Developmental Engineering the Kidney: Leveraging Principles of Morphogenesis for Renal Regeneration

Joydeep Basu* and John W. Ludlow

Multiple methodological approaches are currently under active development for application in tissue engineering and regenerative medicine of tubular and solid organs. Most recently, developmental engineering (TE/RM), or the leveraging of embryonic and morphological paradigms to recapitulate aspects of organ development, has been proposed as a strategy for the sequential, iterative de novo assembly of tissues and organs as discrete developmental modules ex vivo, prior to implantation in vivo. In this article, we focus on the kidney to highlight in detail how principles of developmental biology are impacting approaches to TE of this complex solid organ. Ultimately, such methodologies may facilitate the establishment of clinically relevant therapeutic strategies for regeneration of renal structure and function, greatly impacting treatment regimens for chronic kidney disease. Birth Defects Research (Part C) 96:30–38, 2012. © 2012 Wiley Periodicals, Inc.

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INTRODUCTION

Tissue engineering and regenerative medicine (TE/RM) represents a broad spectrum of methodologies and techniques aiming to repair, augment, and regenerate damaged organs and tissues. Cells, biomaterials, or cell/biomaterial combination constructs may be leveraged to mediate such regenerative outcomes. Such elemental components catalyze the regeneration of complex tissues and organs by recapitulating (ex vivo or in vivo) aspects of organ and tissue development, whether by design or through serendipity. The integration of developmental biology and biomimetic principles into engineering paradigms of organ regeneration has been referred to as “developmental engineering” (Lenas et al., 2009). From this developmental biology perspective, current approaches toward the manipulation of cells and biomaterials for organ regeneration may be classified into three groups based on the extent to which the fundamentals of embryonic morphogenesis are being actively leveraged to create neo-organs and tissues.

(1) In the simplest and most straightforward approach, no a priori knowledge of developmental mechanism is required to achieve a defined regenerative outcome. For example, regeneration of a complete bladder, bladder augment, or bladder derivative may be triggered by implantation of cell seeded, biodegradable, synthetic polymers in canine and human cystectomy models (reviewed by Basu and Ludlow, 2010, 2011). These regenerative constructs do not attempt to mimic any aspect of urologic development. In their current iteration, such constructs do not even recapitulate the cellular composition of the developing or mature bladder. In fact, only smooth muscle cells, which may be sourced from alternates to the primary organ, are required to facilitate the regeneration of a complete, de novo bladder or bladder derivative, upon implantation within the three-dimensional context of a biodegradable scaffold (Basu et al., 2011a). Urothelial cells are not required. These smooth muscle cell-seeded regenerative constructs are matured for 1–2 weeks, to allow time for the smooth muscle cells to deposit extracellular matrix (ECM) upon the scaffold; no additional steps to capture the biology or morphogenesis of the native organ is required. Observed regenerative outcomes are typically explained...
by stating that the cell-seeded construct “stimulates the body's innate regenerative potential” (Basu and Ludlow, 2010, 2011), which can be interpreted to mean that the mechanism of action is essentially unknown.

Constructs that are based on cell-seeded scaffolds derived by decellularization of cadaveric organs indirectly leverage residual bioactivity within the scaffold without knowledge of underlying mechanistic details. For example, decellularized kidney scaffold has been shown to mediate niche-specific localization and differentiation of exogenously applied ES cells along defined renal lineages (Ross et al., 2009). Such methodologies typically attempt to induce neo-organ maturation ex vivo by engineering approaches that simulate aspects of organ physiology, as with the application of pulsatile ventricular load and ventricular stimulation to induce formation of contractile myocardium from scaffolds based on decellularized heart (Ott et al., 2008).

Similarly, cell-based therapies typically mediate regenerative outcomes without clarity on underlying mechanism of action. For example, the injection of certain populations of tubular, epithelial renal cells within remnant kidneys of rodent models of chronic kidney disease has been shown to ameliorate aspects of renal physiology associated with disease outcomes (Kelley et al., 2010). Preliminary data suggest that certain populations of host-specific renal progenitor cells are mobilized in response to the introduction of the bioactive cell population, but how this mobilizing event is translated into observed clinically significant functional outcomes remains undefined (Genheimer et al., in press). Mesenchymal stem cells (MSCs) and related cell types are currently believed to operate mechanistically through “action-at-a-distance” paracrine signaling pathways that stimulate angiogenesis and ameliorate inflammation and fibrosis while simultaneously mobilizing populations of resident stem and progenitor cells (reviewed by Caplan, 2009). Exosomes/microvesicles may also be involved in cell–cell communications pertinent to regenerative outcomes (Camussi et al., 2010).

(2) As TE/RM methodologies become more complex and begin to leverage established developmental mechanisms, elements known to be active in morphogenesis are incorporated within the regenerative platform with the expectation that such elements may modulate regenerative outcomes through mechanisms mimicking their bioactivity during embryonic development. One straightforward example is the incorporation of growth factors and bioactive, ECM-based peptides within electro-spun polymer matrices (Dankers et al., 2011; Ekaputra et al., 2011). Such bioactive factors serve to potentially regulate the proliferation, differentiation, and organization of cell populations interacting with the scaffold matrix. Similarly, the development of strategies for the directed differentiation of stem and progenitor populations along defined developmental lineages is typically based on creating conditions in vitro that attempt to mimic the sequence of inductive growth factors, ECM molecules, and other signaling events that are known to mediate the differentiation of such stem cell populations in vivo. For example, established techniques for induction of hepatocyte-like cells from adipose or bone-marrow stem/progenitor cell populations have been extrapolated from developmental pathways known to be of significance during hepatogenesis (Talens-Visconti et al., 2006).

(3) Finally, TE/RM methodologies may attempt to explicitly recapitulate organ morphogenesis ex vivo through selection and design of three-dimensional environments, ECM, signaling factors, and trans-acting cell populations that mimic modular elements and sequencing involved in morphogenesis of that organ in embryo. In this developmental engineering paradigm, mechanistic knowledge of fundamental morphogenetic pathways is directly leveraged for TE/RM (Lenas et al., 2009). The experimenter attempts to create ex vivo, individual developmental modules that reconstitute the major stages of critical developmental pathways; such modular units may then be combinatorially sequenced to recreate the staged series of salient events that constitute organogenesis in embryo. Although such developmental engineering represents an intuitively appealing and methodologically complex approach to organ regeneration, it remains to be demonstrated if, from a practical perspective, such approaches will become clinically relevant. To this end, we will examine this question directly using the kidney as an illustrative example; TE/RM of kidney represents a significant clinical objective because of the potential to positively impact the current standard of care for chronic kidney disease.

CHRONIC KIDNEY DISEASE

The prevalence of chronic kidney disease has increased >33% between 1996 and 2006 in the US alone (U.S. Renal Data System. Costs of chronic kidney disease [CKD] and end stage renal disease (ESRD). Minneapolis, MN, 2007). CKD is frequently secondary to hypertension and type 2 diabetes, whose prevalence is also rising. Although pharmacological interventions that address primary dysfunctions can slow progression...
of kidney damage and disease, dialysis or whole organ transplantation is ultimately required to achieve sufficient renal filtration to sustain life. Currently, >500,000 people in the United States require dialysis or a kidney transplant to survive, accounting for >$22 billion annually in Medicare costs (6% of the total Medicare budget; Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients: Transplant Data 1998–2007. Rockville, MD: HHS/HRSA/HSB/DOT, 2008). Kidney transplantation is the definitive standard of care for CKD, providing better long-term survival (Wolfe et al., 1999) and cost effectiveness (Lapucai et al., 1996) than dialysis; however, there remains a chronic shortage of organs. Despite increases in both cadaveric and living kidney donors, the rate of transplantation per 100 dialysis patient-years in the United States is actually decreasing (Magee and Pascual, 2004). These realities underscore the need for new treatment paradigms that can alleviate some of the healthcare burden imposed by dialysis and transplant by providing substantial and durable augmentation of kidney functions to slow progression of CKD. TE/RM technologies hold promise for providing next-generation therapeutic options for CKD, especially if they can be initiated effectively after the onset of progressive disease. These clinical realities notwithstanding, kidney provides an excellent illustration of the considerable technical difficulties associated with solid organ regeneration. Numerous specialized cell types, including podocytes, mesangial cells, endothelial cells, fibroblasts, epithelial cells, and numerous stem and progenitor cell populations, are organized across the renal parenchyma into discrete, specialized functional units or nephrons, which serve to selectively filter electrolytes from the vasculature (Vize et al., 2003; Basu et al., 2010). Efforts to trigger regeneration within animal models of ischemic or chronic renal disease have typically centered around the isolation, expansion, and reintroduction of defined populations of mesenchymal, embryonic, or renal stem cells. The rationale here is that these cells are potentially capable of site-specific engraftment and directed differentiation along multiple renal lineages, as well as facilitating the creation of a regenerative microenvironment through paracrine signaling mechanisms (reviewed by Sagrinati et al., 2008; Hopkins et al., 2009). Such cell therapy methodologies have generally had mixed results, with little if any evidence supporting site-specific engraftment and directed differentiation as a mechanism of action by exogenously applied stem or progenitor populations. Introduction of stem cells within the kidney by direct injection into the renal vasculature or renal parenchyma leads to apoptosis or efflux of the majority of applied cells from the target organ within days of implantation (Togel et al., 2007). Uncontrolled differentiation of stem cells that actually do engraft may also represent a significant technical challenge and potential regulatory obstacle against successful commercialization (Kunter et al., 2007). For this reason, populations of committed, primary renal cells and primary renal cell/biomaterial constructs are being investigated for therapeutic bioactivity (Basu et al., 2011b). Constructs composed of tubular epithelial enriched primary renal cells complexed with gelatin hydrogels appear to catalyze nephrogenic events upon microinjection within the renal capsule of healthy adult rodents (Basu et al., 2011b;
see Fig. 1). This de novo nephron formation represents an empirical outcome to a TE/RM intervention observed without clarity of underlying mechanism of action and without any attempts to modulate known pathways of renal development. Alternative strategies that actively leverage developmental mechanisms are also under consideration. However, prior to examining these technologies, a summary of embryonic kidney development is required.

**DEVELOPMENTAL BIOLOGY OF THE KIDNEY**

The mammalian kidney develops from a region of mesoderm known as the intermediate mesoderm, located between the axial and lateral plate mesoderm along the medio-lateral axis of the mammalian embryo. The pronephros, or “first kidney”, represents the initial step of lineage specification in kidney development arising from the intermediate mesoderm. The pronephros is a small, hollow ball of epithelial tubular cells connected to the pronephric duct. The pronephric duct then extends caudally, while the pronephros itself degenerates. The intermediate mesoderm now forms the second kidney, or mesonephros, otherwise known as the mesonephric duct. In females this regresses, but in males, it eventually becomes the epididymis, or the connective tissue between the testis and the bladder. The mesonephric kidney consists of about 30 tubules in mouse. The lateral tip of the mesonephric tubule fuses with the mesonephric duct, opening a passage for the excretory units to the cloaca. The cloaca eventually becomes the bladder and the rectum. Finally, the last kidney or metanephric mesenchyme develops from the ureteric bud, which sprouts out and branches extensively from the nephric duct, with each new growing tip acquiring a cap-like aggregate of metanephric blastema tissue, thereby giving the metanephros a lobulated appearance.

Bidirectional signaling between the ureteric bud and the metanephric mesenchyme is ultimately responsible for mediating the seminal events of nephrogenesis (Figs. 2 and 3). The ureteric bud, an outgrowth of the nephric duct at E10.5, signals to the surrounding metanephric mesenchyme, inducing condensation of metanephric mesenchymal cells around the tips of the invading ureteric bud (Fig. 3A). These mesenchymal condensates then undergo a mesenchymal–epithelial transition (MET), to form primitive epithelial vesicles known as renal vesicles (Fig. 3B). Continued branching of the ureteric bud leads to development of components of the collecting duct system and renal pelvis. Meanwhile, the renal vesicles undergo a systematic series of morphological changes, eventually fusing with the ureteric bud epithelium to form a continuous epithelial tubule, the S-shaped body. Infiltration of the S-shaped body by endothelial cells leads to formation of the glomerular vasculature (Fig. 3C). Continued branching morphogenesis from the ureteric bud epithelia in response to signaling from the neighboring metanephric mesenchyme in turn leads to induction of new aggregates of metanephric mesenchyme at ureteric bud tips and continued nephrogenic events. This iterative process of ureteric bud branching morphogenesis and induction of additional mesenchymal condensates continues along the radial axis of the developing kidney with the youngest nephrons induced toward the periphery (Figs. 2 and 3D).

The molecular genetics underlying branching morphogenesis in the developing kidney and concomitant nephrogenesis are complex and beyond the scope of this article. Briefly, dissection of the molecular sequence of morphogenetic events has been possible through extensive genetic analysis in the mouse. Induction of the ureteric bud is triggered through up-regulation of the secreted growth factor Glial cell-derived neurotrophic factor (GDNF) through its receptor, Ret. Expression of Ret along the pronephric duct is the highest at the site of ureteric bud formation (reviewed by Reidy and Rosenblum, 2009). Knockout mutations of either GDNF or Ret are embryonic lethal and are associated with failure of ureteric budding and consequent abrogation of kidney and ureter formation. Up-regulation of GDNF expression is through the action of transcription factors including Pax2 and Six1, 2, and 4 (Reidy and Rosenblum, 2009). MET during conversion of metanephric mesenchymal condensates to renal vesicles is controlled principally by proteins of the Wnt family, including Wnt9b and Wnt4. To this end, knockouts of Wnt4 die within 24 hr of birth; kidneys are small and abnormal and consist of undifferentiated metanephric mesenchyme (Boyle et al., 2011). Other growth factors modulating aspects of ureteric bud branching and nephrogenesis...
include transforming growth factor (TGF)-β, fibroblast growth factor (FGF)2, FGF7, leukemia inhibitory factor (LIF), and homeodomain transcription factor 1 (LIM1). Multiple interacting signaling pathways are additionally involved in regulating aspects of both ureteric bud branching and nephrogenesis. These include the canonical Wnt/β-catenin pathway, the sonic hedgehog pathway, and the bone morphogenetic protein (BMP) and FGF members of the TGF-β super-family signaling pathway.

Regionalization of the intermediate mesoderm along the anterior/posterior axis is marked by expression of certain key transcription factors, including Pax2, Pax8, and Wilms tumor (WT)1. In WT1 knockout mutations in the mouse, metanephric mesenchyme undergoes apoptosis, and the ureteric bud does not grow out of the pronephric duct (Kreidberg et al., 1993). Similarly, mouse double knockouts of Pax2/Pax8 fail to develop the pronephric duct and lack expression of Lim1, another important regulator of nephrogenesis (Narlis et al., 2007). The WT1 protein is also required for assembly of the podocyte, a specialized epithelial cell acting to maintain the filtration barrier within the glomerulus, reflecting a theme common to many key signaling components of renal morphogenesis—such signaling factors may function simultaneously during multiple pathways of renal development. Finally, numerous components of the ECM are also expressed during renal morphogenesis. These include laminins, proteoglycans, collagens, fibrillins, and cadherins (CADs), as well as ECM degradative enzymes such as the matrix metalloproteinases (MMPs) (reviewed by Kanwar et al., 2004).

Figure 3. Nephrogenesis. A: Signaling from the developing ureteric bud induces condensation of surrounding metanephric mesenchyme. B: Metanephric mesenchymal condensates undergo MET to form renal vesicles. C: Renal vesicles undergo a systematic series of morphological changes, eventually fusing with the ureteric bud epithelia to form a continuous epithelial tubule, the S-shaped body. Infiltration of the S-shaped body by endothelial cells leads to formation of the glomerular vasculature. D: The S-shaped body matures into a functional nephron.
Ultimately, it is the complex interplay between multiple overlapping signaling pathways, the ECM, and ECM modifying factors across the metanephric mesenchyme and pronephric duct that mediates branching morphogenesis of the ureteric bud and subsequent assembly of the nephron (Figs. 2 and 3). This observation notwithstanding, these elementary morphogenetic principles are already beginning to impact strategies for TE/RM of kidney, as we shall discuss in the subsequent sections.

**LEVERAGING DEVELOPMENTAL BIOLOGY PRINCIPLES TO MEDIATE NEO-KIDNEY FORMATION**

Preliminary attempts to induce neo-kidney–like structures leveraged the application of morphogens known to be bioactive in early embryonic patterning in model organisms such as *Xenopus laevis*. Such experiments aimed to recapitulate, ex vivo, some of the earliest embryological events of renal morphogenesis. Treatment of undifferentiated animal pole ectoderm derived from *Xenopus* embryos (“animal cap” assay) with activin A and retinoic acid was shown to induce formation of pronephric–duct–like structures at high efficiency (Osafune et al., 2002). These induced pronephric ducts were histologically similar to native pronephric ducts when examined by electron microscopy and expressed the known pronephric duct molecular markers, *Gremlin* and *c-ret*. Learnings derived from *Xenopus* animal caps have been successfully transferred to mammalian systems. To this end, Kim and Dressler (2005) reported development of a nephrogenic cocktail, composed of activin A, retinoic acid, and BMP7, capable of inducing markers of the intermediate and early metanephric mesenchyme, including *PAX2, WT1, LIM1, GDNF*, and *CAD6*, in mouse embryoid bodies (Kim and Dressler, 2005). Upon injection into developing mouse kidney rudiments, such “primed” ES-cell-derived bodies were shown to contribute to developing renal epithelia with high efficiency. Such acquisition of major developmental markers associated with the intermediate and metanephric mesoderm may not be limited to ES cells. Application of this nephrogenic cocktail can also catalyze the up-regulation of renal markers in cell populations derived from the stromal vascular fraction of adipose (our unpublished observations).

The possibility of using metanephroi harvested from allo- or xeno-grafts has also been explored. In principle, such ectopic metanephroi may be immunologically privileged, owing to the lack of vasculature within the early metanephros. Additionally, transplanted metanephroi are incorporated within the recipient as chimeric organs, thereby presenting, at least in part, vasculature derived from the host (Rogers et al., 1998). In this particular study, whole metanephroi from E15 rats was implanted subcapsularly either within the kidneys of the recipient animal or within the omentum of non-immunosuppressed hosts. Some recipient animals were then subjected to unilateral nephrectomy with or without additional partial contra-lateral renal infarction. At 4–6 week postimplantation, the metanephroi were observed to have become enlarged, vascularized, and had formed mature tubules and glomeruli. Notably, the additional reduction of renal mass postnephrectomy was observed to be important for mediating further enlargement of the transplanted metanephroi (Rogers et al., 1998).

One of the more direct strategies for leveraging developmental biology for renal TE/RM is based on the application of the embryo itself as an “organ factory” for the regeneration of neo-organs. In this approach, rather than attempting to laboriously recreate the three-dimensional milieu of growth factors, tissue architecture, and ECM elements critical for de novo organogenesis, the regenerative construct is introduced within the body of the developing embryo where all the salient elements are already in place. The regenerative construct can then grow and mature as an ectopic neo-organ within the developing embryo in a manner similar to that of any other native organ. Although mechanistic details are not directly leveraged, knowledge of key signaling pathways may nevertheless be incorporated into this type of regenerative platform. One such study leveraged expression of GDNF, a key signaling factor expressed by the metanephric mesenchyme during the preliminary phases of nephrogenesis. Forced expression of GDNF by genetically modified MSC targeted to the site of ureteric bud formation was hypothesized to potentially trigger formation of neo-kidney–like structures in the presence of the native developmental regenerative milieu (Yokoo et al., 2005, 2006). These workers developed a relay culture system for ex vivo maturation of embryonic kidneys. Here, embryos extracted prior to ureteric bud formation were cultured ex utero until formation of a kidney rudiment. At this point, the kidney rudiments could be removed and cultured further ex vivo (Yokoo et al., 2005). Using this system, GDNF-expressing human MSCs were injected into a cultured mouse embryo near the site of ureteric bud formation. Subsequent to relay culture, cells of human origin showing features consistent with tubular epithelial, interstitial, and glomerular cells were found distributed broadly throughout the developing metanephros (Yokoo et al, 2006). Implantation of the human MSC-derived neo-kidney rudiments within the omentum of an adult host facilitated vascularization of the developing neo-organ, leading to functionality as evidenced by production of urine (Yokoo et al., 2006). Application of porcine-derived metanephroi may facilitate the further development of this system for application in human clinical trials (Hammerman, 2004).

Cells derived from the metanephric mesenchyme and ureteric bud have the potential for self-organization; if dissociated and
recombined under the right conditions, they are capable of partial reconstitution of the nephron and collecting duct-like structures, a feature that might potentially be leveraged for renal TE (Geneva et al., 2011). Specifically, in this approach, combination of intact mouse ureteric bud with dissociated metanephric mesenchyme resulted in the induction of nephron-like structures organized near the upper branches of a single collecting duct tree. Alternatively, combination of a reaggregated ureteric bud cyst with reaggregated metanephric mesenchyme catalyzed formation of a single, extensively branched ureteric bud/collection duct system into which de novo induced nephrons appeared to connect (Geneva et al., 2011). As this organization reflects that of native kidney, such neo-kidney rudiments have the potential to be functional, because urine generated by individual nephrons can drain along a common collecting duct. Other populations of primary renal cells have also been shown to have such self-organizing ability (Joraku et al., 2009). Although this property of self-organization has been demonstrated for cells derived from embryonic tissue, it will be important to establish whether this potential exists in renal stem cell populations derived from adult kidney. Should this be the case, and if such renal stem cell populations may be isolated, expanded, and shown to maintain the potential for self-organization into nephrons, collecting ducts, and other renal compartments, this methodology may well prove relevant for clinical trials.

Thus far, the methodologies discussed attempt only minimally to reassemble morphogenic pathways ex vivo, relying instead on leveraging the embryo directly as a source or “factory” for neo-organs. Despite the significant technical difficulties, attempts continue to be made to mimic aspects of nephrogenesis ex vivo. Although still far from being directly relevant for TE/RM of the kidney, such studies serve to create three-dimensional models of morphogenic processes that can be used as research tools to dissect signaling pathways salient to development. In addition, these tools may be used to test the impact of chemical or protein entities for their ability to modulate such ex vivo defined developmental processes. One such mechanistic pathway is epithelial–mesenchymal transition (EMT) and its reciprocal, MET. Both mechanisms play significant roles in embryonic nephrogenesis and the onset of renal disease. Induction of renal vesicles represents an MET event within the metanephric mesenchyme (see Figs. 2 and 3), and the development of tubulo-interstitial fibrosis during progression of CKD is associated with the TGF-β–mediated EMT of tubular epithelial cells (Zeisberg et al., 2002). Cytokines, including TGF-β, have been shown to recapitulate EMT within populations of tubular epithelial cells (Zeisberg et al., 2002), and TGF-β–induced EMT of the human primary tubular epithelial cell line HK2 is now a well-established model system to evaluate the impact of small molecule and protein factors on EMT (Dudas et al., 2009; Hills et al., 2009). Finally, conditioned medium derived from regenerative constructs composed of selected primary renal cell populations complexed with gelatin hydrogels have been shown to attenuate TGF-β–induced EMT in HK2 cells, thereby providing evidence of potential therapeutic bioactivity (Basu et al., 2011b).

The ECM component hyaluronic acid (HA) has been shown to be present throughout the interstitial space of the developing kidney (Song et al., 2006). The specific roles of HA in modulating branching morphogenesis and differentiation of the ureteric bud and metanephric mesenchyme was examined in vitro using cultures of embryonic rat kidneys and isolated ureteric buds. In this system, application of hyaluronidase to whole, cultured embryonic kidneys resulted in a dramatic decrease in branching morphogenesis and overall kidney size. Significantly, when exogenous HA was returned to the ex vivo cultured kidneys, the ability of the ectopic HA to either promote or inhibit branching morphogenesis was contingent upon its concentration and molecular weight. Decreasing the concentrations of ectopic HA increased branching morphogenesis, whereas decreasing the molecular weight of the ectopic HA led to increased branching morphogenesis (Rosines et al., 2007b). Additionally, HA was shown to stimulate ureteric bud differentiation to collecting ducts, as well as MET of the metanephric mesenchyme in a manner independent of molecular weight.

Such data raise interesting possibilities for renal TE/RM. Scaffolds designed to release HA of defined molecular weights at certain concentrations may be implanted within kidney with the expectation of modulating regenerative outcomes. To this end, we have specifically examined the impact of HA particles upon microinjection within the cortico-medullary junction of healthy adult rodent kidneys (Basu et al., 2011b). However, at least within this model system, evidence that ectopic HA facilitated or promoted regenerative outcomes, including de novo nephron and glomerulus formation, was not forthcoming. Nevertheless, the possibility that HA may be leveraged within the context of other types of regenerative constructs to regulate branching morphogenesis during TE/RM of kidney remains a subject for active investigation. The ability to introduce specific biomimetic peptides and defined proteolytic cleavage sites within the context of gel-based biomaterials raises the intriguing possibility of controlling the morphogenesis of tubules, glomeruli, or other renal structural units to modulate defined functional outcomes. For example, polyethylene glycol-based hydrogels engineered with protease degradation sites and controlled densities of arginine-glycine-aspartic acid (RGD) peptide or laminin biologics have been found to regulate epithelial morphogenesis of cysts derived from madin-darby canine kidney (MDCK) cells. Such cysts grown within ligand functionalized
gels demonstrated an increased frequency of lumen formation and unambiguous baso-lateral polarization compared with those grown in unmodified hydrogels (Chung et al., 2008). With these data in mind, a renal augment designed to trigger regeneration of glomeruli, tubules, erythropoietin (EPO) secreting fibroblasts (Paliegé et al., 2010), or other key renal cell populations may potentially be envisioned as an injectable hydrogel containing functionalized matrix optimized to catalyze this defined regenerative outcome. Such methodologies alleviate potential concerns regarding the requirement for major surgical intervention within the diseased organ.

Ultimately, strategies for developmental engineering of kidney aim to identify and isolate key stages of organ morphogenesis as units or modules that can be mimicked ex vivo; such developmental units may then be combined in serially staged sequences recapitulating major defined events in renal development. Such an approach was used to developmentally engineer a vascularized, kidney-like structure de novo from the following individual, ex vivo developmental modules: budding of the pronephric duct, branching of in vitro formed ureteric bud, recombination with metanephric mesenchyme, and concomitant MET. As we have seen above, these modules reflect the salient events in early embryonic nephrogenesis. As is the case during early nephrogenesis, budding of the pronephric duct required the addition of ectopic GDNF. Isolated ureteric buds derived from the pronephric duct ex vivo could continue to undergo branching morphogenesis ex vivo. The branched, in vitro derived ureteric bud was then recombined with freshly dissected metanephric mesenchyme, resulting in induction of nephron-like structures. Importantly, MET within the induced mesenchyme was demonstrated by expression of functional transporter proteins. Finally, implantation of the recombined, ex vivo assembled kidney-like tissue within rodents led to formation of apparently vascularized glomeruli within 14 days (Rosines et al., 2007b). While fascinating, successful translation of this methodology into clinical practice is contingent upon leveraging xenogeneic approaches that provide potential sources of embryologic modules, or, alternatively, upon the isolation and expansion of renal stem and progenitor populations that can capture the salient aspects of this developmental paradigm. As we have seen, the self-organizing potential of renal cell populations lends credence to the possibility that such a scenario may not be entirely fanciful.

The possibility of leveraging such populations of renal (or other) cells instead of embryonic explants would greatly facilitate clinical translation of this type of developmental engineering methodology. Preliminary progress toward this end was provided by studies investigating the potential of rodent renal cell cultures to model kidney progenitor tissue (Rosines et al., 2010). This study specifically explored whether populations of cells could replace the pronephric duct, ureteric bud, and metanephric mesenchyme in the modular developmental engineering paradigm described in Rosines et al. (2007a,b). To this end, hanging cell drop aggregates derived from the adult mouse collecting duct cell line inner medullary collecting duct (IMCD) or a cell line made from the mouse ureteric bud were found capable of forming tubular structures resembling the T-shaped ureteric bud (see Figs. 2 and 3) in response to GDNF. These cellular aggregates were shown to be capable of mediating at least partial MET induction of isolated metanephric mesenchyme and initiation of the preliminary events in tubulogenesis (Fig. 3A, B). However, in the reciprocal experiment, cell aggregates from cell lines derived from metanephric mesenchyme neither could induce ureteric bud branching upon recombination with freshly isolated ureteric bud nor could undergo MET in response to recombination with freshly isolated ureteric bud. Nevertheless, direct culture of isolated pronephric duct with a metanephric mesenchyme derived cell line did lead to induction of ureteric-bud-like structures. Taken together, these data suggest that, at least in principle, certain cell populations may be leveraged to mimic embryologic rudiments, such as the pronephric duct, ureteric bud, and metanephric mesenchyme. Reconstituting these developmental modules and their signaling interactions ex vivo may therefore represent a feasible strategy for TE/RM of the kidney.

**FUTURE PERSPECTIVES**

The growing incidence of CKD presents a significant public health threat whose impact is only predicted to grow. TE, cell-based therapies, and other RM technologies represent one potential response. Ultimately, such therapeutic interventions seek to recapitulate key pathways of embryogenesis within the context of the diseased adult, wherein they are referred to as “regeneration.” Regenerative outcomes may be achieved by leveraging developmental pathways with little understanding of underlying mechanistic detail. Given successful therapeutic results, such knowledge may even be regarded as irrelevant. However, as we have seen, the ability to isolate, dissect out, and manipulate development modules salient to the kidney is creating novel methodologies for renal TE/RM. Such strategies attempt to directly mobilize knowledge of developmental biology for application in renal TE/RM. Evaluation of these methodologies within preclinical models of renal disease will be of significant interest in the coming years.

**REFERENCES**


