Piping for beginners

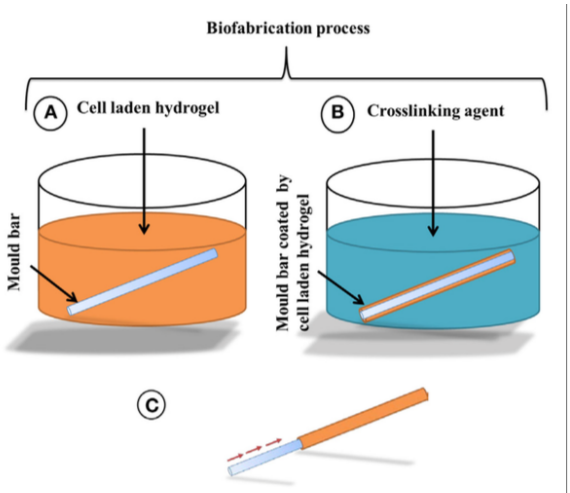
Many scientific papers are very complicated, largely because the data come from a long series of intricate, multi-part experiments each of which uses a sequence of arcane techniques: to read such a paper and understand it properly can take hours. Sometimes, though, people publish techniques that are really useful and so simple they can be understood in minutes. We have just been involved in publishing a very simple method that arose through a collaboration between three laboratories; the lab of Wenmiao ('Will') Shu at Heriot-Watt University, just south of Edinburgh Bypass, my lab and that of John Mullins, both in the University of Edinburgh.

The context is a research project being pursued by Chris Mills, a PhD student working with John and me. The mini-kidneys this lab is able to produce from stem cells show many realistic anatomical features (see the review in the links below) but they do not have a ureter. Chris decided to do something about this, and the PhD thesis he is about to submit for examination is effectively the development and comparison of several alternative methods for making a ureter and connecting it to the growing organ. The most successful technique is something we are writing up for publication right now and, following usual rules, I will wait until the manuscript has passed peer review and been accepted before discussing it. One of the other strategies was a simple one based on classical tissue engineering: the aim was to produce a tube of about the size of an embryonic ureter, composed of ureter-type cells on a scaffold that would hold them in a tubular shape. This is where Will's group came in: Will is an experienced tissue engineer and his laboratory has excellent facilities for making many different shapes of scaffolds by processes such as 3D printing, and he was keen to work with us on the problem and to lead the engineering side.

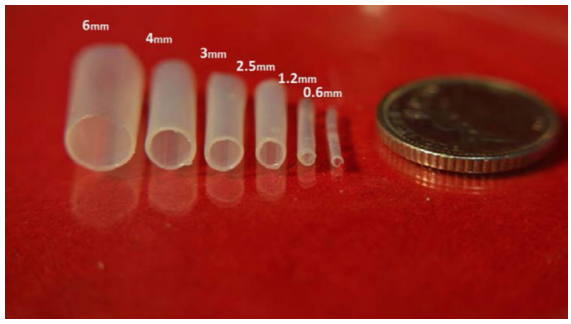
Although 3-D printing a tube is in principle an easy thing to do, existing technologies are ideal for making tubes the size of adult arteries and veins and the very small dimensions of an embryonic ureter created problems. Quite simply the printing mechanisms could not cope with making something so fine without either leaving small holes in the walls or blocking up the lumen (space) down the middle. It was all getting rather depressing, but then Atabak Tabriz, a PhD student working with Will, had a brainwave.

The hydrogels from which we were wanting to make the tubes are made of a plant-derived polymer called alginate. In the absence of divalent cations such as calcium or barium, alginate flows as a

thick liquid but, in the presence of either of these ions, it solidifies into a gel. Atabak hit on the gloriously simple idea of dipping a thin metal rod in a solution of alginate, withdrawing it quickly so that a coating of alginate remained, and dipping it into a solution containing barium chloride or calcium chloride so that it solidified. Then he repeated the process over and over again to build up the thickness of the coating on the metal and, after this, he simply pulled the tube off its rod as one might pull a mitten off a hand. The result was a fine tube of alginate hydrogel just the size we needed.



Atabak's clever idea for making an alginate tube by dipping a metal rod alternately in solutions of alginate and of calcium or barium chloride.



It might have been possible to use the alginate tube as a surface on which to grow cells but Chris had already established that ureter cells do not like growing on alginate, even when the alginate has been combined with peptides that would be expected to make it more cell-friendly. We therefore explored the idea of suspending cells in the alginate solution so that they would be incorporated directly into the walls of the tube. Among the cell types tested were ones from this lab that had been engineered to switch on a gene encoding a red fluorescent protein when treated with a drug. Cells incorporated successfully into the walls of the tube and would switch on their fluorescent gene on drug treatment, demonstrating that they were alive and responsive. What is more, tests with drugs

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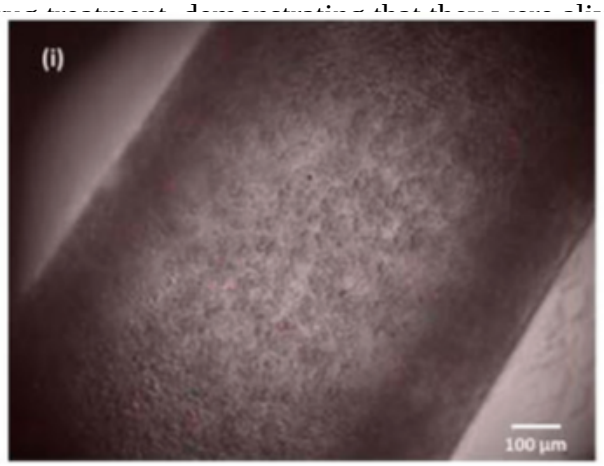

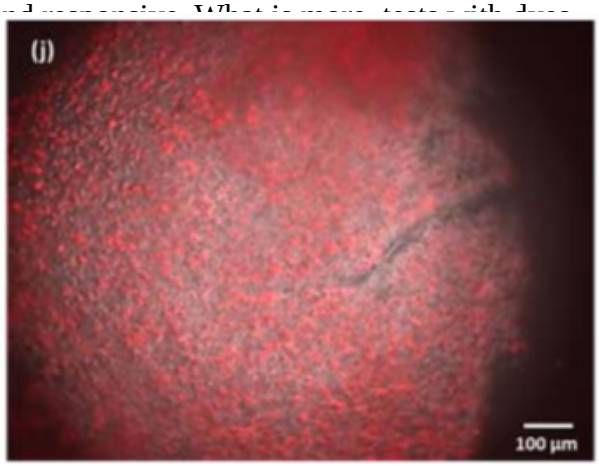

Cells visible within the tube walls, with and without the drug that switches on the gene for a red fluorescent protein.

The reason that this way of making tubes was worth publishing is that it is so simple. Most biology labs do not have fancy 3D printers, but we all have beakers and it is hardly difficult to get hold of a metal rod. Atabak's brainwave makes the fabrication of tubes laden with living cells accessible to anyone with a basic biology lab, and the technique is simple enough that anyone can learn it in minutes.

Why, given how well this worked, did we not end up regarding it as the best way to make a ureter? There were two issues. One was that, while cells were happy to live in the tube, they could not easily rearrange themselves to make the rather complicated multi-layer tissues of a real ureter: the alginate simply got in the way too much. The other was that even if we had made a realistic ureter this way, we would have had the problem of how to connect it to the kidney. But, even though the technique was a dead-end for Chris, it may be valuable for all sorts of other people wanting other sorts of cell-laden tubes.

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February 2017

Links:

- Review of kidney engineering: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3928531/
- Atabak's paper: http://journal.frontiersin.org/article/10.3389/fbioe.2017.00013/abstract