Engineered kidneys: principle, progress and prospects

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MINI REVIEW

Engineered kidneys: principles, progress, and prospects

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There is an urgent need for new ways to treat end-stage renal disease: by promoting regeneration *in situ*, by repopulating decellularized donor organs with a patient's own stem cells, or by making entirely new kidneys. There are two broad strategies for making new kidneys: precision engineering by positioning everything exactly – for example, by 3D printing – or supporting cells' self-organizing ability. We describe the latter approach, which begins with a suspension of renogenic stem cells and produces a small kidney with nephrons, a collecting duct system, active transport, and an ability to integrate with host vasculature. Many problems have to be solved before these kidneys are directly clinically useful, including size, maturation, provision of a ureter, and production from human-induced pluripotent stem cells. Even the existing engineered kidneys, if they can be made from human rather than animal cells, may be useful for assays for adverse drug reactions that will be free of the problems of extrapolating from animal tests to predicted human responses.

Keywords: *kidney*; *renal*; *tissue engineering*; *organogenesis*; *stem cell*; *renal replacement*

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End-stage renal disease is a serious and common morbidity. Patients are treated by dialysis or transplantation: dialysis is a medium-term lifesaver but is uncomfortable, restrictive, and time-consuming for the patient. Transplantation is much more satisfactory, and a transplant provides the full range of renal function, though immunosuppressive drugs can produce serious side effects. The main problem is availability, both in terms of the total number (in most countries, the number of organs offered is less than half of the demand) and in terms of an individual finding a close enough immunological match. Various strategies are being pursued to improve availability, from social (e.g. moving to presumed consent) to immunological (developing techniques to promote toleration of less precise tissue type matches). Nevertheless, a serious transplantation gap remains.

There is therefore much interest applying stem cell–based regenerative medicine to renal failure. There are four potential approaches: pharmacological encouragement of regeneration *in situ* by endogenous stem cells, regeneration *in situ* by application of exogenous stem cells, repopulation of a decellularized postmortem kidney with fresh stem cells, and construction of a complete, new, transplantable kidney. This review will concentrate only on the fourth, but excellent reviews on the first three approaches can be found elsewhere (1–4).

Engineering a kidney *de novo*

Kidneys are anatomically complex. Human kidneys have 100,000–2,000,000 nephrons, each of which consists of a glomerulus and a sequence of tubular segments that drains into a multibranched collecting system. The segments of the nephron are arranged in an anatomically precise way, starting in the cortex, then dipping down into the medulla and back: this arrangement is necessary for efficient water recovery. There is also an extensive blood system that makes very fine filtration structures in the glomeruli and a countercurrent loop parallel to the medullary loops of the nephrons. In addition, stromal and neural cells support the tubules and relay physiological signals within the kidney and beyond. The task of engineering a kidney is therefore challenging – far more so than, for example, making a sheet of cartilage.

Approaches to meeting this challenge can be divided into two types. In one, spatial information is provided

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from outside. A model of the desired organ might, for example, be programmed into a computer connected to a three-dimensional (3D) printer that can print extracellular matrix loaded with the correct type of cell. Simple biological structures have been made by electrospinning and 3D printing (5, 6), but nothing anywhere near as complex as a kidney has yet been described in the peer-reviewed research literature, although a very promising account of unpublished work presented at a meeting has appeared in the blog of a learned journal (7).

The other strategy relies on cells' own capacity to organize themselves, as they do in embryonic development. In this approach, engineers' involvement is limited to providing a permissive environment (chemical and physical). This approach is the main topic of this review.

The first experiments in renal self-organization used the only certain source of renogenic stem cells: disaggregated embryo kidneys (alleged renal stem cells made from embryonic stem (ES) and induced pluripotent stem (iPS) cells do exist, but they have been identified only through markers, and not their developmental potential has not been fully verified). Making a suspension of mouse fetal renogenic cells and simply reaggregating them, in the presence of drugs that suppress anoikis, are sufficient for a substantial degree of self-organization to take place. Nephron progenitors form and produce distinct, correctly ordered segments, and collecting duct stem cells make multiple small collecting duct trees (8). A serial technique, in which a first, multi-tree kidney is engineered as above, and then just one of those small trees is combined with a suspension of fresh nephrogenic stem cells, results in the arrangement of nephrons around a single tree, as they should be (9).

Recent years have seen the development of a new culture system that allows intact fetal kidney rudiments to produce distinct cortex and medulla, and loops of Henle, in culture (10). In this system, the engineered organs behave similarly (Fig. 1a) (11). Furthermore, when transferred to chick egg chorioallantoic membrane (12) or adult rat hosts (13), engineered kidneys become vascularized. Their proximal tubules also display active transport in culture (Fig. 1b).

The next challenges

Though excited by the complexity and realism of the organs that can be engineered using this approach, we readily acknowledge that they are currently far from being clinically useful. In no particular order, they have the following problems: they are small; they are flat (only about 100 μm thick, although millimeters in diameter); they have no exit for urine; they are murine, not human; and they come from fetal stem cells (human fetuses cannot ethically be harvested for making new organs).

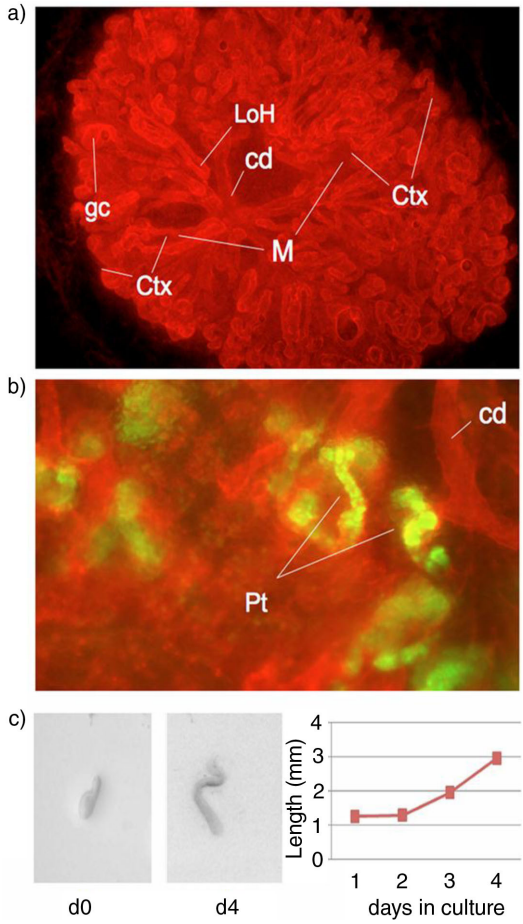

Fig. 1. Recent progress in renal tissue engineering. (a) shows an engineered 'fetal kidney' made by reaggregation of a suspension of isolated fetal renogenic stem cells, followed by culture in an environment conducive to organotypic development. The organ formed has an outer cortex (Ctx), with nephrons with glomerular clefts (gc). The nephrons send loops of Henle (LoH) into the otherwise nephron-free medulla (M), while a branched collecting duct system (cd) ramifies throughout. The red stain is anti-laminin, which shows basement membranes of all renal tubules. There is not, though, any ureter through which the kidney can drain. (b) shows an assay for a key renal physiological function in engineered kidneys – active organic anion uptake by proximal tubule cells. Here it is demonstrated by concentration of 6-carboxyfluorescein (green) in the proximal tubule (Pt) cells: the red channel shows peanut agglutinin, which stains renal basement membranes. (c) demonstrates that fetal ureters can survive and grow *in vitro*, elongating markedly between day 0 and day 4 of culture. An important next step is to engineer these from simple cell suspensions and connect them to engineered kidneys.

Small size and flatness may be mitigated by growth inside the eventual host. The engineered kidney seemed to become more 3D in the rat host experiment (13), and effort might be applied usefully in promoting *in vivo* growth in all directions. Lack of a urine exit might be solved

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by combining the existing engineering technique with techniques for tissue engineering ureters: there are now promising ways of growing natural (fetal) ureters in culture (Fig. 1c). We are working collaboratively on the problem of replacing fetal mouse stem cells with reprogrammed mouse ES or iPS cells. The first stage is to use mixtures of allegedly reprogrammed ES or iPS cells and the fetal renogenic cells to test the developmental potential of the reprogrammed cells (14), then to go to all ES- or iPS-derived systems. Some progress has been made toward both of these aims (15, 16).

Applications other than renal replacement

Even when we (or any other group) can make engineered kidneys, whether by self-organization or by printing, there will need to be extensive safety testing before they can be applied clinically to real human patients. There is one potential application that does not need any advance beyond what we have already achieved, except that it requires the engineering to be done with reprogrammed human iPS cells rather than fetal mouse ones. Engineered human kidneys in culture (even small and flat one) could give the pharmaceutical and chemical industries a much more realistic *in vitro* assay for assessing the risks of nephrotoxicity in humans. This is important. Especially in the elderly, a significant proportion of renal disease is iatrogenic, mostly caused by adverse reactions to drugs, particularly nonsteroidal anti-inflammatory drugs and antibiotics, or other drugs in complex combinations (17). Currently, preclinical risk assessment for nephrotoxicity is based on a combination of crude human cell line assays and animal testing. The cell lines currently available reflect natural kidney function only poorly (18). Primary cultures of human proximal tubule cells are more realistic in metabolic terms (19), but they are hard to obtain, relying on postmortem or resection material that comes directly from a hospital. Animal tests are physiologically realistic for that animal but not necessarily for humans. Meta-analyses of the predictive power of animal testing for human toxicity (i.e. 'adverse drug reaction') has shown that human responses are predicted correctly about 10–50% of the time (20, 21). This is a terrible statistic: not only does it mean that a vast amount of resources is wasted on developing drugs that have to be withdrawn following phase I–IV trials, but also it means that some candidate drugs that would in fact be safe and effective in humans are wrongly abandoned because of alarming animal data. Having small but physiologically realistic human kidneys in culture would be a major step forward.

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References

1. Panigrahy D, Kalish BT, Huang S, Bielenberg DR, Le HD, Yang J, et al. Epoxyeicosanoids promote organ and tissue regeneration. Proc Natl Acad Sci USA. 2013; 110: 13528–33.
2. Yoshida M, Honma S. Regeneration of injured renal tubules. J Pharmacol Sci. 2014; 124: 117–22.
3. Little MH. Regrow or repair: potential regenerative therapies for the kidney. J Am Soc Nephrol. 2006; 17: 2390–401.
4. Song JJ, Guyette JP, Gilpin SE, Gonzalez G, Vacanti JP, Ott HC. Regeneration and experimental orthotopic transplantation of a bioengineered kidney. Nat Med. 2013; 19: 646–51.
5. Jayasinghe SN, Warnes G, Scotton CJ. Bio-electrosprayed living composite matrix implanted into mouse models. Macromol Biosci. 2011; 11: 1364–9.
6. Faulkner-Jones A, Greenhough S, King JA, Gardner J, Courtney A, Shu W. Development of a valve-based cell printer for the formation of human embryonic stem cell spheroid aggregates. Biofabrication. 2013; 5: 015013.
7. Topf J. Kidney Week 2013: Need and organ? Print an organ. Am. J. Kid. Dis. Blog 2013. http://ajkdblog.org/2013/11/12/kidney-week-2013-need-an-organ-print-an-organ/
8. Unbekandt M, Davies JA. Dissociation of embryonic kidneys followed by reaggregation allows the formation of renal tissues. Kidney Int. 2010; 77: 407–16.
9. Ganeva V, Unbekandt M, Davies JA. An improved kidney dissociation and reaggregation culture system results in nephrons arranged organotypically around a single collecting duct system. Organogenesis. 2011; 7: 83–7.
10. Sebinger DD, Unbekandt M, Ganeva VV, Ofenbauer A, Werner C, Davies JA. A novel, low-volume method for organ culture of embryonic kidneys that allows development of cortico-medullary anatomical organization. PLoS One. 2010; 5: e10550.
11. Chang CH, Davies JA. An improved method of renal tissue engineering, by combining renal dissociation and reaggregation with a low-volume culture technique, results in development of engineered kidneys complete with loops of Henle. Nephron Exp Nephrol. 2012; 121: e79–85.
12. Davies JA, Chang CH. Engineering kidneys from simple cell suspensions: an exercise in self-organization. Pediatr Nephrol. 2014; 29: 519–24.
13. Xinaris C, Benedetti V, Rizzo P, Abbate M, Corna D, Azzollini N, et al. In vivo maturation of functional renal organoids formed from embryonic cell suspensions. J Am Soc Nephrol. 2012; 23: 1857–68.
14. Siegel N, Rosner M, Unbekandt M, Fuchs C, Slabina N, Dolznig H, et al. Contribution of human amniotic fluid stem cells to renal tissue formation depends on mTOR. Hum Mol Genet. 2010; 19: 3320–31.
15. Mae S, Shono A, Shiota F, Yasuno T, Kajiwara M, Gotoda-Nishimura N, et al. Monitoring and robust induction of nephrogenic intermediate mesoderm from human pluripotent stem cells. Nat Commun. 2013; 4: 1367.
16. Takasato M, Er PX, Becroft M, Vanslambrouck JM, Stanley EG, Elefanty AG, et al. Directing human embryonic stem cell differentiation towards a renal lineage generates a self-organizing kidney. Nat Cell Biol. 2014; 16: 118–26.
17. Chronopoulos A, Cruz DN, Ronco C. Hospital-acquired acute kidney injury in the elderly. Nat Rev Nephrol. 2010; 6: 141–9.

18. Jenkinson SE, Chung GW, van Loon E, Bakar NS, Dalzell AM, Brown CD. The limitations of renal epithelial cell line HK-2 as a model of drug transporter expression and function in the proximal tubule. Pflugers Arch. 2012; 464: 601–11.
19. Brown CD, Sayer R, Windass AS, Haslam IS, De Broe ME, D'Haese PC, et al. Characterisation of human tubular cell monolayers as a model of proximal tubular xenobiotic handling. Toxicol Appl Pharmacol. 2008; 233: 428–38.
20. Fletcher AP. Drug safety tests and subsequent clinical experience. J R Soc Med. 1978; 71: 693–6.
21. Archibald K, Coleman R, Foster C. Open letter to UK Prime Minister David Cameron and Health Secretary Andrew Lansley on safety of medicines. Lancet. 2011; 377: 1915.

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