Ian's pipe dreams coming true

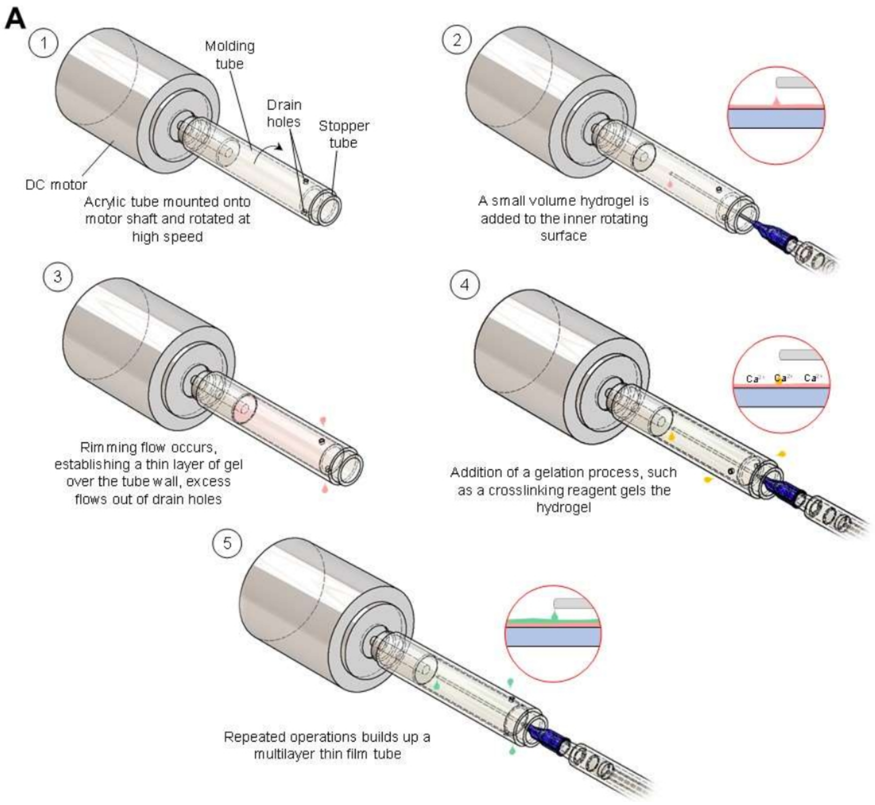
The US senator Ted Stevens once famously defined the Internet as 'a series of tubes' (a definition that makes more sense the more one knows about the physical infrastructure of the Internet). The human body is another object that could be defined mostly as 'a series of tubes': most solid tissues are permeated by tubes that carry blood and lymph, and organs such as the kidney consist almost entirely of tubes of one kind of another. For this reason, there is great interest in finding better ways of bio-engineering tubes for use in repairing bodies, or in creating realistic proxies for body tissue for testing drugs and other medical devices.

This lab has some ‘form’ in tube bioengineering, particularly in collaboration with the lab of Will Shu, now at the University of Strathclyde (see the blog entry ‘Piping for beginners’). A few years ago, Ian Holland joined my lab from Will's, and began an imaginative project on tube bioengineering that he pursued alongside his main project of building systems suitable for optogenetic control of development. His aim was to address two big problems in existing methods of bioengineering, such as 3D printing. One problem is in making tubes down at the millimetre scale instead of centimetre scale: this is important because arteries, veins, fallopian tubes and ureters are of this size and very few tubes are centimetres across. The other problem is that these tubes are composed of multiple layers, some of which are only around 15 micrometers thick. Typically, a tube has a very thin lining one or two cells thick, some connective tissue around 50 micrometers thick, and layers of muscles up to a few hundred micrometers thick, with more connective tissue around the outside. Engineering something like that demands a technology capable of laying down layers accurately at a range of thicknesses, each of which can carry cells at or near physiological densities ('near' because cells can multiply in situ). With some elegant lateral thinking, Ian has effectively solves these problems.

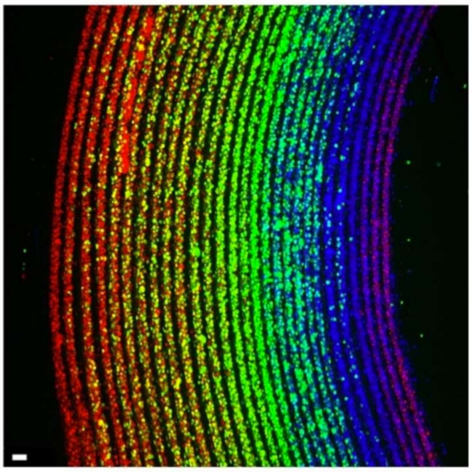
His system does not rely on printing, but instead on the centrifugal force[1] effect used in 'wall of death' fairground rides, and a behaviour of fluids known as 'rimming flow'. His apparatus consists of a hollow plastic tube attached to an electric motor that can spin the tube on its long axis at precisely controllable speeds. The tube is equipped with drain holes through its side, at one end: these turn out to be very important to working at all. With the tube spinning, the experimenter drips a few drops of a 'liquid hydrogel' (for example, a solution of collagen) into the hollow middle of the

[1] Please don't write in with school teacher-inspired assertions that there is no such thing as centrifugal force. Whether it makes sense to call it centrifugal or centripetal depends on your reference frame.

spinning cylinder. The liquid spreads when it hits the tube, and centrifugal force flattens it into a layer. The thickness of this layer is controlled by the viscosity of the liquid and the magnitude of the centrifugal force, which is determined by the rotational speed of the motor and the diameter of the plastic tube. Excess liquid leaves via the drain holes, and decorates the lab ceiling (or did until Ian was persuaded to rig up a catching device). Once a layer has gelled, another can be applied, building up an eventually thick wall, layer by layer. The next layer might be the same as the last, or made of a different material, and it could be made under the same or a different speed of rotation. We have named the whole process 'RIFLE', the acronym standing for for 'rotational internal flow layer engineering'.



Importantly, the forces involved in the RIFLE process are very gentle: there is none of the violent shear involved in forcing cells through a nozzle of a 3D printer. For this reason, cells can be suspended in the liquid hydrogel and end up in the solidified tube, still entirely alive and happy. Different layers can have different types of cell.   Ian demonstrated this very clearly by using preparing three batches of cells so that each batch fluoresced with a different colour: red, green or blue. He then made different mixtures of the cells to approximate to colours in the rainbow. Finally, he



Scale bar = 100µm

engineered a RIFLE tube so that each layer incorporated one of the mixtures to create a 'rainbow wall' in the tube.

The description of the RIFLE technique in this blog piece is necessarily brief: interested readers can find a full protocol in the research paper we have just published (see 'links' below), and there is also a patent pending. We have submitted applications for grants to extend this work in two directions, one in engineering a specific tissue using RIFLE, and the other to turn a Heath-Robinson / Rube Goldberg machine that is a sprawl of wires and controllers on a lab bench, into a normal piece of lab equipment that could be manufactured easily and sold and used by biologists without any particular mechanical engineering skill. Please with us luck with the applications!

Jamie Davies, Edinburgh, July 2023

Links:

Ian's paper: Holland I, Shu W, Davies JA (2023) Stratified tissue biofabrication by rotational internal flow layer engineering. *BioFabrication* 2023 Jul 19;15(4). **doi: 10.1088/1758-5090/ace2ed**.