Are Current Strategies for Building a Human Kidney Misguided? Speculative Alternatives

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Suppose that one were asked to design a human organ, the principle functions of which are to maintain external balance for a variety of ions and solutes and to allow for the elimination of molecules that are deleterious to bodily function. Would one come up with a design for an organ that would filter 180 L of fluid per day, only to then reclaim 98%–99% of this volume, allowing 1–2 L to escape from the body? Add to this the fact that such a reclamation function would be a major energy-consuming process. Well, this is the nature of the kidney that has been handed down to humans through the tortuous process of evolution and it now appears that there exists a widespread effort to mimic this evolutionary folly and to build a biologic kidney of similar design, using the powerful capabilities of modern biology.[1] Strategies to build renal organoids, to transplant kidney primordia, or to repopulate decellularized nephron structures are among the gamut of approaches currently under consideration. It is strategies such as these that are questioned below and for which an alternative is suggested.

UNDERSTANDING THE SHORTCOMINGS OF THE EVOLUTIONARY BIOLOGY OF THE HUMAN KIDNEY

In his exquisitely written "From Fish to Philosopher," published in 1953, Homer Smith provides an explanation for why the human kidney has suffered this fate.[2] Briefly, he argues that a multicellular organism, living in a salty, fluid environment that is in osmotic equilibrium with the body fluids, needs only an excretory route for delivering substances into the environment that are detrimental to the organism. This was achieved by an excretory tubule comprised of cells able to secrete the small volumes of fluid and molecules that needed to be disposed of. Because these organisms evolved in increasingly dilute, hypotonic surroundings, progressively larger volumes of water were osmotically attracted into the body fluids, yielding volumes that could no longer be eliminated by the secretory tubule. And so, a biologic filter, the glomerulus, evolved, which was connected to the tubule and which could accommodate the excretion of this excessive fluid load.[2,3]

The glomerulus is a wondrous structure, composed of capillaries endowed with a fenestrated endothelium and enveloped by interlocking epithelial cells that allow water and dissolved ions and small molecules to pass through them, while restricting the passage of larger molecules. On dry land, this meant ultrafiltering about 100 ml/min in adult humans, which translates into 180 L/d. Failure to reabsorb even small percentages of this amount would lead to death in a matter of hours. Not to be outsmarted, evolution married a complex reabsorptive and secretory tubular system attached to the glomerulus, to reclaim this filtered fluid and solute load back into the body and to secrete for excretion those ions and solutes that need to be eliminated.[2,3]

The tubular part of the human nephron is comprised of about 25 different cell types, each endowed with a set of specific transport functions. Because these cells are subject to regulatory controls which include hormones and extracellular fluid composition, it is the kidney, which serves as the homeostatic organ, which maintains the constancy of the internal environment, as promulgated by Claude Bernard.[4]

WHY ATTEMPT TO BUILD A HUMAN KIDNEY?

The simple answer to this question is: To replace the function of a chronically diseased, nonfunctioning kidney in a way that does not require a patient to be hooked up to a machine or to receive a kidney transplant from a very small pool of donors. Current attempts to address this monumental task include strategies to limit deterioration of nephron function and to enhance healing, repair, and regenerative processes. When all else fails, could one build a new kidney for a patient with failing renal function, using the power of modern regenerative biology? This is a question under serious consideration at the moment.[1] The laudable goals of understanding the biologic mechanisms of disease progression, healing, and regeneration

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will all benefit from this research agenda. But it could be argued that spending a vast amount of time and effort attempting to mimic our evolved kidney with a hyper-functioning glomerulus and with multiple tubular, interstitial, and vascular cell phenotypes may be misguided. There is simply no need for most of these elements.

A SIMPLIFIED ALTERNATIVE STRATEGY FOR BUILDING A REPLACEMENT KIDNEY

We know from our experience with hemodialysis that removal of a few liters of fluid per day is adequate to match fluid intake and to excrete adequate amounts of ions and small molecules. If an organ could be created to generate only that volume of ultrafiltrate which is required to match the amount of ingested water, this would take care of volume homeostasis.

However, regulation of the ionic composition of the extracellular fluid would not be achieved this way. So, for instance, if the concentration of a given ion such as potassium is elevated, there would be the need for a separate mechanism to sense and to drive potassium excretion when the native kidneys could no longer achieve this. The same would apply to a large number of solutes. This would have to be a "secretory" organ. (It is of interest that the chronically diseased human kidney contains such tubules [aglomerular nephrons], so clearly depicted in the elegant dissections of Jean Oliver.[5] Such tubules could only be secretory in function.[6])

The two separate organs that would thus be needed to replace the function of a diseased kidney would be: (*1*) A filter that allows 1–3 L/d of fluid to be removed from the body. (*2*) An excretory organ that could sense biologic signals and up- or downregulate secretory transport systems for a variety of ions and solutes in response to such signals.

There would be no need be for a connection between filter and tubule, because there is no need for reabsorption of fluid.

OPTIONS FOR BUILDING A BIOLOGIC FILTER

To allow for the ultrafiltration of reasonable amounts of fluid from the blood, a fenestrated endothelium would be a prerequisite.[7] Isolating such endothelial cells from glomeruli in large enough numbers could be difficult. An alternative would be to look for another fenestrated endothelium which could be created from induced pluripotent stem cells. The choroid plexus is built from such endothelial cells, allowing for the escape of about a liter of cerebrospinal fluid per day. Both glomerular and choroid plexus endothelia require VEGF for maintaining the integrity of their fenestrae.[7,8] Expansion of such cells *ex vivo* in three-dimensional systems would be feasible and the goal would be to create a large hemangioma-like structure, which is fed and drained by one or more blood vessels. If at least one of these feeding vessels were endowed with vascular smooth muscle cells responsive to vasoactive hormones, some degree of *in vivo* pharmacologic regulation could be achieved. Given the small volume of ultrafiltrate required, loss of macromolecules would be limited, and the glycocalyx lining the fenestrae of the endothelium[7] should restrict protein leakage to some extent.

Hemangioma-like structures have been created *in vitro*[9] and *in vivo*[10] in experimental animals. It would be possible to bioengineer a capsule containing such a hemangioma. This bioabsorbable artificial capsule, replaced permanently by fibrous tissue, could be connected by a tunnel of urinary bladder wall to the bladder with the internal space remaining patent. (Surgically created breast implants use this technology.)

Filtrate from the enclosed vascular tuft would thus enter the bladder on a continuous basis. Its performance would be analogous to the procedure of continuous venous-venous hemofiltration. A limitation would be the accumulation in the body fluids of small molecules, which are normally excreted convectively, but which would be excreted in insufficient quantities at the substantially lowered level of ultrafiltration.

To what extent such molecules, such as urea and creatinine, would be deleterious *per se* is not easy to predict. An equilibrium state would be reached with a 10–20-fold elevation of extracellular concentrations of such molecules, at which point excretion in 1–3 L of filtrate alone would be adequate to match the rate of production. Such high concentrations may not necessarily be deleterious to the recipient. (A rough calculation indicates that serum urea nitrogen would have to rise to around 200 mg/dl to achieve a steady state between excretion and production on a moderately low-protein diet). However, alternative approaches to reduction of plasma urea levels, using encapsulated genetically engineered live cells administered orally, may well become feasible for human use in the near future.[11]

(There is the possibility that functional kidney organoids[12] or chimeric kidneys, in which functional nephrons are inserted into native kidneys,[13] could be created in the future to achieve the above goal. These would be advantageous if they were to be hormone-generating; *e.g.*, erythropoietin.

However, the need for multiple connections to arteries, and the adequate total filtration surface area required,[14] make these options less feasible).

OPTIONS FOR BUILDING A SECRETORY ORGAN

Filtration alone would not eliminate "potentially injurious" ions and molecules which would have to be removed by secretion.

There are examples of primitive excretory organs, such as the shark rectal gland, which can be mimicked.[15] To create an excretory organ, a pouch of colonic or bladder epithelium, containing genetically engineered epithelial cells with multiple secretory transport systems, could be constructed. This is entirely feasible using modern regenerative and stem cell technologies.[16] As an example, "gut-on-a-chip" experiments have shown that colonic epithelia *in vitro* form folds that increase the surface area exposed

to the lumen.[17] An array of engineered cell types could be created from induced pluripotent stem cells, each designed to deliver specific ions and solutes into the lumen of the colon or the urinary bladder. Quantitative considerations would determine the nature and size of such an organ.

MAJOR CHALLENGES AND OPPORTUNITIES

Important limitations of the above approaches are quantitative and would have to be matched by some degree of control of intake into the body. For instance, without the ability to concentrate or dilute the urine, control of volume intake matched to the level of ultrafiltration would be needed.

The major challenge, however, would be the bioengineering of the simplified filtration and secretory organs, something that is becoming increasingly feasible in the era of synthetic biology. Nonetheless, the currently more daunting and challenging task of designing a nephron, with its sequential processes of filtration, tubular absorption, and secretion, will be infinitely more difficult to achieve and an approach analogous to the "deconstructed" kidney model proposed herein may well be a more practical option.

DISCLOSURES

None.

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Persistent Underrepresentation of Kidney Disease in Randomized, Controlled Trials of Cardiovascular Disease in the Contemporary Era

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Over the last few decades, studies have consistently demonstrated an increased risk of ischemic heart disease, heart failure, and arrhythmias in patients with kidney disease (KD).[1] The presence of KD also confers significantly greater risk for poor outcomes in patients with cardiovascular disease (CVD).[2] But despite the established association between KD and CVD, randomized, controlled trials (RCTs) investigating CVD have historically excluded subjects with preexisting KD.[3–5]

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