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Self-Organization as a Tool in Mammalian Tissue Engineering

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1. Introduction

The end goal of most efforts in tissue engineering is the production of an artificial tissue or organ that is as similar as possible to the corresponding natural structure. So far, most approaches to this have involved combining cells with artificially-sculpted, spun or printed scaffolds. The approach works well for anatomically-simple, matrix-rich structures such as connective tissue, both in culture and in vivo. The visually-striking example of an engineered 'human ear' on the back of a mouse (Cao *et al.*, 1997) brought much public attention to the idea. Scaffold-based tissue engineering has since found valuable clinical use in the production of new cartilage (Andereya *et al.*, 2006), ligaments (Vunjak-Novakovic *et al.*, 2004), vessels (Lovett *et al.*, 2010), bladder wall (Atala, 2011) and nipple (Cao *et al.*, 1998).

Some of the most significant clinical requirements for effective tissue engineering concern not matrix-rich, simple tissues such as connective tissue, but very intricately-arranged complex organs that consist of many cell types, precisely located and in intimate contact with one another. Outstanding amongst these, in terms of clinical urgency, is the kidney, a fragile organ that regenerates itself very poorly, and which is damaged irreversibly by a large range of toxins, including some medicines. The demand for transplantable kidneys far exceeds their supply: in the UK alone, there are about 6,500 people on the waiting list, many leading fairly miserable lives in which they spend many hours per week hooked up to a dialysis machine.

Being able to engineer organs such as kidney and pancreas promises a very positive impact on the lives of many patients, particularly if the engineering could be done from the patient's own stem cells. There are, though, significant problems in extending scaffold-based techniques to organs such as these. The kidney, for example, consists of at least sixty-four distinct cell types (Little *et al.*, 2007) and these are arranged not haphazardly but in very precise order along intricately folded and branched tubules, vessels and stroma (Fig 1). Even if a scaffold could be laid down by some highly-developed three-dimensional printing process to pattern accurately the basement membranes of each of a hundred thousand nephrons, ten thousand collecting ducts and a corresponding number of vessels, it is difficult to see how cells would enter each tube in the appropriate order to populate each segment with the correct type of cell. Kidneys do not normally develop by cells moving into a pre-made scaffold, so there is no reason to suppose that their cells would have evolved the ability to do this even if a scaffold could be provided for them. In normal life, there may be some limited movement of cells along tubules

as they are replenished from stem cell populations (Lindgren *et al.*, 2011) but here the movement is from a stem cell niche part way along each nephron (at the 'neck' of the Bowman's capsule), not from the open end of the ureter, artery and vein, which could be the only openings in an anatomically-realistic 3-D printed kidney.

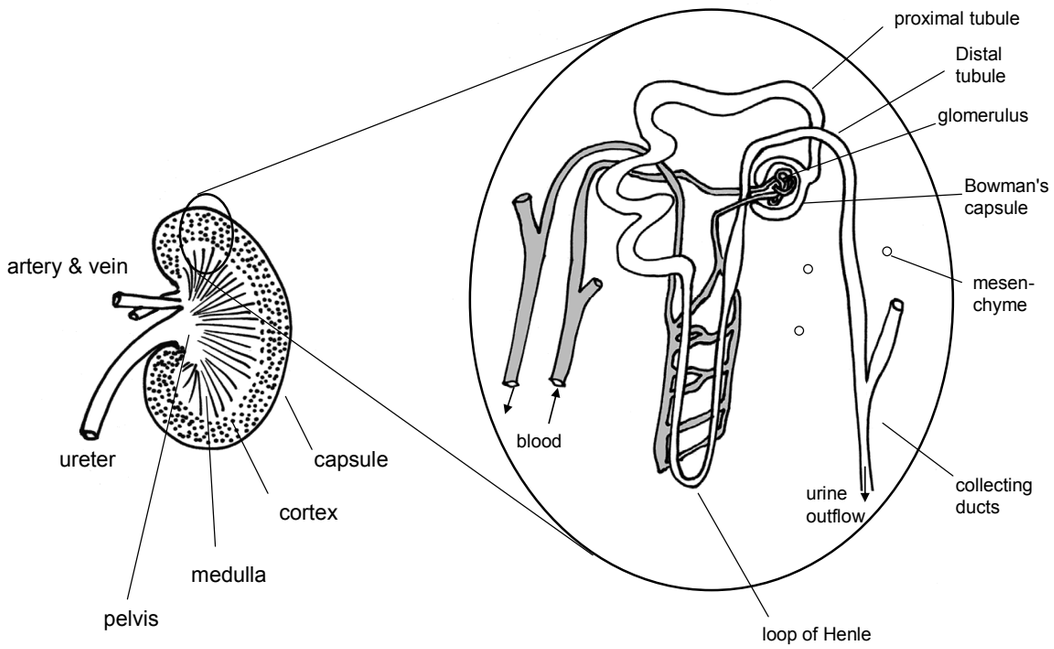

Fig. 1. The basic anatomy of the kidney (in this case mouse: human kidneys have multiple segments). The figure on the left shows the gross anatomy of the organ, while the detail shows one of the c. 2,000 nephrons of the organ (100,000 – 1,000,000 in human). Many of the tissue types present in the nephron include several different types of cells, for example the glomerulus contains at least five, and in each case these cell types are organized precisely.

It may turn out to be possible, one day, to print the cells and the matrix together at the required resolution to put everything in the correct location at the time of manufacture. There are formidable technical challenges to this endeavour, however, and it is sensible to explore alternatives.

The approach that is most radically different to developing ever more intricate 3-D micro-fabrication techniques is to minimize the requirement for engineering by making maximal use of cells' inherent abilities to organize themselves. The underlying idea is to work, as far as possible, with the flow of normal development; to engineer a system to turn a simple suspension of cells, such would be produced by stem cell culture, into a properly-arranged early foetal form of the organ in question, and then to engineer an environment that lets that "foetal organ" grow and mature in the usual manner. There are three main challenges in this; (a) production of the correct type of committed stem cells in the first place, (b) self-organization of a suspension of these cells into a "foetal organ" and (c) transplant of that into a recipient in a way that allows it to grow and mature. Most of this chapter will concentrate on step (b), engineering by self-organization, but steps (a) and (c) will be discussed briefly towards the end.

2. The self-organizing abilities of cells

The self-organizing abilities of cells arise from two broad classes of mechanism; biophysical and developmental (Davies, 2005). Self-organization through simple biophysics makes no demands on there being a 'developmental programme' or on cells responding to each other's signals; instead it works purely on the current properties of the cells. Different cell types bear different types and quantities of molecules that mediate adhesion between cells or that mediate adhesion between cells and matrix. As always, if the components of a system (cells, in this case) are free to change their relationships, they will tend towards an arrangement that minimizes free energy. By definition, unbound adhesion molecules are in a state of higher free energy than bound ones (if this were not true, they would not promote adhesion because they would not naturally bind), and the difference in free energy is greater for higher affinity interactions. Free energy is therefore minimized by a state in which the maximum high-affinity binding is able to take place.

When only one cell type is present, free energy is minimized either by cells sticking together as much as possible in an aggregate, as happens when cell-cell affinity is greater than cell-matrix affinity (Fig 2a), or by cells dispersing themselves so that they are completely surrounded by matrix, as happens when cell-matrix affinity is higher (the two-dimensional equivalent of this is the spreading of cells on a culture dish to which they are highly adhesive). Where cell-cell affinity is higher than cell-matrix affinity, and where two cell types are present, differences in the affinity of cells for their own kind and for the other kind can drive cell sorting (Steinberg, 1962b). Where each cell type has a higher affinity for its own kind than for another, mixtures of cells will spontaneously separate, the cell type with the highest mutual affinity being surrounded by their less adhesive neighbours (Fig 2b) (Steinberg, 1962a; Foty & Steinberg, 2005). Where each type of cell sticks better to the other type and one type is much less common than the other, the resulting arrangement will be a 'salt-and-pepper' mixture in which the less common type is surrounded by the more common. Where the numbers are equal, stripes would be predicted to result.

The situation becomes more complicated still where cells are polarized, as simple epithelia are, so that their apical surfaces are barely adhesive, their lateral surfaces are strongly adhesive to similar cells and their basal surfaces are most adhesive to matrix. Here, epithelial cells tend to produce cysts or tubes in which lateral cell-cell contacts are maximized, as are basal cell-matrix interactions. The direction of the apico-basal polarity of cells in the walls of these cysts depends on the availability of suitable matrix: if an adhesive matrix is available, they will polarize basal side outwards; if it is not, they will polarize basal side inwards (Wang *et al.*, 1990). Any mesenchymal cells present will be located on the outside of the cysts (Fig 2c).

It cannot be over-emphasized that the arrangements described above arise from simply biophysics and have nothing to do with any 'developmental programmes'. To emphasize this point, it is worth noting that the arrangements emerge in computer simulations that model the current state of the cells but include no information whatever about any changes in gene expression (Takano *et al.*, 2003; Krupinski *et al.*, 2011; Agarwal, 1995). Of course, a 'developmental programme' may underlie the reason that different cells express different adhesion molecules in the first place, but that is as far as it goes: once established, those differences are enough to drive cells into specific, predictable arrangements (as long as they are free to move).

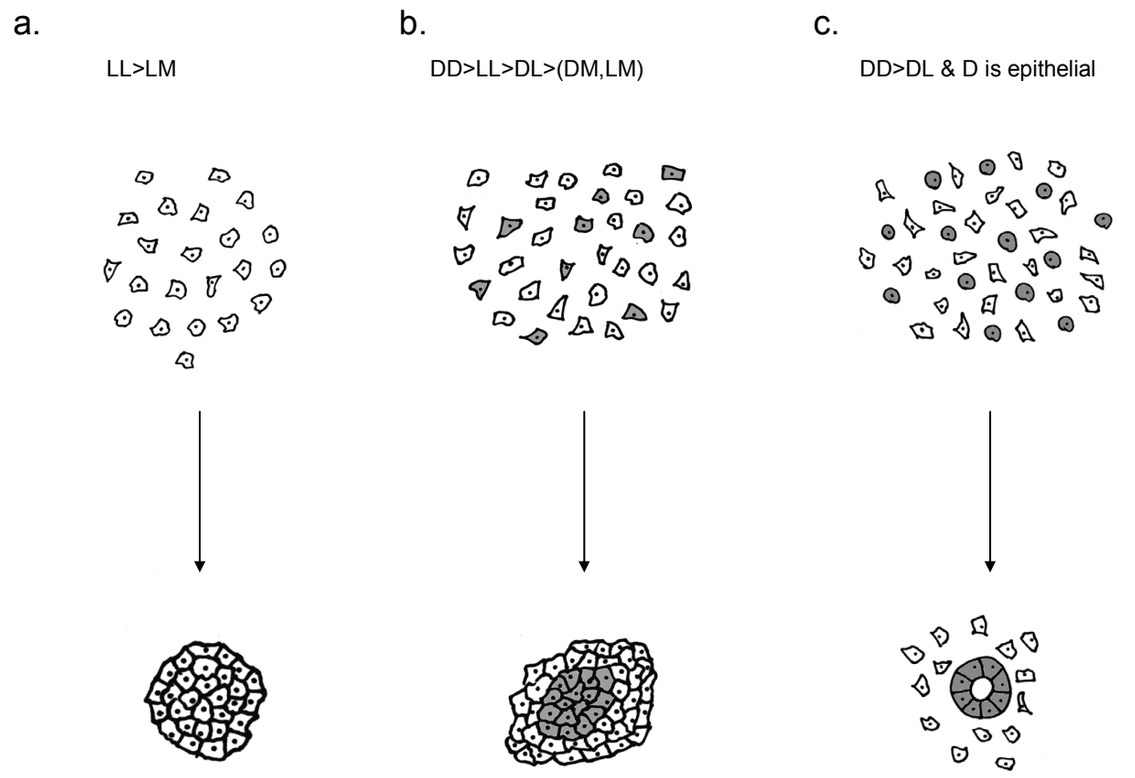

Fig. 2. Adhesion-mediated cell sorting. (a) depicts a group of identical cells, drawn in a light colour and called 'L' for this reason, whose mutual adhesion (LL) is greater then their adhesion to the matrix (LM): these cells aggregate together to maximize their areas of contact and minimize unbound adhesion molecules. (b) depicts a mixture of cells, dark (D) and light (L). In this case, the mutual adhesion of dark cells (DD) is stronger than the mutual adhesion of light cells (LL), but any cell-cell interactions are stronger than cell-matrix ones. Under these circumstances, the cells aggregate, but with the dark cells in the middle. (c) Where the dark cells are epithelial, and primed to achieve apico-basal polarization when in contact with one another, the result is an epithelial cyst surrounded by mesenchyme. References for these effects are given in the main text.

The second class of self-organizing mechanism is more classically developmental and involves cells changing their states over time. In vertebrates, at least, rather little developmental change is cell-autonomous. Rather, most is controlled by signals received from the environment, which is dominated by the secretions of other cells. The cells of a developing organ multiply, die, move and differentiate in response to the signals of other cells around them. The behaviours available to a cell are set by its current internal state (eg gene expression), but which of these potential behaviours are triggered, how much and when, are determined by neighbours. In the kidney, for example, the branching epithelium that will become the urine collecting duct system grows only when it receives signals, such as glial cell-derived neurotrophic factor (GDNF) (Sainio *et al.*, 1997), from the cap of mesenchymal cells that covers each branch tip. The cells of that mesenchymal cap proliferate in response to signals, such as Wingless homologue 9b (Wnt9b) (Karner *et al.*, 2011)

produced by the developing collecting duct system. At first sight, this mutual encouragement has the look of a positive feedback system, and so it is when everything is in balance. If the sizes of the tissues happen to be out of balance, however, it can work as negative feedback that steers the system back where it should be. If a collecting duct tip has branched too early, for example, and there is not enough cap mesenchyme to properly service two new branches, there will not be enough of a signal from it to maintain proliferation in the new collecting duct tips, and they will therefore be forced to wait until proliferation of the cap mesenchyme has caught up. Similarly, if the cap mesenchyme has become too large, its further proliferation will have to wait until the branches have caught up again.

Negative feedback processes such as the one outlined above are very common in organ development: in the kidney alone, there are similar processes to balance production of excretory nephrons and stroma and to balance production of vessels and glomeruli (Davies & Fisher, 2002). The self-correcting nature of these systems is an important element of natural self-organization of tissues in foetal life, reducing the need for unrealistic accuracy and making the growth of very large animals, such as us, possible. Importantly for the context of this chapter, they mean that a tissue engineer may not have to produce an engineered "foetal organ" that is exactly, 100% identical to what would exist in a real foetus. Rather, there is reason to hope that even a rough approximation to an early foetal organ will be enough to kick-start the cells' own abilities to correct errors and converge automatically on the normal anatomy.

This, then, is the theoretical background to a method of tissue engineering that aims to allow cells to do almost everything for themselves, with the minimum of human intervention. The rest of this chapter will describe the progress we have made in applying these principles to the problem of engineering "foetal kidneys". Given that the approach will work with the normal processes of development, it is necessary describe briefly how kidneys normally develop in an embryo before considering using this information for engineering purposes.

3. Normal kidney development

In its earliest stages, the anatomy of a normal foetal kidney rudiment is very simple: it consists of an unbranched epithelial tube, the ureteric bud (itself a side-branch from the Wolffian duct, outside the kidney), surrounded by mesenchyme. Over the next few days of normal development, the ureteric bud will undergo rounds of branching and growth to produce a tree-like collecting duct system. The mesenchyme close to the bud tip will condense to form a 'cap' over the tip. As the tips divide, the cap will tear in two, so that each new tip carries away a small cap of its own. Some of the signals that ensure these two components keep pace with each other and maintain co-location have been described in the section 2 of this chapter. As well as maintaining itself, the cap also sheds groups of cells that will undergo a mesenchyme-to-epithelial transition: these will become excretory nephrons (Fig 3).

For much of renal development, roughly two nephrons form for each collecting duct branching event, although the first-formed nephrons later disappear. The nephrons form first as small cysts, and these then undergo a stereotyped series of morphogenetic events, progressing through the so-called 'comma-shaped' and 'S-shaped' stages. As they do, they become segmented into Bowman's capsule, proximal tubule, Loop of Henle and distal tubule. The distal tubule connects to the nearby collecting duct, and the Loop of Henle

extends towards the inside, or medulla, of the organ. Within the Bowman's capsule, cells become specialized for urine filtration. As they do so, they secrete vascular endothelial growth factor (VEGF), which acts as a chemo-attractant for endothelial cells, bringing capillaries to what will become the glomerulus of each nephron (Tufro, 2000). Similar chemo-attraction, based on angiopoietin, is thought to attract blood vessels to the Loops of Henle (Kolatsi-Joannou *et al*., 2001).

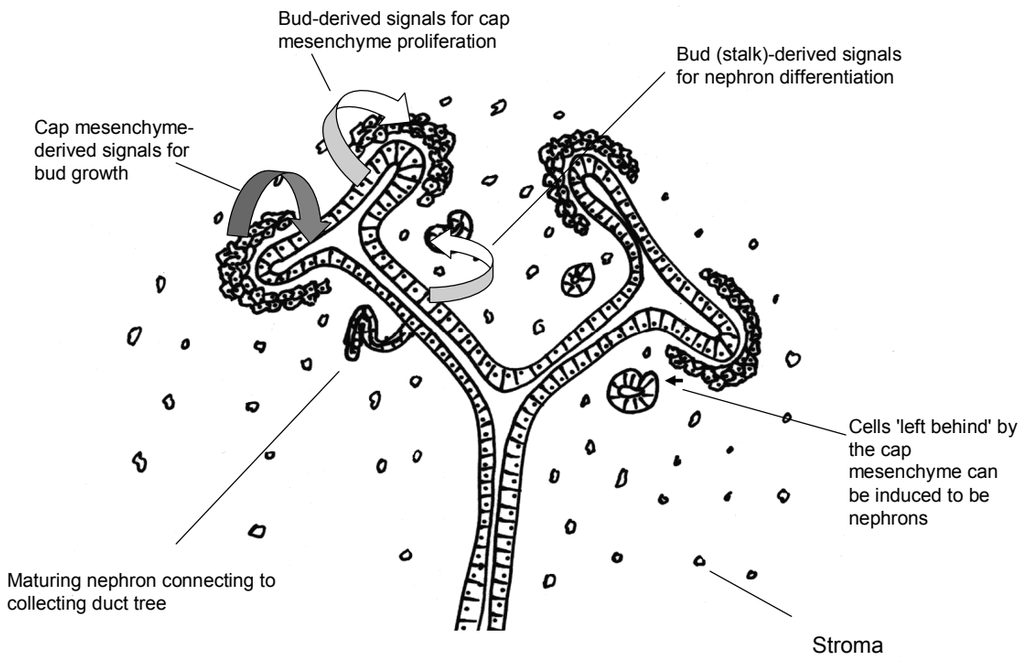

Fig. 3. A very simplified diagram of kidney development, showing how nephrons derive from cap mesenchyme, and showing how signalling between the branching ureteric bud/ collecting duct tree and the mesenchyme patterns the organ and ensure that the compartments proliferate in pace with each other

If a kidney rudiment is removed from a mouse and maintained in simple organ culture conditions, almost all of the above events happen normally, if a little slowly. The exception is the formation of a system of blood vessels, probably because they normally enter the kidney as a branch from the aorta (as the future renal artery) and from the vena cava (as the future renal vein): there is some evidence for an endogenous source of endothelia too (Loughna *et al*., 1998). The fact that a kidney rudiment will mature basically normally in culture is very important to the topic of this chapter, because it implies that the few cell types involved 'know' everything they need to know, without any need for information to be provided by the rest of the embryo. All we may need to engineer is the very simple bud-and-mesenchyme combinations from which the kidney develops, and then let nature take it course.

4. Engineering a kidney from a suspension of renogenic cells

The ultimate goal of this work is the production of clinically-useful kidneys from appropriately committed stem cells. In this context, 'appropriately committed' means one

population identical to the stem cells that maintain the mesenchyme (some of whose daughters give rise to nephrons etc) and another population identical to the stem cells in the ureteric bud (that give rise to new bud tips and the stalks they lay down behind them). There have been some encouraging developments towards directing mouse embryonic stem (ES) cells toward a renal fate (Kim & Dressler, 2005), but there is not yet a reliable method for producing the required cell states at high efficiency.

To experiment with ways of engineering "foetal kidneys" from suspensions of stem cells, we therefore began not with ES cells, but with cells isolated directly from early foetal kidney rudiments, which are therefore known to be in exactly the required state. The stage chosen was E10.5-E11.5, when the ureteric bud and mesenchyme are present but no nephrons have yet begun to form. Depending on definitions, a kidney rudiment of this stage can be considered to consist of either two cell types (metanephrogenic mesenchyme and ureteric bud), or of three (metanephrogenic mesenchyme, ureteric bud tip and ureteric bud stalk). Given that we have shown ureteric bud tip and stalk cells to be inter-convertible in both directions (Sweeney *et al.*, 2008), this chapter will consider there to be just two cell types. We begin by using enzymes to disperse cells of kidney rudiments into a simple suspension of isolated cells, to simulate a harvest from a culture dish or FACS machine that would be the output of a reliable method for programming stem cells to become renogenic, when such a method is finally developed.

The immediate goal is therefore to allow these cells to come together to re-create something sufficiently like a real kidney rudiment that it can go on and develop as if it really were one. The mesenchymal and epithelial cells are known to express very different sets of adhesion molecules (see www.gudmap.org). It is therefore a reasonable assumption that if they are simply brought together into a random lump, then biophysical mechanisms might lead to their sorting out into epithelial cysts and surrounding mesenchyme. This turns out to be true: if the suspension of cells is re-aggregated by simple centrifugation and then cultured on a polycarbonate filter (which is not adhesive enough for cells to spread out on it, so they remain as a lump), epithelial cells find one another and form one or more cysts, surrounded by mesenchyme (Unbekandt & Davies, 2009). There is, though, a very great loss of cells in this process, probably because cells separated form their normal cell-cell and cell-matrix contacts tend to undergo elective cell death in a process called anoikis (Frisch & Screaton, 2001).

Inhibition of the Rho-dependent kinase, ROCK, is known to protect some cell types from elective cell death (Watanabe *et al.*, 2007). In particular, ROCK inhibitors used to study the effect of the Rho-ROCK-myosin pathways on renal tubule morphogenesis also result in reduced apoptosis (Meyer *et al.*, 2006). Together, these observations suggest that pharmacological inhibitors of ROCK might be effective in preventing the massive death in kidney rudiment cell dispersal and re-aggregation experiments. So it proved: inhibition of ROCK using the drugs H1152 or Y27632 resulted in very much improved survival, resulting in re-aggregates with many epithelial cysts expressing ureteric bud markers, surrounded by mesenchyme (Unbekandt & Davies, 2009). This proved beyond reasonable doubt that the mixed cells from kidney rudiments are able to organize themselves back into their basic tissue types. Unfortunately, the system as described so far has two major defects from the point of view of making a more mature kidney: first, the mesenchyme cells fails to form nephrons and second, there are many ureteric 'cysts' rather than one branched ureteric bud.

Our studies on the effect of ROCK inhibition on the morphogenesis of renal epithelia had already suggested that nephron formation requires normal ROCK function at various

critical stages, including the very earliest (Lindstrom and Davies, unpublished). Nephron formation does not begin in a normal foetal kidney until about one day later than the stages at which kidneys were harvested for the dispersal and reaggregation experiments described in the paragraph above. The danger of anoikis would be expected to be highest when cells are dispersed, before they have come together again to find suitable neighbours, while the danger to nephron formation would be relevant only after cells have found each other well enough for the signalling loops that induce nephron formation to be up and running. This reasoning suggested that it might be possible to use ROCK inhibition for temporary 'life support' during the first 24h of the reaggregation experiment, and then to remove it and rely on cells' new contacts to keep them alive and healthy. When this is done, the result is good survival and good formation of nephrons. The nephrons go through all of the normal morphological stages of development, such as the comma and S-shaped stages, and they connect to nearby ureteric bud/ collecting duct 'cysts'. Furthermore, they express markers for Bowman's capsule, proximal tubule, distal tubule etc at the expected times and in the correct places (Unbekandt & Davies, 2009).

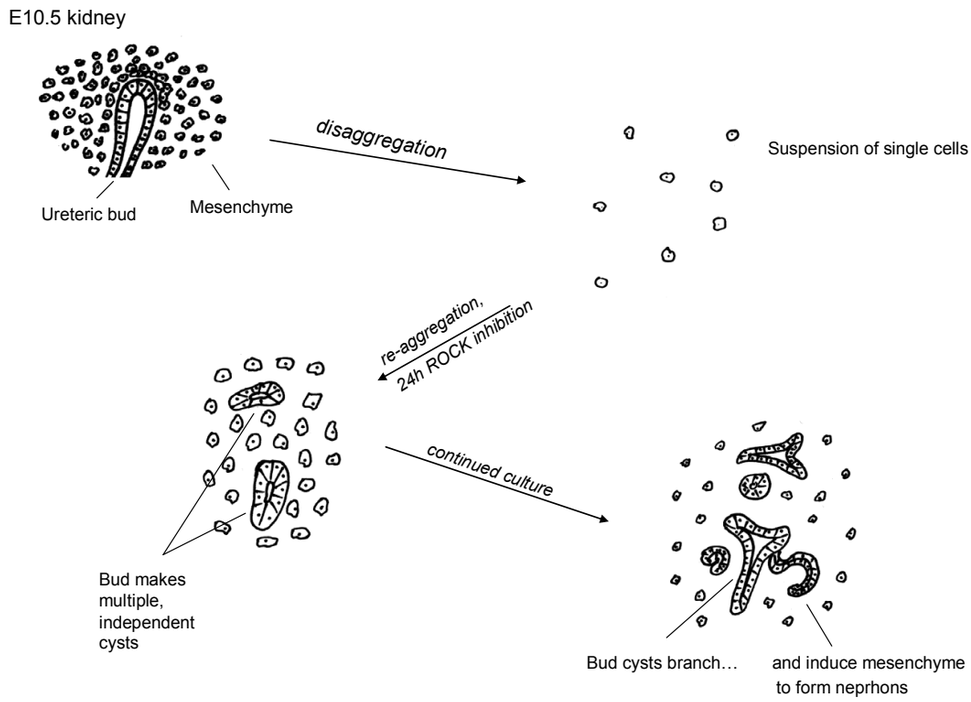

Fig. 4. The basic method of reconstructing renal tissue by re-aggregation of dispersed progenitor cells. The method illustrated here, based on Unbekandt and Davies 2010, has the disadvantage that multiple small ureteric bud 'tree-lets' are formed: an improved method is illustrated in Fig 6.

Under the conditions just described, many of the small ureteric bud 'cysts' form extended tubules that branch, each becoming a 'tree-let'. (Fig 4) This suggests that the basic cell biology of ureteric bud/collecting duct morphogenesis is running normally, but the presence of many 'tree-lets' rather than one tree is abnormal and would be functionally useless for two reasons. The first is that, in a normal kidney, nephrons drain their urine to the branches of a single

collecting duct tree and these branches drain in turn to the original 'trunk' of the tree, the ureter. Having nephrons connecting to lots of small, isolated 'tree-lets' would provide no means of egress for urine. The second reason is more subtle, but still critical. The architecture of the growing collecting duct tree imposes a large-scale order on the kidney, in particular a cortex in which blood-filtering glomeruli are located and a medulla into which the Loops of Henle dip, and through which the collecting ducts pass down from the cortex toward the ureter. When the kidney is working, the cortical interstitium is of normal salinity but the medulla is very salty, and this saltiness is critical to the kidney's ability to recover water and to concentrate urine. If a kidney had its nephrons scattered everywhere, without the clear cortico-medullary distinction imposed by arrangement around a single collecting duct tree, concentration of urine could not take place.

The fundamental problem with the simple reaggregation system is that small epithelial cysts are stable. Once enough epithelial cells have come together to form a small cyst, there is no reason for them to leave in favour of moving again to find a larger mass (indeed, the biophysics of the situation would prevent them from leaving, which would entail breaking energetically-favourable mutual adhesions). This is an example of the well-known physical phenomenon of a local minimum: a system that has reached a moderately energetically-desirable state but cannot reach an even more desirable state, because all possible routes between these two states would involve being in a temporarily less desirable one (Fig 5). Multiple small aggregates therefore form, and the system is stuck in this state.

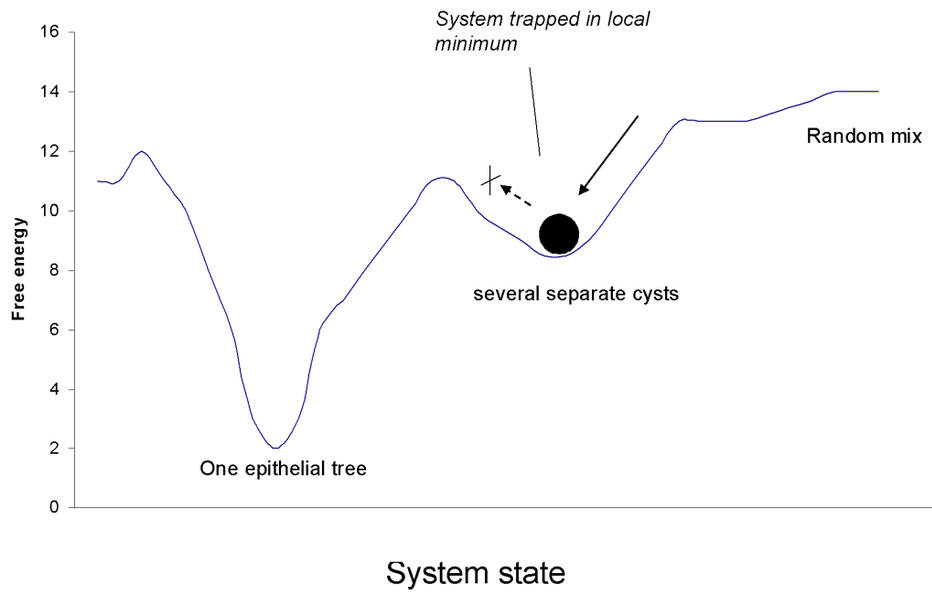

Fig. 5. The problem of the system being trapped in a local minimum. In re-organizing themselves from a random mix into one in which epithelial cells adhere to one another in separate small cysts surrounded by mesenchyme, the system succeeds in lowering its free energy. In order to reach the most favourable possible state (lowest on the diagram), though, the cells of the cysts would have to let go of one another and travel to join in with another cyst. This would mean a temporary move to a less favourable state than they initially found. The system therefore becomes trapped into a state that is moderately favourable, but not the best possible.

The idea of solving this problem by placing only a very few epithelial cells in the culture to begin with, although theoretically-possible, is unlikely to be useful because the total volume of the culture would have to be very small to allow the epithelial cells to find one another at all by random wandering (there is no evidence for long range chemotactic attraction between these cells, and no reason to suppose from normal development that such attraction should exist). Also, making an extremely small rudiment is not likely to be maximally useful from the point of tissue engineering. We have therefore devised a system of serial culture, in which multiple cysts are allowed to form in the first culture, and one of these is then combined with a fresh suspension of mesenchyme cells for a second culture (Ganeva *et al.*, 2011). When this is done, the cyst develops into a branching tubule. The overall result is an engineered "foetal kidney" that is arranged, as it should be, around a single branched ureteric bud/ collecting duct system (Fig 6) (Ganeva *et al.*, 2011).

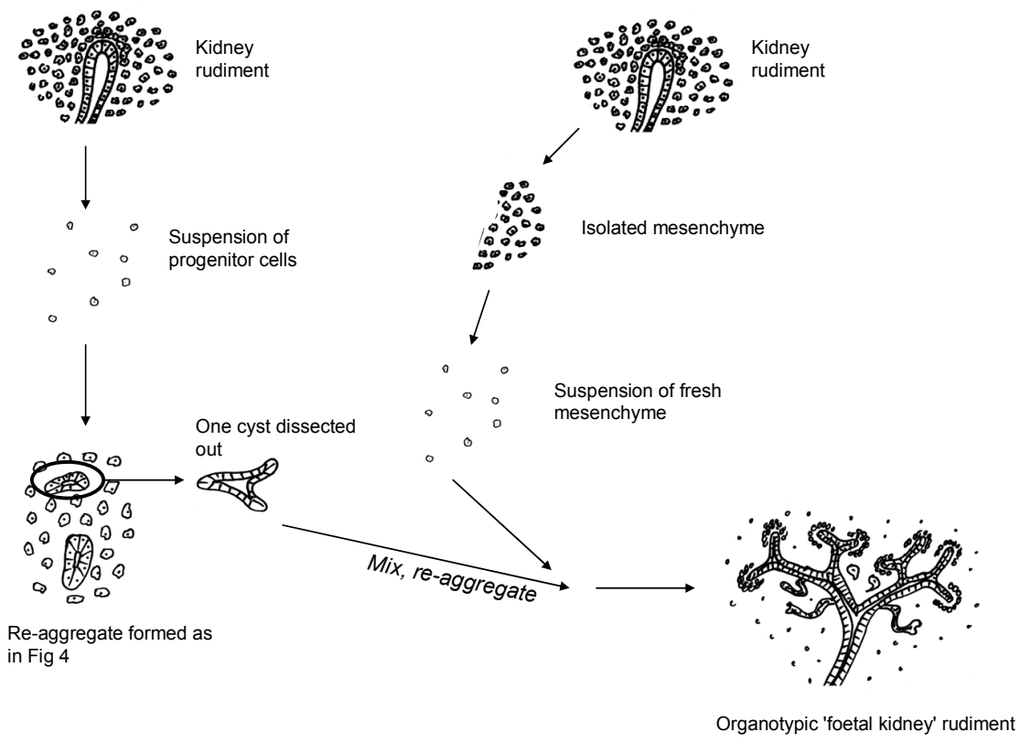

Fig. 6. An improved method for reconstructing kidney rudiments by re-aggregation from progenitors (Ganeva et al. 2011). A conventional re-aggregation is first performed, as in Fig 4, and then just one ureteric bud cyst is isolated from it and combined with a suspension of fresh renal mesenchyme cells free of ureteric bud. Under these circumstances, the result is a kidney organized normally around a single ureteric bud/ collecting duct tree.

5. Connecting to a blood supply

Like intact foetal kidney rudiments grown in organ culture, the engineered "foetal kidneys" produced by the method describe above lack a vascular system. This is a major omission for an organ, the main purpose of which is to filter blood and which receives about a fifth of the cardiac output. Normal developing kidneys attract endothelial ingrowth by secreting

molecules such as VEGF, particularly from specialized cells that will go on to form the filtration barrier in the glomeruli (Tufro, 2000). If a mouse kidney rudiment is cultured on the chorioallantoic membrane of a bird's egg, this chemoattraction is sufficiently strong to induce ingrowth of blood vessels from this membrane: indeed, the ease with which quail cells can be distinguished from those of mouse allowed this system to be used to demonstrate that glomerular endothelia arise from ingrowing vessels rather than from the nephron itself (Sariola *et al.*, 1983).

Our preliminary data suggests that self-organized "kidney rudiments" produced by the re-aggregation method described here can attract blood vessels in a similar way. When they are cultured on a chick egg chorioallantoic membrane, the rudiments attract ingrowth of vessels that is clearly visible at a gross level and sections of the resulting hybrid tissue show the formation of what appear to be glomeruli.

6. Self-organized organ rudiments in a clinical context

The production of a tiny analogue of a normal foetal kidney is clearly a long way from the functional, adult organ that is actually required. Is effort in this direction therefore a waste of time compared with efforts to build an adult organ directly?

I would argue that it is anything but a waste of time, for several reasons. The first is that we *know* that it is possible for a kidney to build itself from its foetal form, at least in a foetal environment, because that is what happens in normal life: enthusiasts for direct engineering can only *hope* that their aim is possible in any environment at all. The second is that we already know that it is possible to transplant normal foetal kidney rudiments into an immature or adult recipient and have those rudiments grow and become functional. So far, function is partial and not enough to sustain life for a long period (Rogers & Hammerman, 2004; Rogers *et al.*, 1998) , but these are early days and the technique continues to improve. Given how well kidneys grow in culture, in isolation from the foetal environment, the problems are probably not those of missing signals from the rest of a foetal body. It is more probable that they are to do with what is a very immature organ having to stand blood pressures and levels of blood oxygenation far in excess of the foetal situation while it is growing, and then having too poor a blood supply to function in its ectopic location. These problems may be soluble by surgery, possibly moving the graft from place to place as it grows, so that it begins its life in a protected site with low blood flow and pressure but ends up grafted to the renal artery.

The third reason is that the time-course of many renal diseases is very slow; in these conditions, a child is typically diagnosed before the age of four with a condition that will require transplantation only in their teens or twenties. There is therefore plenty of time for a transplanted 'foetal' kidney to grow to mature function before the host kidney completely collapses. Indeed, with even partial function being shared, the host kidney may last a great deal longer.

Formidable challenges certainly remain. There is a pressing need to improve the functional maturation of transplanted foetal kidneys, and to test the self-organized rudiments the same way. There is also, of course, the need for a method for production of renogenic stem cells but even this is probably easier than finding methods for the production of the many different cell types that would be needed for direct engineering of an adult organ.

7. Potential for application to other organs

There is every reason to suppose that the techniques that we have applied to the kidney can be applied equally well to other visceral organs of the basically glandular type – that is, organs that consist of many repetitions of fine-grained anatomy. Lung, pancreas, prostate, mammary gland and salivary gland all develop in a very similar way and are organized around a branching epithelial tree, while liver and testis development are not wholly dissimilar although their tubes form by a hollowing-out process rather than invasive branching. In each case, the organ begins to develop from two cell types, epithelium and mesenchyme, that have self-renewing stem cell properties and that differ markedly in their expression of adhesion molecules. A suspension of such cells would be expected to sort into epithelial ducts surrounded by mesenchyme, and the signal exchange between such tissues would be expected to mimic that found in normal development and therefore to organize the growth of an engineered 'foetal organ'. We have indeed shown this to be the case with lung (Unbekandt and Davies, 2010).

Whether the engineering of other 'foetal organs', followed by their transplantation to mature in situ, is likely to be clinically useful is governed mainly by the likelihood of their being able to mature. Kidneys are unusual, in that many renal diseases progress slowly and a patient with very weak renal function can be kept alive for very long periods by dialysis, allowing time for a transplanted rudiment to mature before any demands are made of it. The same may be true of pancreas in the context of diabetes, where the patient's existing pancreas maintains exocrine function and injected insulin substitutes for endocrine function until the transplanted rudiment is ready to take over. Similarly, mammary gland function is obviously dispensible (being absent in the male half of the population anyway). It is, on the other hand, harder to see how the scheme would work for lungs, for which there is no adequate long-term substitute.

Engineering gonads by re-aggregation of dispersed cells offers intriguing possibilities in fields other than medicine. At the cell suspension stage it is possible to mix cells from different sources: the result is a fine-grained chimaeric organ (Unbekandt and Davies, 2010). It is also, in principle, possible to subtract cells from the suspension stage by FACS or magnetic sorting. Putting these ideas together, it might be possible to disaggregate the cells of an early gonad rudiment from the foetus of a common (domestic) animal, sort away the germ cells, replace them with germ cells from a rare, endangered species that does not breed well in captivity, and transplant the resulted engineered gonad rudiments back in to a domestic host. In this way, the rare genome of a highly-endangered species might be propagated in domestic animals which are happy to mate in captive conditions, to produce offspring of the endangered species. Placental immunology and other physiological considerations will almost certainly impose limits on how different the species involved might be before an embryo cannot be carried, but the approach may work between related species if there is nothing else to be done. Obviously, conservation of species by protection of habitat is a much better course of action, but realistically many habitats are being lost and will take a long time to be regenerated even if there is the will. In this interval, a 'Noah's Ark' based on propagation of genomes in surrogate domestic animals might provide an emergency stop-gap solution.

8. Conclusion

In summary, data obtained so far suggests that the idea of using cells' own abilities for self-organization to produce an immature organ progenitor, with a view to placing this in a host

to mature, is a viable avenue of research. This is especially true for organs whose failure is slow enough to allow time for transplanted organs to grow. In making maximal use of cells' own abilities and in deliberately minimizing the requirement for detailed engineering, the approach contrasts with many others in this book. Which will win out in the end, or whether the techniques will co-exist for different problems, remains to be seen.

9. Acknowledgements

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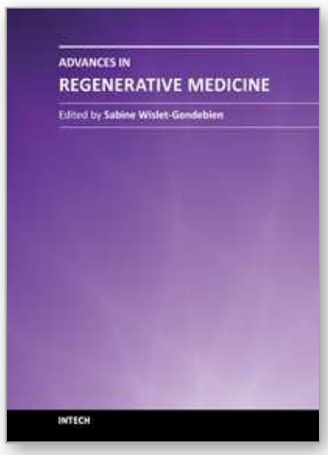

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Even if the origins of regenerative medicine can be found in Greek mythology, as attested by the story of Prometheus, the Greek god whose immortal liver was feasted on day after day by Zeus' eagle; many challenges persist in order to successfully regenerate lost cells, tissues or organs and rebuild all connections and functions. In this book, we will cover a few aspects of regenerative medicine highlighting major advances and remaining challenges in cellular therapy and tissue/organ engineering.

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