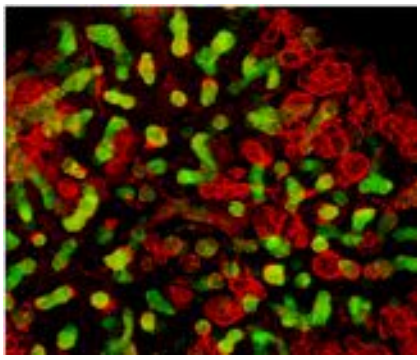
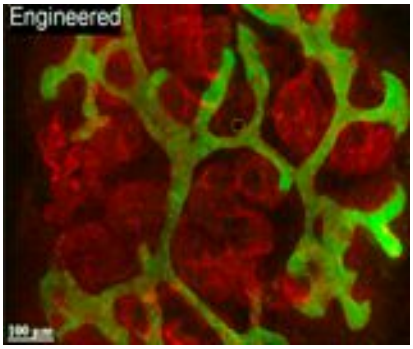
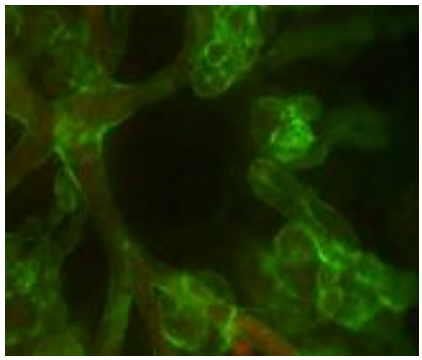
Chris takes the you-know-what out of kidney engineering.

A recurring topic in these blogs is our long-term project to make kidneys from stem cells. Our first faltering steps, in 2010, led by the post-doc Mathieu Unbekandt, resulted in the production of lots of realistic components of the kidney but they were arranged in a higgledy-piggledy way, as in the left-hand figure below. An improvement made by a PhD student, Veronica Ganeva, brought some order, with the excretory nephrons of the kidney being arranged around a single tree-like urine collecting duct system (middle picture, below). A further improvement made by another PhD student, C-Hong Chang, took the kidney to a more mature stage, with distinct outer and inner parts (cortex and medulla), and loops of Henle dipping down towards the centre of the kidney, as they should (right-hand figure, below).

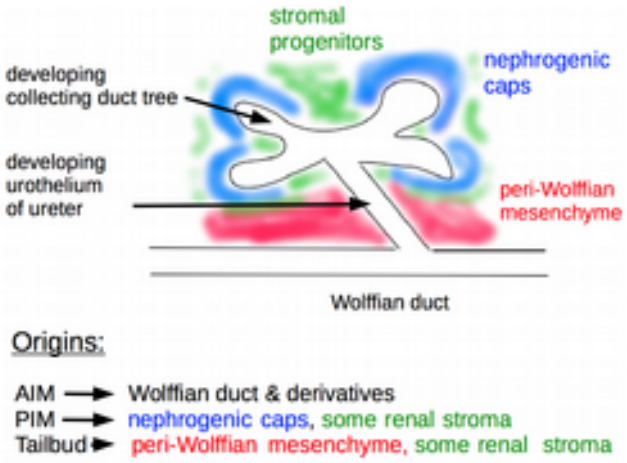
		
Mathieu, 2010 (red nephrons and small collecting ducts arranged haphazardly – the colours are just stains applied afterwards)	Veronica, 2011 (red nephrons arranged around a green collecting duct tree)	C-Hong, 2012 (a higher magnification view, showing loops of Henle)

One really obvious feature was missing from these engineered kidneys, though: the collecting duct tree had no exit tube for urine, and obviously having one would be essential for a working organ. Four years ago, Chris Mills came to my lab to study for his PhD, and bravely took on the challenge of finding a way of adding an exit tube.

He tried to do this in two very different ways. One was to engineer an artificial (but living) tube and somehow add it to one of our engineered kidneys, perhaps just by stabbing it into the heart of the urine collecting duct tree and hoping the structures would make a good seal. Overall, this approach turned out to be disappointing, although his collaborative work in making tubes did get him a joint publication (see the blog ‘Piping for beginners’). The problem was that technology for making

scaffolds for living tubes has not yet been miniaturised enough for the scale of our engineered organs.

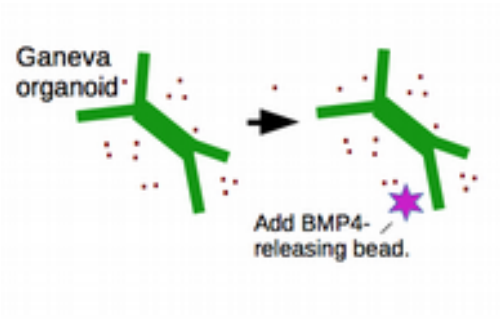
The alternative, and as it turned out more successful, approach was to use our knowledge of natural embryonic development to try to persuade one of the collecting duct tree branches in our engineered kidneys to turn into a ureter instead. This is not as crazy as it may sound, because the ureter and the collecting ducts have a common origin. Just before kidneys begin to form in the embryo, the Wolffian duct (a pipe running down each side of the embryo) emits a side-branch. This side-branch grows through a thin layer of loose-packed cells, the peri-Wolffian mesenchyme (shown red in the diagram opposite), and enters the area that will form the kidney (blue and green in the diagram), where it starts to branch to make the collecting duct tree. The part that remains outside the kidney, in the peri-Wolffian mesenchyme, does not make collecting duct but makes ureter instead. That the same tube can make two things, in two different environments, suggests that its choice may be governed by influences from its surroundings.

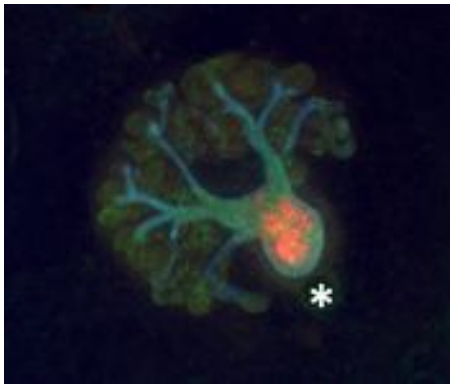


About a decade ago, Derina Sweeney, then a PhD student in my lab and now at Cambridge, worked with me to explore this idea, and we showed that transplanting the part of the tube that would normally make a ureter to the environment of the kidney caused it to make collecting ducts and participate fully in making kidney tissue. Later, Brenner-Anantharam and colleagues in New York showed that treating kidneys with BMP4, a molecule usually made by peri-Wolffian mesenchyme, causes ureter-like character to spread up into the older collecting ducts. Could this mean that a collecting duct might be persuaded to become a ureter?

Chris addressed this question directly, by transplanting small pieces of collecting duct into the peri-Wolffian mesenchyme, and showing that it would then express proteins such as uroplakin, which are characteristic of the ureter and absent from collecting ducts. Having shown with this experiment, and a series of others, that collecting ducts can change careers when exposed to persuasive growth factors like BMP4, he went on to apply a local source of BMP4 right next to one

collecting duct in an organoid. The result was that this particular collecting duct stopped branching and behaving in a kidney like way, and instead swelled up and made uroplakin (stained red in the picture below). Far from the BMP-soaked bead, the kidney formed normally. The overall result was a kidney-shaped kidney (at last), with a clear exit.





There is more to a ureter than a tube with uroplakin – in particular, various muscles are needed and we still have to find a way of making these. But Chris' work has given us a big step forward, and the idea may be very useful in the engineering of other glandular organs too.

Earlier this week, Chris had his PhD *viva-voce* exam, and passed subject to a few small corrections to his thesis (this outcome is pretty normal), so he should become Dr Mills at the next available graduation ceremony, and hopefully go on to build his own independent scientific career.

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Links

Chris' paper: https://www.ncbi.nlm.nih.gov/pubmed/29093551

Derina's paper: https://www.ncbi.nlm.nih.gov/pubmed/18579677