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Review Article

The Future of Regenerative Medicine: Urinary System

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Regeneration of tissues and organs is now within the technological reach of modern medicine. With such advancements, substantial improvements to existing standards-of-care are very real possibilities. This review will focus on regenerative medicine approaches to treating specific maladies of the bladder and kidney, including the biological basis of regeneration and the history of regenerative medicine in the urinary system. Current clinical management approaches will be presented within the context of future directions including cell-based regenerative therapies.

Introduction

Current technological advances with progenitor cells have matured to the point that diseased or missing organs can now be regenerated *de novo* in adults. Harnessing the body's ability to regenerate using regenerative medical technologies can be contemplated at multiple levels:

- Cellular level regeneration—cell delivery to reconstitute function.
- Tissue level regeneration—implantation of scaffold materials (e.g., natural or synthetic) which frequently contain an active biological component (e.g., cellular or trophic biological molecule).
- Organ regeneration—implantation of complex biomaterial and biological component.

Current standards-of-care for patients with upper urinary tract damage, for example kidney failure, frequently depend on extracorporeal dialysis devices or whole organ transplantation in the attempt to augment or replace the patients' renal function. Unfortunately, these current renal replacement approaches are insufficient at fully replicating organ function.[1,2] Although transplantation technologies have dramatically improved, challenges remain associated with finite organ supplies, morbidities, and the cost burden associated with organ rejection and immune suppression.

In cases of lower urinary tract maladies, surgical procedures, many of which are over 100 years old, are frequently used to reconstruct bladder and urethral tissues by using parts of other organ systems. These procedures often rely upon the use of gastrointestinal tract, oral mucosa, or even skin to replace malformed, damaged, or cancerous urinary organs. However, even under the most favorable conditions, it is not unusual for the patient to have to endure multiple surgical procedures, experience metabolic disorders, and suffer from absorption of urine, stricture, and stone formation, thus rendering them partially or completely incapacitated and with a poor quality of life.[3] The ability to regenerate urinary system tissues and organs holds the potential to substantially improve existing standards-of-care and patient quality of life. While this review focuses on the bladder and kidney, it is important to realize that key learnings from studies on urinary system regeneration may be more broadly applied to regeneration of the lung, gastrointestinal tract, heart, liver, and the central nervous system (reviewed in Refs.[4,5]). Fundamental to these applications is the use of a synthetic or natural scaffold seeded with some type of host cells, which upon implantation, facilitates tissue regeneration.

Biological Basis of Regeneration

Regeneration is a fundamental process of biological systems to replace damaged, lost, or failing tissues and organs. There is, however, considerable variability in how much function is restored in the damaged tissue dependent upon the organ system. The molecular mechanisms that control the functional restoration of tissue damage have only recently begun to unfold. The inductive signals that initiate repair or regeneration, the cellular cues that control the activation and proliferation of cellular precursors, and finally the regulators of differentiation and morphogenesis are common to a specific regenerating cell or tissue. The ontogenic patterning of the tissue and the specific pathophysiologcial processes of the underlying disease also influence the regenerative outcome. The degree of tissue repair ranges from regeneration of a damaged cell, to the replacement of selective cell populations, to organ hypertrophy, and finally to the more complex structures that require intrinsic morphogenetic machinery (e.g., blastema) to replace a part or all of an organ. Mistakenly, stem cells and regenerative medicine are often considered synonymous. Although stem cells may well have a role in a regenerative process, any particular

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cell or constituent of a healthy tissue is insufficient to regenerate an organ. To this end, understanding components of the healing process form the biological basis of regenerative medicine. Significant components include the mesenchymal elements consisting of a blood supply, nerves, and intercellular stromal components of connective tissue and the parenchymal components that compose the fundamental active portion which provides specificity and organismal need for that particular organ.

Tissue turnover is a normal homeostatic process accomplished by replacement of those components that are normally lost through cellular senescence–stem cells in bone marrow replacing blood components and stem cells lining the small intestinal crypts replacing lumen components are examples of stem cells that naturally replenish cellular constituents. Compared to this normal physiological tissue turnover, regeneration is defined as the ability of a tissue to reconstitute normal function after the impact of a disease process. Although more complex forms of regeneration associated with blastema formation are well characterized in lower vertebrates (e.g., newt limb regeneration),[6] epimorphic regenerative processes in the adult human are not as well understood.[7] However, regenerative medicine therapies are highlighting the plasticity of adult human tissues, such as the complex structure and functional processes recently shown to underlie the reparative regeneration in the urinary tract.[8–10]

Blood supply is paramount to successful tissue regeneration; the new tissue will require blood vessels to maintain integrity. New vessels can form by differentiation and morphogenesis of precursor cells endogenous to the tissue or by addition to a regenerative product. Stimulation of differentiation and morphogenesis most likely occurs by the action of growth factors and angiogenic factors such as vascular endothelial growth factor-A, fibroblast growth factor-2, and transforming growth factor-beta.[11,12] These angiogenic cytokines can also induce tissue remodeling,[13] another key element in tissue regeneration.

Aspects of developmental biology, the process by which progenitor cells specialize and organize into a three-dimensional framework, also play a role in tissue regeneration. In this regard, the kidney has proven to be a model developmental system.[14] Mutation of the Notch2 gene results in deletion of all proximal cell types and structures of the kidney.[15] The Wnt gene family is arguably the most studied as its members function in a variety of biological processes including embryonic development and pathogenesis[16] and renal regeneration. Mutations in Wnt genes result in aberrant development of metanephric structures,[17] mesenchymal to epithelial transitions,[18] and arresting of nephron patterning.[19]

While our understanding of the cytokines and genes involved in tissue remodeling and development is incomplete, what is known may have profound influence on improving regenerative medicine approaches to treating bladder and kidney disease. One could envision a bioengineering approach, utilizing cells and a synthetic biomaterial that would recruit stem and progenitor cells to an area in need of regeneration. This may be accomplished by selecting a specific cell type that expresses cytokines involved in angiogenesis, cell differentiation, and proliferation or stimulates neighboring cells to do so. Alternatively, using a biomaterial that mimics extracellular matrix may influence cell migration and differentiation.[20]

Regenerative Medicine for the Urinary Tract

Commercial efforts to develop regenerative medical technologies have begun to address unmet medical needs for patients suffering from various maladies of the urinary tract, which we are defining here to include kidneys and bladder, as illustrated in Figure 1. One currently available urinary tract product for prostate cancer demonstrates the potential of using autologous cellular components.[21] This product not only represents personalized medicine for cancer therapy, but also the clinical utility of making biological therapies using autologous cells to stimulate healing mechanisms that include immune system activation. Providing improved patient outcomes helps alleviate some of the devastating consequences of prostate cancer, a strategy that highlights the need for collection, transport, and use of autologous cells as a potent biological active in harnessing the body's ability to heal.

Additional autologous cell-based products for urinary tract healing and regeneration are also working their way through clinical development, targeting neurogenic bladder[22] and bladder cancer[5] patient populations. These products catalyze the body's ability to regenerate urinary tissue and parts of the urinary bladder. Providing clinical benefit by only partial replacement of diseased tissues indicates that it is now possible to contemplate regeneration of entire organs such as the bladder. Such technologies have been developed to the point that they have been demonstrated to work in large mammals–historically considered unable to regenerate urinary tissue, let alone an entire organ.[23,24]

Regenerative medicine technologies currently in clinical trials have emerged by understanding regenerative pathways nascent in mammals that share many of the same innate healing pathways as humans. The idea of inducing *de novo* tissue and organ formation to regenerate form and function was considered unrealistic not that long ago, yet regenerative medical products are working their way toward commercialization. Such rapid progress elicits several questions:

- What types of regenerative products might today's medical students and practitioners anticipate routinely using in practice?
- What changes in medical practice will likely occur as regenerative medicine becomes more broadly implemented?
- How might advances in regenerative medicine change the infrastructure of and relationship between established fields of practice?

This review presents some answers to these questions by looking at the development of regenerative medicine therapies for patients with conditions for which the standards-of-care has not significantly improved for decades. Table 1 summarizes the more common intervention strategies. Early successes in the field of regenerative medicine were gained in relatively simple tissue structures such as flat surfaces (e.g., chondrocytes, or two-dimensional cellular structures) where limited organ function was required. Autologous cell-based regenerative technologies to augment or replace failing three-dimensional internal hollow organs such as the bladder can avoid many of the serious morbidity complications associated with using heterologous tissues such as the intestinal tract to reconstruct the urinary system.

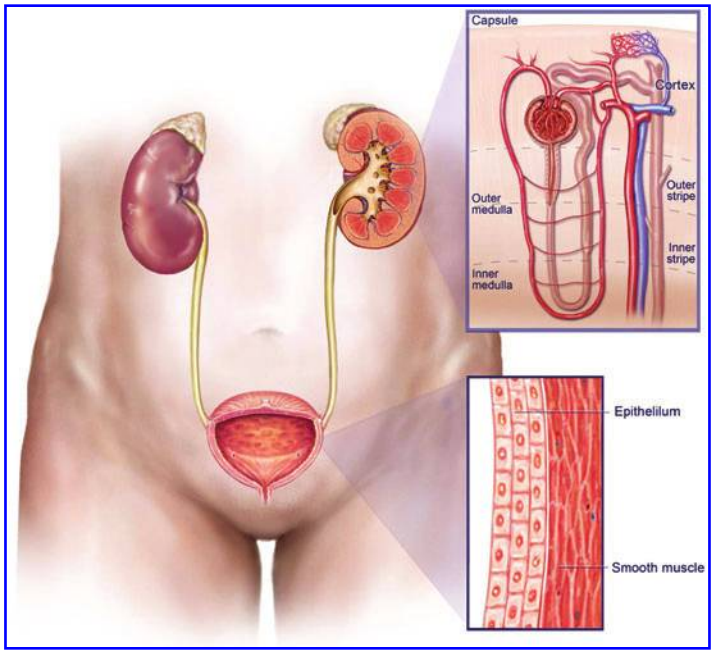

FIG. 1. Schematic diagram of kidney (solid organ) and bladder (tubular organ) of the urinary tract system. Upper inset shows the compartments of a nephron, the functional unit of the kidney. Lower inset depicts the epithelial and smooth muscle cellular composition of the bladder wall.

The History of Urinary System Regenerative Medicine

Neurogenic bladder

An impairment of function involving the bladder and/or external urethral sphincter results from congenital or traumatic damage to the nerves supplying the bladder leading to a clinical condition referred to as neurogenic bladder.[25] Patients with neurogenic bladder may suffer from inappropriate and involuntary bladder contractions during the filling phase. For some patients, the urethral sphincter fails to relax during bladder contraction (voiding phase), leading to functional obstruction of the bladder outlet. As a result of these dysfunctions, the bladder muscle hypertrophies, leading to high intravesical pressure, reduced bladder capacity, and incontinence. Moreover, transmission of this high intravesical pressure to the upper urinary tract may result in hydronephrosis and/or vesicoureteral reflux that may damage the kidney and lead to end-stage renal disease.

Current clinical management approaches

Neurogenic bladder usually causes difficulty or full inability to pass urine without the use of a catheter or other means of voiding assistance. Clinical management strategies for neurogenic bladder patients vary depending on the cause of nerve damage and the type of voiding dysfunction that results.[26] If the problem is urinary retention (the bladder does not empty when full), it may be necessary to use a catheter to empty the bladder at regular times. In the case of urine incontinence, there are a range of treatments depending on the cause of leakage. If the bladder involuntarily squeezes out urine at the wrong time, then drugs can help the bladder stay relaxed and store urine longer; however, many patients do not respond or respond with serious side effects to these agents, including tachycardia, visual accommodation, and cognitive function.[27] If urine leakage happens because the sphincter is not working, a surgically-placed artificial sphincter can help control leakage. However, for

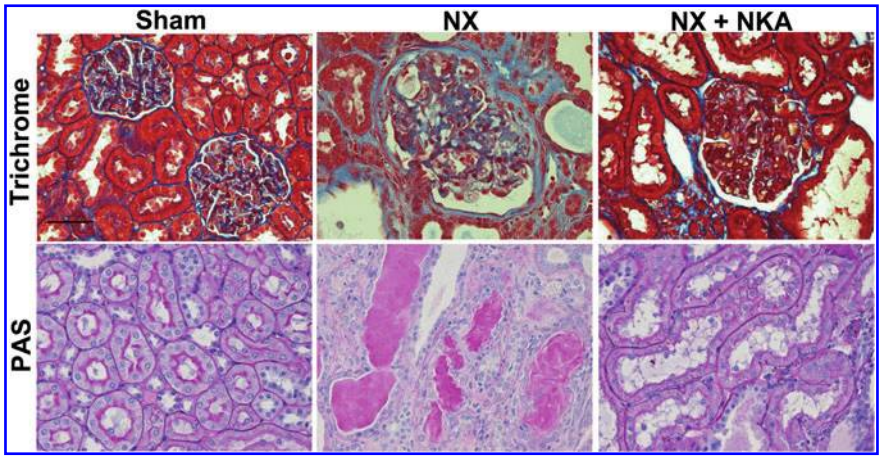

FIG. 2. Comparative renal histopathology 24 weeks after treatment with constitutive adult and renal-specific cell populations. Samples are from control rats which were sham nephrectomized (sham), 5/6 nephrectomized rats (NX), and 5/6 nephrectomized rats treated with constitutive adult and renal-specific cell populations delivered into the remnant kidney (NX + NKA). Animals were sacrificed at 24 weeks post-treatment and kidney tissue recovered and prepared for histological examination. Representative sections (stain indicated) from nephrectomized remnant kidney tissue show moderate to marked glomerular and tubulointerstitial injury, as evidenced by glomerulosclerosis, tubulointerstitial fibrosis (Trichrome), and accumulation of protein casts in the tubular lumen (PAS), compared with samples taken from control and treated animals.

Table 1. Intervention Strategies

	Intervention	*Bladder*	*Renal*
Urinary Tract	Pharmacological	Anti-neoplastic Neurostimulation	RAAS modulators
	Device	Catheterization	Dialysis
	Surgery	Ileal-conduit diversion	Kidney transplant
	Regenerative	Neo-Urinary Conduit	Renal assist device Neo-kidney augment

RAAS, renin-angiotensin-aldosterone system.

extensive damage or where a patient does not respond to less invasive procedures, a long-time approach has been bladder augmentation involving the surgical implantation of intestinal segments into the existing bladder. This procedure is associated with multiple comorbidities including metabolic disorders, stone formation, frequent infections, mucus in the urine leading to outflow obstructions, and the risk of cancer at the augmentation site.[3,28]

Bladder cancer

Another clinical challenge of the lower urinary tract is bladder cancer which encompasses a broad spectrum of malignancies of this organ. The most common type of bladder cancer originates from the internal epithelial lining of the bladder, the so-called transitional epithelium,[29] is referred to as transitional cell carcinomas. Less common bladder cancer types include squamous cell carcinoma, adenocarcinoma, sarcoma, and small cell carcinoma.[30] While a majority of patients present with superficial urothelial tumors that involve little to no metastasis of the smooth muscle compartment, approximately 25% of all bladder cancers will invade the detrusor musculature. Many of these patients will initially present as invasive cancers with metastatic potential. Invasive cancers often require multimodality therapy involving some type of chemotherapy, surgery (i.e., radical cystectomy), and construction of urinary diversion after the bladder is removed.[31]

Current clinical management

After bladder removal, a route for the safe exit of urine from the body must be constructed. All currently available surgical options for construction of urinary diversions involve the use of a segment of small or large intestine.[32] Serious and common postoperative complications of radical cystectomy result from the use of the gastrointestinal tract to construct the urinary diversion and are similar to the comorbidities associated with using gastrointestinal tissue for bladder augmentation.

Regenerative clinical management approach to both neurogenic bladder and bladder cancer

Although neurogenic bladder and bladder cancer arise from very different etiologies, they both can be treated by using intestinal tissue as either a surrogate for urine storage or a vessel for urine transport from the ureters to the outside of the body. As mentioned above, use of such tissue in managing these two pathologies is fraught with serious side effects which negatively impact patient quality of life. As such, one benefit of regenerative medicine approaches for bladder augmentation/replacement or urinary diversion is avoiding the use of gastrointestinal tract tissue. Regenerative medical technologies for bladder augmentation include composite cystoplasty, whereby cultured autologous urothelium sheets are combined with de-epithelialized bowel or vascularized uterine smooth muscle at the time of surgery,[33–38] and implanting a synthetic biocompatible scaffold material seeded with autologous urothelial and smooth muscle cells cultured from bladder tissue harvested by biopsy.[8,9,22,39–41] In both approaches, since the bladder cells came from the patient's own tissue, graft versus host disease and other complications that could arise from the use of allogeneic tissues or organs were not of concern.

Focusing on the aforementioned approach of implanting a synthetic scaffold seeded with autologous bladder-derived cells, *de novo* regeneration of partial bladder augments has been successful in humans and animals[8,9,22,39–41] and full bladder replacement has been achieved in animals.[23,24,42] Bladder augment and bladder replacement implants each consisted of a synthetic biodegradable scaffold seeded with autologous cells to form a Construct.[8,9,22,23,39,40] Following *in vivo* implantation, Constructs seeded with urothelial and smooth muscle cells, or seeded with smooth muscle cells only, elicited a regenerative response that reconstituted a native-like multilayered bladder wall with vasculature.[9,22,24,39] For bladder cancer patients, *ex vivo* expansion of autologous cells from bladder biopsies carries the risk that expanded cultures could contain cancerous urothelial cells; therefore, even single-cell (smooth muscle cells only) strategies using bladder tissue as the source of autologous cells still require a sensitive screening methodology to avoid reintroducing cancerous urothelium back into the patient at Construct implantation. Although a screening methodology with a lower limit of detection of one cancerous urothelial cell in a mixture containing one million nontumor smooth muscle cells exists,[43] identifying an alternate source of smooth muscle cells is a preferred course of action. Using stem cells as the alternate source carries with it requirements by regulatory agencies to monitor and control differentiation, making them for now a less practical alternative.[5] A strategy that demonstrates sensitivity to these regulations was to isolate fully differentiated and phenotypically uniform smooth muscle cells from adipose tissue and use these cells to seed Constructs. Following implantation, bladder tissue regeneration was observed that resulted in tissue that was histologically indistinguishable from native tissue.[44]

Acute and chronic kidney diseases

The elegant and elaborate renal architecture composed of the nephron and surrounding parenchyma supports the diverse functions of the kidney, including metabolic waste removal, elimination of foreign compounds, maintaining

acid-base balance, blood and interstitial electrolyte homeostasis, erythropoietic and bone mineral regulation, and maintaining blood pressure. Emerging scientific understanding indicates that solid organs of humans have the capacity to repair and in some cases regenerate.[45] The capacity for the injured kidney to replace denuded tubular epithelia following an acute insult such as ischemia or sub-chronic levels of nephrotoxins has been well described.[46] Our understanding of the kidney's reparative ability following complex secondary causes of renal injury, such as diabetic nephropathy, are incomplete. However, recent evidence suggests that regenerative capacity exist within the native diseased kidney.[10,47]

Current clinical management approaches

Patients with chronic kidney disease (CKD) ultimately require renal replacement therapy including dialysis or kidney transplantation. Remarkably, pharmacological antagonism of the angiotensin II pathway (Angiotensin-converting enzyme inhibitors and angiotensin-II type 1 receptor blockers) has been shown to preserve renal function by restricting glomerular hypertension and protein trafficking in both diabetic and non-diabetic animal models.[51] It remains to be seen whether the handful of pharmaceuticals and biologics in clinical development for CKD will significantly impact survival. Regenerative therapy may well offer CKD patients hope of maintaining stable renal function by delaying and perhaps avoiding dialysis and the burden of immunosuppression following transplant.

Regenerative clinical management approach for acute and CKD

The regenerative capacity of the kidney has been shown to exist within constitutive cell populations and progenitor cells of extrarenal origin.[48–51] A recent study evaluating the delivery of constitutive adult and renal-specific cell populations directly into the kidneys of terminally progressive CKD animals demonstrated not only significant improvement in survival, but the treated animals also restored erythropoiesis, electrolyte and mineral balance, and improvements to protein handling.[10] Histological findings, as shown in Figure 2, support the serological and survival benefits reported. Follow-up studies in multiple animal models of CKD using autologously-sourced cells support the regenerative potential of these bioactive cell populations (unpublished data). One basis for considering progenitor cells as therapeutics comes from an understanding of their role during embryonic nephrogenesis.[51,52] Mammalian nephron formation occurs during gestation through interactions between two intermediate mesodermal populations, the epithelium of advancing ureteric tips and the cap mesenchyme, a condensed population of self-renewing progenitor cells. The condensed mesenchyme gives rise to the nephron, the functional unit of the kidney, and the early ureteric tissue gives rise to the collecting duct system. A specialized nephrogenic zone persists in some adult vertebrate animals; a self-renewing population of nephron progenitor cells allows the zebrafish to regenerate nephrons *de novo* after injury throughout their lifespan.[53] Importantly, a self-renewing population of nephron progenitor cells does not appear to persist after birth in humans. Nonetheless, renal-specific progenitor populations that persist into adulthood may be able to reinitiate some aspects of this developmental program.[48,52]

Future direction

In addition to today's standard-of-care that includes pharmaceutical agents, biologics, and devices, treatment choices for patients with CKD may soon include cell-based regenerative therapies. Several companies are actively developing cell-based therapies and the hope for new therapeutic approaches that slow or reverse the sequelae of renal disease or restore renal function is becoming a reality. Translational studies are assessing the safety and efficacy of bioactive cells in the context of comorbid diseases, such as diabetes and hypertension. Future clinical studies will elucidate and potentially harness the clinical benefits of candidate cell-based products that might include progenitor cell populations in the fight against chronic diseases of the kidney.

Conclusions

Inevitably, regenerative medical technologies will bring important new classes of therapeutic options for physicians facing the daunting challenge of organ failure in the urinary system. With the potential to catalyze tissue and organ regeneration to address organ and tissue failure of the urinary tract, today's practitioner will be presented with new medical opportunities that may enable them to delay or even eliminate the need for renal dialysis or replacing an entire urinary bladder after cystectomy from such devastating diseases like bladder cancer. These products will undoubtedly change medical practice by bringing therapies to patients and provide unimaginable benefit-a cure, not just a treatment.

Regenerative medicine products represent the ultimate in personalized medicine and may well offer improved quality of life by potentially eliminating co-morbidities and even reducing mortality associated with diseases of the urinary systems. By accessing regenerative medicine products, the 21st century physician, can replace outdated and century old approaches with modern technologies that will not only heal but also regenerate diseased and failing organs of the urinary tract. As regenerative medical products make their way through development and become commercially available, disease diagnosis and treatment paradigms may well shift from long-term management of organ failure to cure, thereby achieving the dream of patients, physicians, and the regenerative medicine industry.

Disclosure Statement

Authors declare an equity interest in Tengion, Inc.

References

1. Lysaght, M.J., Vonesh, E.F., Gotch, F., Ibels, L., Keen, M., Lindholm, B., Nolph, K.D., Pollock, C.A., Prowant, B., and Farrell, P.C. The influence of dialysis treatment modality on the decline of remaining renal function. ASAIO Trans **37,** 598, 1991.
2. Oberbauer, R., Kreis, H., Johnson, R.W., Mota, A., Claesson, K., Ruiz, J.C., Wilczek, H., Jamieson, N., Henriques, A.C., Paczek, L., Chapman, J., and Burke, J.T. Long-term improvement in renal function with sirolimus after early cyclosporine withdrawal in renal transplant recipients: 2-year

results of the Rapamune Maintenance Regimen Study. Transplantation **76,** 364, 2003.
3. Khoury, J.M., Timmons, S.L., Corbel, L., and Webster, G.D. Complications of enterocystoplasty. Urology **40,** 9, 1992.
4. Basu, J., and Ludlow, J.W. Tissue engineering of solid and tubular organs—an industry perspective. In: Winslet-Gendebien, S., ed. Advances in Regenerative Medicine. Rijeka, Croatia: InTech Publishing, 2011, pp.235–260.
5. Basu, J., and Ludlow, J.W. Platform technologies for tubular organ regeneration. Trends Biotechnol **28,** 526, 2010.
6. Kumar, A., Godwin, J.W., Gates, P.B., Garza-Garcia, A.A., and Brockes, J.P. Molecular basis for the nerve dependence of limb regeneration in an adult vertebrate. Science **318,** 772, 2007.
7. Stappenbeck, T.S., and Miyoshi, H. The role of stromal stem cells in tissue regeneration and wound repair. Science **324,** 1666, 2009.
8. Atala, A., Bauer, S.B., Soker, S., Yoo, J.J., and Retik, A.B. Tissue-engineered autologous bladders for patients needing cystoplasty. Lancet **367,** 1241, 2006.
9. Jayo, M.J., Jain, D., Wagner, B.J., and Bertram, T.A. Early cellular and stromal responses in the regeneration of a functional mammalian bladder. J Urol **180,** 392, 2008.
10. Kelley, R., Werdin, E.S., Bruce, A.T., Choudhury, S., Wallace, S.M., Ilagan, R.M., Cox, B.R., Tatsumi-Ficht, P., Rivera, E.A., Spencer, T., Scott Rapoport, H., Wagner, B.J., Guthrie, K., Jayo, M.J., Bertram, T.A., and Presnell, S.C. Tubular cell-enriched subpopulation of primary renal cells improves survival and augments kidney function in rodent model of chronic kidney disease. Am J Physiol Renal Physiol **299,** F1026, 2010.
11. Goumans, M.J., Zwijsen, A., van Rooijen, M.A., Huylebroeck, D., Roelen, B.A., and Mummery, C.L. Transforming growth factor-beta signalling in extraembryonic mesoderm is required for yolk sac vasculogenesis in mice. Development **126,** 3473, 1999.
12. Poole, T.J., Finkelstein, E.B., and Cox, C.M. The role of FGF and VEGF in angioblast induction and migration during vascular development. Dev Dyn **220,** 1, 2001.
13. Buschmann, I., Heil, M., Jost, M., and Schaper, W. Influence of inflammatory cytokines on arteriogenesis. Microcirculation **10,** 371, 2003.
14. Dressler, G.R. Advances in early kidney specification, development and patterning. Development **136,** 3863, 2009.
15. Cheng, H.T., Kim, M., Valerius, M.T., Surendran, K., Schuster-Gossler, K., Gossler, A., McMahon, A.P., and Kopan, R. Notch2, but not Notch1, is required for proximal fate acquisition in the mammalian nephron. Development **134,** 801, 2007.
16. Merkel, C.E., Karner, C.M., and Carroll, T.J. Molecular regulation of kidney development: is the answer blowing in the Wnt? Pediatr Nephrol **22,** 1825, 2007.
17. Kreidberg, J.A., Sariola, H., Loring, J.M., Maeda, M., Pelletier, J., Housman, D., and Jaenisch, R. WT-1 is required for early kidney development. Cell **74,** 679, 1993.
18. Carroll, T.J., Park, J.S., Hayashi, S., Majumdar, A., and McMahon, A.P. Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. Dev Cell **9,** 283, 2005.
19. Stark, K., Vainio, S., Vassileva, G., and McMahon, A.P. Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. Nature **372,** 679, 1994.
20. Badylak, S.F. Regenerative medicine and developmental biology: the role of the extracellular matrix. Anat Rec B New Anat **287,** 36, 2005.
21. Burch, P.A., Croghan, G.A., Gastineau, D.A., Jones, L.A., Kaur, J.S., Kylstra, J.W., Richardson, R.L., Valone, F.H., and Vuk-Pavlovic, S. Immunotherapy (APC8015, Provenge) targeting prostatic acid phosphatase can induce durable remission of metastatic androgen-independent prostate cancer: a Phase 2 trial. Prostate **60,** 197, 2004.
22. Jayo, M.J., Jain, D., Ludlow, J.W., Payne, R., Wagner, B.J., McLorie, G., and Bertram, T.A. Long-term durability, tissue regeneration and neo-organ growth during skeletal maturation with a neo-bladder augmentation construct. Regen Med **3,** 671, 2008.
23. Bertram, T.A., Christ, G.J., Aboushwareb, T., Wagner, B.J., Jain, D., Ludlow, J.W., Payne, R., Jarapu, Y., Turner, C., and Jayo, M.J. Total regenerated urinary bladders are structurally and pharmacologically similar to native canine bladder tissue. J Urol **179,** 76, 2008.
24. Bertram, T.A., Christ, G.J., Andersson, K.E., Aboushwareb, T., Fuellhase, C., Solex, R., Wagner, B.J., Jain, D., Ludlow, J.W., Payne, R., and Jayo, M.J. Pharmacologic response of regenerated bladders in a preclinical model. FASEB J **23,** 291, 2009.
25. Yoo, J.J., Olson, J., Atala, A., and Kim, B. Regenerative medicine strategies for treating neurogenic bladder. In Neurol J **15,** 109, 2011.
26. Benarroch, E.E. Neurol control of the bladder: recent advances and neurologic implications. Neurology **75,** 1839, 2010.
27. Sexton, C.C., Notte, S.M., Maroulis, C., Dmochowski, R.R., Cardozo, L., Subramanian, D., and Coyne, K.S. Persistence and adherence in the treatment of overactive bladder syndrome with anticholinergic therapy: a systematic review of the literature. Int J Clin Pract **65,** 567, 2011.
28. Castellan, M., Gosalbez, R., Perez-Brayfield, M., Healey, P., McDonald, R., Labbie, A., and Lendvay, T. Tumor in bladder reservoir after gastrocystoplasty. J Urol **178,** 1771, 2007.
29. Wu, X.R. Urothelial tumorigenesis: a tale of divergent pathways. Nat Rev Cancer **5,** 713, 2005.
30. Madeb, R., Golijanin, D., Knopf, J., and Messing, E.M. Current state of screening for bladder cancer. Expert Rev Anticancer Ther **7,** 981, 2007.
31. Konety, B.R., Joyce, G.F., and Wise, M. Bladder and upper tract urothelial cancer. J Urol **177,** 1636, 2007.
32. Daneshmand, S., and Bartsch, G. Improving selection of appropriate urinary diversion following radical cystectomy for bladder cancer. Expert Rev Anticancer Ther **11,** 941, 2011.
33. Cross, W.R., Eardley, I., Leese, H.J., and Southgate, J. A biomimetic tissue from cultured normal human urothelial cells: analysis of physiological function. Am J Physiol Renal Physiol **289,** F459, 2005.
34. Fraser, M., Thomas, D.F., Pitt, E., Harnden, P., Trejdosiewicz, L.K., and Southgate, J. A surgical model of composite cystoplasty with cultured urothelial cells: a controlled study of gross outcome and urothelial phenotype. BJU Int **93,** 609, 2004.
35. Scriven, S.D., Trejdosiewicz, L.K., Thomas, D.F., and Southgate, J. Urothelial cell transplantation using biodegradable synthetic scaffolds. J Mater Sci Mater Med **12,** 991, 2001.
36. Southgate, J., Hutton, K.A., Thomas, D.F., and Trejdosiewicz, L.K. Normal human urothelial cells *in vitro*: proliferation and induction of stratification. Lab Invest **71,** 583, 1994.

37. Turner, A., Subramanian, R., Thomas, D.F., Hinley, J., Abbas, S.K., Stahlschmidt, J., and Southgate, J. Transplantation of autologous differentiated urothelium in an experimental model of composite cystoplasty. Eur Urol **59,** 447, 2011.
38. Turner, A.M., Subramaniam, R., Thomas, D.F., and Southgate, J. Generation of a functional, differentiated porcine urothelial tissue *in vitro*. Eur Urol **54,** 1423, 2008.
39. Oberpenning, F., Meng, J., Yoo, J.J., and Atala, A. *De novo* reconstitution of a functional mammalian urinary bladder by tissue engineering. Nat Biotechnol **17,** 149, 1999.
40. De Filippo, R.E., Bertram, T.A., Jayo, M.J., and Seltzer, E. Adaptive regulation of regenerated bladder size after implantation with Tengion Neo-Bladder Augment: Early clinical outcomes and preclinical evidence. J Urol **181,** 267, 2009.
41. Seltzer, E., Tillinger, M., Jayo, M.J., and Bertram, T.A. Role of biomechanical stimulation (cycling) in neo-bladder regeneration—translational basis for clinical outcomes. J Urol **181,** 282, 2009.
42. Bertram, T.A., Christ, G.J., Wagner, B.J., Jain, D., Aboushwareb, T., Ludlow, J.W., Payne, R., Jarapu, Y., Turner, C., and Jayo, M.J. Total urinary bladder regeneration with restoration of native structure and pharmacological response. FASEB J **22,** 581, 2008.
43. Genheimer, C., Guthrie, K., Shokes, J.E., Bruce, A.T., Quinlan, S.F., Sangha, N., Ilagan, R.M., Basu, J., Burnette, T., and Ludlow, J.W. Increased sensitivity for cancerous urothelial cell detection within a heterogeneous population of bladder smooth muscle cells using a two-step MACS/qRT-PCR approach. Appl Immunohistochem Mol Morphol **19,** 184, 2011.
44. Basu, J., Guthrie, K., Ilagan, R., Sangha, N., Genheimer, C., Quinlan, S., Payne, R., Rapoport, S., Knight, T., Wagner, B.J., Rivera, E., Jayo, M.J., Jain, D., Bertram, T.A., and Ludlow, J.W. Functional *de novo* Neo-Urinary Conduit from porcine peripheral blood and adipose-derived smooth muscle cells. Presented at the 7th Annual Meeting of the International Society for Stem Cell Research, Barcelona, Spain, 2009.
45. Orlando, G., Baptista, P., Birchall, M., De Coppi, P., Farney, A., Guimaraes-Souza, N.K., Opara, E., Rogers, J., Seliktar, D., Shapira-Schweitzer, K., Stratta, R.J., Atala, A., Wood, K.J., and Soker, S. Regenerative medicine as applied to solid organ transplantation: current status and future challenges. Transplant Int **24,** 223, 2011.
46. Lin, F., Moran, A., and Igarashi, P. Intrarenal cells, not bone marrow-derived cells, are the major source for regeneration in postischemic kidney. J Clin Invest **115,** 1756, 2005.
47. Humphreys, B.D., Valerius, M.T., Kobayashi, A., Mugford, J.W., Soeung, S., Duffield, J.S., McMahon, A.P., and Bonventre, J.V. Intrinsic epithelial cells repair the kidney after injury. Cell Stem Cell **2,** 284, 2008.
48. Hopkins, C., Li, J., Rae, F., and Little, M.H. Stem cell options for kidney disease. J Pathol **217,** 265, 2009.
49. Little, M.H., and Bertram, J.F. Is there such a thing as a renal stem cell? J Am Soc Nephrol **20,** 2112, 2009.
50. Little, M.H., and Rae, F.K. Review article: Potential cellular therapies for renal disease: can we translate results from animal studies to the human condition? Nephrology (Carlton) **14,** 544, 2009.
51. Benigni, A., Morigi, M., and Remuzzi, G. Kidney regeneration. Lancet **375,** 1310, 2010.
52. Hendry, C., Rumballe, B., Moritz, K., and Little, M. Defining and redefining the nephron progenitor population. Pediatr Nephrol **26,** 1395, 2011.
53. Diep, C.Q., Ma, D., Deo, R.C., Holm, T.M., Naylor, R.W., Arora, N., Wingert, R.A., Bollig, F., Djordjevic, G., Lichman, B., Zhu, H., Ikenaga, T., Ono, F., Englert, C., Cowan, C.A., Hukriede, N.A., Handin, R.I., and Davidson, A.J. Identification of adult nephron progenitors capable of kidney regeneration in zebrafish. Nature **470,** 95, 2011.

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