and that has two cell types, endothelial cells, astrocytes, but then we'll try to add neurons back and other types of brain cells. But we're not going to model a long bone and the effects of compression on your bone or your spine or consciousness but the trick is kind of simplifying down to the key functional elements that are important for either physiology or disease and seeing what we can replicate in a way that's amenable to analysis. There's certain things we can't do.

DYANI LEWIS
You're listening to Up Close. I'm Dyani Lewis and my guest is bioengineer, Professor Donald Ingber. We're talking about organs-on-chips.
Don, can you create organs-on-chips that replicate what might be going on at different stages in an animal's development?

DONALD INGBER
That's a very interesting question. We have not done that. We definitely have interest in modelling organs from a child versus an adult for example, because it's very hard to do clinical trials in children but we could get cells from children and see if we can demonstrate functionality with paediatric devices. There are people that have used microfluidic systems to look at fertilisation and actually select for sperm that are more effective at fertilising and taking the eggs out that get fertilised. Development is a very dynamic process and it really involves remodelling of the interfaces. So the way we have it right now, I think we could probably visualise development but we haven't approached it yet.

DYANI LEWIS
Computational biology has come a really long way in recent years. How do organs-on-chips compare with or perhaps complement computational approaches to understanding what's going on in biological systems?

DONALD INGBER
Well first off in our project to do human body-on-chips we have a computational component, we have collaborators at a company called CFDRC (CFD Research Corporation) and the idea is to use the data we generate. If you put a drug in this human body and let's say you deliver an oral drug to the gut-on-a-chip and you can measure its levels and its metabolism throughout the whole system, then you watch it being broken to metabolise in the liver and it comes out of the liver and you can see some of it cleared by being peed out through the kidney and you can measure the outflow from the kidney as well as what goes to the next organ, you can look at that as toxicity in the heart and then you can see if it has an effect on the bone marrow or on the liver.
We'll have all these measurements. That is basically what drug companies do and a lot of animal testing has to do what they call pharmacokinetics, pharmacodynamics, how the drug is cleared, the temporal time course that helps to find dosing so when they go to the clinical trials what dose do you use, what regimen? That we're going to use computational approaches. That's a key part of this whole system.
In terms of computational biology in general, my group's done a lot of that work in the past as well. Computational models are only as good as the data that they use to build the model and I do think that because we get this window on molecular scale properties and a lot of computational models are based on molecular behaviours, we may be able to get more rigorous numbers and data that can feed back to the computational models. And so I think people would find it useful in the future.

DYANI LEWIS
Don, is there anything that we can do on a chip that we couldn't otherwise do?

DONALD INGBER
Yes, I think one of the nice things about the chips is that there's certain things that are just unethical to do in humans but need to be done. One example is in the United States Congress has given the FDA, the Food and Drug Administration, funding to try to develop countermeasures to radiation toxicity as well as chemical and biothreat type things so that they could stockpile therapeutics in case of an emergency. And the animal models are not good. There's no way you're going to expose humans to lethal doses like a reactor plant disaster. But we actually got funding from the FDA and we're working with the bone marrow-on-a-chip and the gut-on-a-chip which are major sites of toxicity.
We can get very similar responses to for example the bone marrow-on-a-chip, exposing it to radiation versus the whole animal and looking at what the bone marrow in the animal responds. Whereas the standard culture dishes don't look anything like it. And we've also shown some of the known drugs that have some effect as a countermeasure we could see in these devices. That's an example where the FDA's extremely excited about it.
We also are looking into potentially testing infectious agents like SARS (severe acute respiratory syndrome), MERS (Middle East respiratory syndrome), influenza, noravirus. Noravirus you can't culture. In addition they can't study it to figure out how to develop therapeutics so we're just about to explore whether we can grow that in the gut-on-a-chip, which would be an amazing thing. Same thing with these other viruses. Many viruses are human specific so I think those types of things would be really powerful.

DYANI LEWIS
At what point will we be able to actually say yes this technology is a really good stand-in for animal models and therefore we no longer need those animal models?

DONALD INGBER
What I have been told by the FDA, all we have to do is show that they're as good or better than animal models but have to be robust statistically and repeatable and so forth. But because the animal models are often so poor, if you can just be as good as them and be human they would view that as something that's valid to move them on. This is not going to replace all animal models and it's not going to do it all at once but I do think you'll see one animal model being replaced at a time. So it will be a progressive one and it's not going to work for certain things but it will work for others.
I think if we could do one animal model that's a major positive because worldwide there's a major effort in reducing and replacing animal models. And it's not going to come in one fell swoop.

DYANI LEWIS
Surely I guess pharmaceutical companies would be very keen on a technology that saves the kind of waste of backing drugs that aren't going to be winners.

DONALD INGBER
Yes, the catchphrase in the pharmaceutical industry is they have to learn to fail

quickly and cheaply because right now they're failing very expensively and over long timeframes. They want to be able to know how do I prioritise, if I have a thousand compounds that look interesting and narrowed it down to 10, how do I prioritise which one I'm going to put my half a billion dollar investment into and 10 years of our company's effort? Or if there's a problem with that, how do I choose the second one to move up? These types of systems might be very useful for that. They may be useful if you see toxicity to figure out what is that toxicity and which of our other compounds don't have it or have less of it?
That's where I think the real value, we call it the high value high content screens. Not massive screening, they know how to do that already.

DYANI LEWIS
Don, it's certainly an extraordinarily exciting area of research and thank you for being on Up Close today to tell us about it.

DONALD INGBER
OK thank you. My pleasure.

DYANI LEWIS
Professor Donald Ingber is Founding Director of the Wyss Institute for Biologically Inspired Engineering at Harvard University. He's also the Judah Folkman Professor of Vascular Biology at Harvard Medical School and Boston Children's Hospital, and Professor of Bioengineering at the Harvard School of Engineering and Applied Sciences. If you'd like more information or a transcript of this episode, head to the Up Close website.
Up Close is a production of the University of Melbourne, Australia. This episode was recorded on 4 June 2014. Producers were Kelvin Param, Eric van Bemmel and myself, Dr Dyani Lewis. Audio engineering by Kelvin Param. Thanks also to Anne Rahilly for coordinating this interview. Up Close is created by Eric van Bemmel and Kelvin Param.
Until next time, good bye.

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