Renal engineering

Renal engineering: strategies to address the problem of the ureter.

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Abstract

Current techniques for making renal organoids generate tissues that show function when transplanted into a host, but they have no ureter through which urine can drain. There are at least 4 possible strategies for adding a ureter: connecting to ta host ureter; inducing an engineered kidney to make a ureter; making a stem-cell derived ureter; and replacement of only damaged cortex and outer medulla, using remaining host calyces, pelvis and ureter. Here we review progress: local BMP4 can induce a collecting duct tubule to become a ureter; a urothelial tube can be produced directly from pluripotent cells, and connect to the collecting duct system of a renal organoid; it is possible to graft ES cell-derived ureters into host kidney rudiments and see connection, smooth muscle development and spontaneous contraction, but this has not yet been achieved with all components being derived from ES cells. Remaining problems are discussed.

Keywords

Organoid, renal replacement therapy, kidney, metanephros, ureter, urothelium, smooth muscle, stem cell, ES cell.

Introduction

Though not part of the kidney, the ureter is essential to its function, providing the only route by which urine can drain from the kidney to the bladder. A ureter, or a functional surrogate for one, will therefore be an essential feature of any technology that replaces a patient's failing kidneys with a new one made from stem cells [1]. Worrying about this now may seem premature, when the field of kidney engineering has such a long way to go before clinical application, but there are two good reasons for not putting the ureter off until later. The first is that the ureter is not a simple structure: it is an active, peristaltic tissue that has stem cell-containing urothelium with nuclei of distinct cell types in distinct layers, a basement membrane, two layers of smooth muscles, nerves, blood vessels and adventitia (Fig 1a) [2-4]. Assuming that engineering one will be trivial may therefore be a mistake and it would be wise to tackle this in parallel with the kidney, especially as engineered ureters may be directly useful for ureter disorders (reviewed by Woolf [5]). The second reason is that choices of which strategy to use in renal engineering may be guided by the details of whatever method of ureter engineering works best, if ureter work ends up proceeding more quickly.

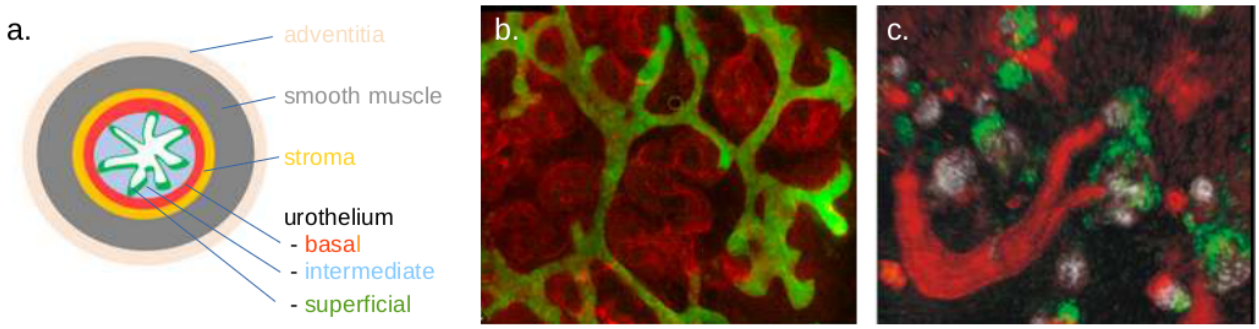

Figure 1: Foundations for this research: the ureter, and kidney organoids. (a) depicts the layers fo the adult ureter. (b) shows an organoid made by the Ganeva method, from ex-fetu renogenic cells (from our earlier paper [8]). Green shows the collecting duct tree, red nephrons. (c) shows an organoid made by a similar method from mouse ES-derived renogenic stem cells. Red shows the collecting duct tree (Cytokeratin 8), green glomerular podocytes (WT1), white proximal tubules of nephrons (Jagged 1). This image is from unpublished work from this lab.

At the time of writing, renal organoids made entirely from human pluripotent cells lack large scale anatomical organization necessary for renal function (eg cortex, medulla, nephrons connected to a single collecting duct tree) [6, 7], but organoids engineered with murine cells are a lot more advanced in regard to these features. A method for making 'Ganeva'-type organoids, with nephrons connecting to the branches of a single collecting duct tree (Fig1b), has been available for organoids made from ex-fetu renogenic cells since 2011 [8]. The method has been adapted for use with a mix of ex-fetu stromal progenitor cells, and mouse ES cell-derived nephron and collecting duct progenitors in 2017 [7] and also seems to work when all three cell types are differentiated from mouse ES cells (Fig 1c: our own data, manuscript in preparation). We therefore feel it is reasonable to take for granted that engineered kidneys of the future will possess a properly arranged collecting duct system leading from all nephrons to a common renal pelvis, and we will take this as a starting point.

This review will deliberately exclude discussion of production of adult-sized ureter/ bladder tissue by 'classical' tissue engineering techniques that seed cells on an engineered or a decellularized natural scaffold (e.g. [9 - 11]. These methods are much more applicable to repairing damaged adult tissue than for newly engineered organs that will probably be transplanted when still small and immature and be allowed to mature in situ [12]. They have been reviewed elsewhere [13]

Strategies for adding a ureter:

We are aware of four main strategies for providing an engineered kidney with a ureter, none of which has yet matured enough for clinical use, even experimentally. The strategies are; (i) direct use of the host ureter, (ii) inducing an

engineered kidney to produce its own ureter, (iii) making a stem cell-derived ureter and connecting it to the engineered kidney, and (iv) use of the host calyces, pelvis and ureter by connecting engineered renal tissue within the host kidney itself.

(i) Direct use of the host ureter

Direct use of a host ureter might be one way to solve the problem of an absent or too-short ureter in an engineered kidney graft. The technique has been used by researchers who have transplanted natural foetal kidneys into adult hosts (an important research step in the path to being able to transplant engineered foetal kidneys). Rogers and Hammerman [12] connected the ureter of a rat E15 metanephros transplanted into the omentum of an adult host rat and allowed to grow, to the ureter left by a unilateral nephrectomy. 20 weeks later, they removed the remaining host kidney and showed that the graft was producing urine and could support life of the recipient (though not indefinitely). Marshall and colleagues showed that it was possible to join multiple metanephroi to one host ureter [14]. Yokote and colleagues [15] grafted pig metanephric rudiments, still connected to their cloaca, into the omentum of syngeneic porcine hosts and allowed them to grow. The transplanted cloaca developed into a small bladder, which began to fill with urine. One kidney of the host was then removed and its ureter sutured to this cloaca-derived bladder, and evidence was obtained for the production of concentrated urine by the graft, which was much healthier than metanephroi grafted with no means of drainage.

In all of these cases, the uretoureterostomy depended on the grafted kidney having at least a short ureter of its own, and as far as we can tell, nobody has connected a ureter directly into the pelvis or collecting duct system of a transplanted foetal kidney or organoid. In this sense, techniques to use the host

ureter for part of the drainage system do not solve the fundamental problem of having a ureter in the graft at all, but they at least indicate that we need only a graft-derived ureter long enough to make the uretouretostomy connection, and not one long enough to reach all the way to the bladder. This simplifies the requirements of kidney-ureter engineering.

(ii) Inducing an engineered kidney to make its own ureter

When transplanting donor kidneys into a recipient, surgeons currently work with organs that have lengths of their artery, vein and ureter projecting from a kidney in which these three tubes are fully and correctly internally located and connected. Existing surgical techniques could be transferred most easily if a kidney engineered from stem cells also came with its own 'plumbing' properly attached. This poses an interesting challenge to renal engineers because, during normal development, none of these structures arises from the kidney. All invade the metanephrogenic area from outside and then ramify within it, the ureteric bud first and the blood vessels soon after, these vessels apparently following the ureteric bud's lead [16].

The ureter arises from the ureteric bud, which emerges from the Wolffian duct, crosses a zone of peri-Wolffian mesenchyme, and then enters the metanephric mesenchyme in which it branches repeatedly to form the collecting duct. A number of classical reciprocal transplantation experiments have shown the choice of ureteric bud cells to make unbranched ureter or branched collecting ducts is determined by the mesenchyme that surrounds them [17, 18]. Critical to this is the different signalling environment created by these mesenchymes; the peri-Wolffian mesenchyme being rich in BMP4 and the metanephric mesenchyme rich in GDNF and the BMP antagonist, Gremlin [19-23]. Mills and colleagues [24] used this plasticity to induce a renal organoid to produce its own (immature) ureter.

They began by making a 'Ganeva-style' organoid as described in the Introduction and Fig 1b, and placed a slow-release bead loaded with BMP4 next to one of the branches of the developing collecting tree in the organoid. That duct branched no more and induced no nephrons, but it thickened and expressed the ureter marker, uroplakin. Far from the bead, the rest of the tree continued to branch and it induced nephron formation. The result was the formation of an immature 'kidney' with a single collecting duct tree rooted in a ureter trunk (Fig 2a). Beads loaded with irrelevant proteins did not have this effect. This result suggests a possible approach to making kidneys with ureters attached, but it is important to note that all that has been achieved so far is to make a short urothelium at the base of the tree: it is not an elongated tube, it has no muscle layers and therefore shows no peristalsis. It is at most a foundation for further work.

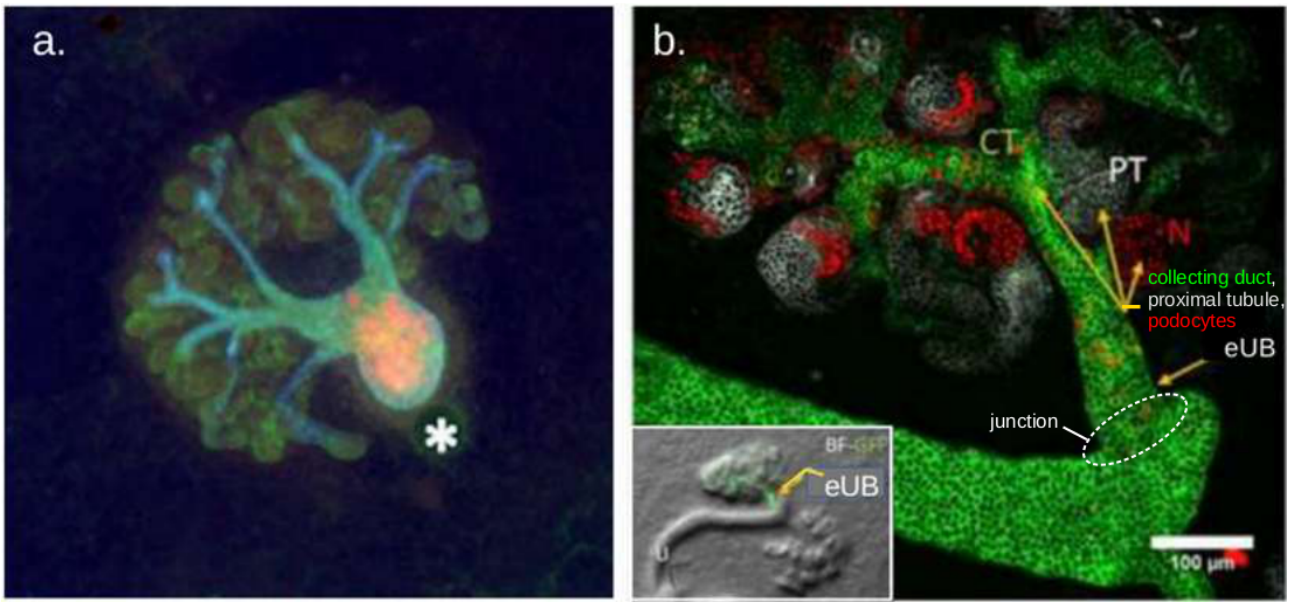

Figure 2: Two ways of adding a ureter to a kidney rudiment. (a) is from our paper [24]: a BMP4-soaked bead (*) was placed adjacent to one collecting duct of a Ganeva-type organoid (e.g. Fig 1b) and this tubule did not branch but became larger and expressed the urothelial marker, uroplakin. Colours: orange = uroplakin; green = collagen IV to mark all basement membranes; blue = cytokeratin 8 to mark collecting ducts. (b) shows the effect of grafting an engineered ureteric bud (eUB) engineered from GFP+ mouse ES cells, into a cultured mouse kidney rudiment near its ureter (see inset). The portion of the graft near the ureter joined to that ureter, while the other end branched and induced nephron formation in the host mesenchyme. Effectively, this formed a 'micro-kidney' graft

that connected to the host in terms of urine drainage. Colours: green = cytokeratin 8; red = WT1 (podocytes); white = jagged 1 (proximal tubules). This image is from [29].

(iii) Making an exogenous ureter and connecting it

A different approach might be to engineer a ureter from pluripotent cells, at a scale appropriate for the engineered kidney, and to connect the two micro-surgically before transplantation of the complete unit. This might be combined with approach (ii), to extend the length of an integral ureter. Engineering a ureter from pluripotent cells, independent of the kidney, also has the advantage that the technology might be used to help patients with purely ureter-related problems, and this aspect might reach maturity long before engineered kidneys do.

The foundations for ureter engineering were laid by Taguchi and Nishinakamura, who developed a method to differentiate ureteric bud cells from mouse ES cells [6]. We used this method, and then transferred the ureteric bud progenitor cells not to a metanephric mesenchyme-type environment that would turn them into collecting duct, but to cells obtained from the peri-Wolffian mesenchyme. This resulted in their differentiating into a non-branching urothelium, expressing Uroplakin, Krt5, Krt15, and NP63 in anatomically correct locations [25]. The overall anatomy of the urothelium was, however, more like a rounded cyst than a tube. Strikingly, the differentiating urothelium organized peri-Wolffian mesenchyme cells around it to differentiate into smooth muscle, and this contracted rhythmically at a frequency broadly similar to that seen in natural cultured ureters undergoing peristalsis.

(iv) Using the host ureter, calyces and pelvis

So far, this review has focused on the ureter aspects of using a complete engineered kidney as a means of renal replacement therapy. Some researchers are focusing instead on repairing a failing kidney by adding stem cells to make new tissue in situ directly or to secrete factors that induce host cells to carry out endogenous repair [26, 27]. A possible 'middle way', replacing sections (eg pyramids) of a failing kidney with new engineered sections, has been largely ignored because of the perceived difficulty of making the correct 'plumbing' connections deep within the kidney. We already know, from experiments involving transplantation of renal organoids below the renal capsule, that renal vessels are capable of entering organoids, connecting to glomeruli and filtering blood-borne tracers into the organoid's nephrons [28], so the blood connections may not be a problem. What about the ureter?

In experiments in which we were transplanting ureteric bud epithelia engineered from mouse ES cells into host foetal kidneys in culture, to test their plasticity in terms of ureter/ collecting duct differentiation, we found rare examples in which the graft seemed to connect to and integrate into the host ureter-collecting duct tree. Investigation of this revealed that frequency of making connections could be greatly promoted (to 50%) by making a micro-incision into the host tubule near the site of grafting. When the graft is made near the ureter, the graft differentiates into ureter locally, recruiting muscle progenitor cells and contracting in sync with the host, but can become collecting duct further away. This can be used to create a grafted 'micro-kidney' connected to the host (Fig 2b) [29].

It must be stressed that these experiments have been performed only in culture, and with grafts only to very early foetal kidneys. We have no information at all about whether such a connection could be made to the pelvis or calyces of an adult kidney, but the observation at least raises the possibility of a strategy that

replaces sections of a failing kidney, plumbing them to the existing calyces and collecting duct. As with all of the strategies discussed, more work is needed before it is clear whether the idea of replacing just parts of a kidney may be useful, or just a dead-end.

Conclusions and challenges

The clearest conclusion to be drawn form the foregoing is that the field of engineering ureters from pluripotent cells is in its earliest stages [30]. There is a method to make well-differentiated and organized urothelia from mouse ES cells. These can organize ex-fetu smooth muscle progenitor cells to form spontaneously contractile muscle layers around them, but there is not yet a reliable method to produce these muscle layers from ES cells. Importantly, the urothelia produced by this method are essentially spherical, cyst-like objects, some oblate but none that would be called 'tubes'. These observations lead naturally to the two most pressing challenges: to make all layers of the ureter from pluripotent cells, and to find a way of persuading the cyst to become a tube. The former will probably be achieved by the usual method of 'walking' pluripotent cells through the sequence of signalling environments experienced by embryonic cells that end up being the cell type of interest. The latter, extension into a tube, may be more problematic. One possible method might be application of mechanical tension to drive orientated cell division by Hertwig's rule, effectively slowly 'pulling' the cyst into a pipe. Another challenge, worth addressing because it offers a completely different way forward, is to examine ways of achieving an even higher rate of connection between graft and host ureters, and to determine whether this connection works only in very young hosts or may work in the adult kidney.

From a clinical point of view, there is also the challenge of transferring the knowledge gained from mouse pluripotent stem cells to human. This is not trivial

problem, and it is already clear from the kidney itself that protocols optimized in the mouse do not transfer directly to human [7]. Working in mouse has real advantages, such as the ease of obtaining natural tissues as 'controls', a wide range of genetic techniques and strains, and the possibility of extending to in vivo tests in a well understood model. For this reason, many researchers continue to work in mouse to shape a basic strategy rather than beginning with human ES or iPS cells. But it must be acknowledged that this puts off a humanization problem that must one day be faced if patients are to benefit from current research.

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Competing interests

None of the authors has any competing interests.

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Figure legends

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