Tissue engineered extracellular matrices (ECMs) in urology: Evolution and future directions

N.F. Davis¹ E.M. Cunnane² ³ F.J. O’Brien³ J.J. Mulvihill² M.T. Walsh²

1: Department of Urology and Transplant Surgery, Beaumont Hospital, Dublin, Ireland
2: School of Engineering, Bernal Institute, Health Research Institute, University of Limerick, Limerick, Ireland
3: Tissue Engineering Research Group, Department of Anatomy, Royal College of Surgeons in Ireland, Dublin 2, Ireland

Address correspondence to:
N.F. Davis
Department of Urology and Transplant Surgery, Beaumont Hospital, Dublin 9, Co Dublin, Ireland.
Fax: +353 1 809 3962.
Email: nialldavis@rcsi.ie
Abstract

Autologous gastrointestinal tissue has remained the gold-standard reconstructive biomaterial in urology for >100 years. Mucus-secreting epithelium is associated with lifelong metabolic and neuromechanical complications when implanted into the urinary tract. Therefore, the availability of biocompatible tissue-engineered biomaterials such as extracellular matrix (ECM) scaffolds may provide an attractive alternative for urologists. ECMs are decellularised, biodegradable membranes that have shown promise for repairing defective urinary tract segments in vitro and in vivo by inducing a host-derived tissue remodelling response after implantation. In urology, porcine small intestinal submucosa (SIS) and porcine urinary bladder matrix (UBM) are commonly selected as ECMs for tissue regeneration. Both ECMs support ingrowth of native tissue and differentiation of multi-layered urothelial and smooth muscle cells layers while providing mechanical support in vivo. In their native acellular state, ECM scaffolds can repair small urinary tract defects. Larger urinary tract segments can be repaired when ECMs are manipulated by seeding them with various cell types prior to in vivo implantation. In the present review, we evaluate and summarise the clinical potential of tissue engineered ECMs in reconstructive urology with emphasis on their long-term outcomes in urological clinical trials.

Keywords

Tissue engineering, Regenerative medicine, Reconstructive urology, Extracellular matrix, Stem cells, Biomedical engineering
Introduction

More than 400 million people worldwide suffer from congenital and acquired anomalies of the urinary tract that may necessitate surgical intervention. Surgical repair for end stage upper and lower urinary tract defects often necessitates autologous, vascularised, mucus-secreting gastrointestinal tissue to either replace the diseased organ or to augment inadequate tissue.\(^1\) Postoperatively, the compliance of the bowel is often sufficient to restore the basic shape, structure and function of the urinary tract, however lifelong postoperative complications are common.\(^1\), \(^2\) Long-term comorbidities that result from interposition of gastrointestinal tissue in the urinary tract are classified as metabolic or neuromechanical and their incidence approaches 100% due to the mucus-secreting nature of the bowel epithelium.\(^3\)

Tissue engineering in urology focuses on developing alternative strategies for reconstructing injured, diseased and congenitally absent cells, tissues and organs within the urinary tract.\(^4\) Throughout the twentieth century synthetic scaffold biomaterials were investigated in reconstructive urology as alternatives to gastrointestinal tissue. In the 1950s non-biodegradable synthetic materials like polytetrafluoroethylene (PTFE), silicone, rubber, polyvinyl, and polypropylene were applied but were found to rapidly encrust with prolonged urinary contact.\(^5\) Additionally, these materials were susceptible to bacterial colonisation and foreign body reactions.\(^6\) The debilitating comorbidities associated with autologous gastrointestinal tissue and synthetic biomaterials may be mitigated by the availability of tissue-engineered readily available, porcine derived extracellular matrix (ECM) scaffolds.
Tissue engineered extracellular matrices (ECMs)

ECMs are decellularised, biocompatible, biodegradable biomaterials usually derived from porcine organs. They are prepared by mechanical, chemical and enzymatic treatments to yield tissue that is minimally immunogenic but retains its basic structural elements. Collagen, glycosaminoglycans, fibronectin, laminins and other intrinsic growth factors are retained after the preparation process to allow for host derived cellular attachment, growth and differentiation.

Appropriately prepared urological ECMs aim to provide a biologically active tissue/organ substitute that can effectively integrate into host tissue, maintain urinary tract compliance and functionally replace a defective urinary tract segment after a pre-established duration. An integrated tissue-engineered ECM should restore or preserve the normal function of the urinary tract structure it is augmenting or replacing. Furthermore, it should be biocompatible to minimise rejection and inflammatory reactions.

Urological ECMs

In urology, porcine small intestinal submucosa (SIS) and porcine urinary bladder matrix (UBM) are selected as the more favourable ECMs for tissue reconstruction. Their harvesting sites differ; however both can be utilised as interposition grafts within the urinary tract as they support ingrowth of native tissue, proliferation and differentiation of multi-layered urothelial and smooth cells layers while maintaining a robust connective tissue layer to provide mechanical support to the urinary tract. SIS is harvested from porcine small bowel and contains significant amounts of collagen type I with lesser amounts of collagen types III, IV, V and VI. UBM is
harvested from the porcine urinary bladder and contains an intact basement membrane layer with large quantities of collagen type VII.\textsuperscript{13}

**Manipulating tissue-engineered ECMs**

Currently, there are two techniques for manipulating biodegradable ECM scaffolds after their preparation process. These approaches are referred to as unseeded and seeded techniques. The unseeded method involves the use of a bare ECM scaffold in vivo to provide a framework for ingrowth and regeneration of native tissue. In general, unseeded ECMs are reserved for reconstructing smaller (i.e. $\leq 1$ cm) urinary tract segments\textsuperscript{14} (Fig. 1). The seeded method requires the in vitro culture and expansion of various cell types on an ECM scaffold to create a composite tissue for grafting in vivo (Fig. 2).\textsuperscript{11, 15, 16} Seeded cells function in a paracrine manner once implanted and recruit endogenous cells to regenerate the native tissue across the scaffold (Fig. 3).\textsuperscript{17, 18} Structural, functional and biological properties of cell-seeded ECM scaffolds should be accurately characterised in vitro to confirm that the ECMs mechanical and physiological properties are similar to the intended urinary tract segment that is being replaced.\textsuperscript{9, 19, 20, 21}

**Urinary tract applications of tissue-engineered ECMs**

Repairing defective urinary tract structures with ECM scaffolds has been widely reported with many successes and failures described. Unseeded ECM scaffolds are effective for repairing small urinary tract defects through the release of stimulatory growth factors. Cell seeded or composite ECMs are more robust than unseeded ECMs and are therefore capable of repairing larger urinary tract segments and structures.\textsuperscript{22, 23} At the turn of the millennium most studies on urological ECMs were described in animal models. Recently, phase 1 and phase 2 human clinical trials are
becoming more prevalent with some encouraging short- and intermediate-term follow-up results.\textsuperscript{22, 24} Recent progress in whole organ tissue-engineering strategies in urology is an exciting development that may be clinically translatable soon.\textsuperscript{2}

**Kidney**

Renal transplantation is the definitive treatment option for patients with end-stage renal disease (ESRD).\textsuperscript{25} However, only a minority of patients with ESRD receive kidney transplants due to a global shortage of deceased donor organs.\textsuperscript{26} Researchers are currently developing three-dimensional (3D) cell culture techniques to construct tissue-engineered kidney ECMs in vitro as potential alternatives to renal allografts.\textsuperscript{2, 27, 28} Preliminary studies have demonstrated the feasibility of decellularising and recellularising renal ECMs in rat models.\textsuperscript{29} However, development of an intricate and sophisticated vascular network has limited clinical progression.\textsuperscript{30, 31} Vascular endothelial cell-seeding techniques have recently been pioneered to address this limitation by coating the decellularised vasculature of porcine kidney ECMs with an endothelial lining. Pre-seeding with a precursor to a vascular endothelium enhances vascular patency of the implanted scaffold in vivo.\textsuperscript{28, 32} Challenges such as difficulties in expanding sufficient quantities of cell lines in vitro and thrombosis in vivo are 2 areas that need to be addressed to ensure the feasibility of these approaches in the near future.\textsuperscript{32, 33, 34, 35}

**Ureter**

Reconstruction of long ureteral segments may necessitate autologous ileum to restore the mechanism for draining urine from the kidney to the urinary bladder.\textsuperscript{36} Early studies on porcine ureters reported encouraging histopathological outcomes when SIS was utilised to replace a 7 cm ureteral segment after two thirds of the
periphery of the upper third of the left ureter was excised. Vascularised segments of collagen and smooth muscle were found in the 8 porcine models at autopsy after 7 weeks. There was no macroscopic or microscopic features of anastomotic strictures, ureteral stenosis or inflammation. The control group was the contralateral ureter where a Davis ureterotomy was performed. There was no evidence of epithelial regeneration at 7-weeks in the control group and all porcine models had radiological evidence of ureteral stenosis. Subsequently, porcine SIS was utilised to replace a 2 cm ureteral deficit (encompassing half the circumference) in 9 porcine models. Again, there were no macroscopic or microscopic features of stenosis at autopsy after 9 weeks. Histopathology demonstrated regenerated urothelial and smooth musculature at the site of the implanted SIS. Discouraging findings were described in other studies where complete circumferential ureteral defects were replaced. Shalhav et al. reported a 100% failure rate when acellular SIS replaced the ureter in 6 Yucatan mini pigs after 12-weeks.

More recently, Zhao et al. investigated the cell seeded approach by isolating adipose derived stem cells (ADSCs) from rabbits and stimulating the cell line to differentiate into a smooth muscle cell (SMC) phenotype. SMCs were then seeded onto a vascular extracellular matrix (VECM) and implanted into rabbit models to replace a 3 cm full circumferential ureteral defect. Histopathology demonstrated an organised smooth muscle layer with a stratified differentiated urothelium and no evidence of ureteral stricture disease after 16 weeks' follow-up. The success of this recent novel approach is attributable to an abundant cell source and improved implantation techniques.
**Bladder**

The urinary bladder can be compromised by neoplastic diseases and debilitating medical conditions such as bladder exstrophy, spinal cord injury, myelomeningocele, multiple sclerosis and interstitial cystitis. Bladder augmentation with a tissue engineering approach has been described in 22 patients to date in 3 different clinical trials.\(^{22, 24, 42}\) Cell-seeded ECM scaffolds cultured in conjunction with biodegradable synthetic materials did show early promise for augmenting or replacing the urinary bladder as exemplified in a phase 2 clinical trial study by Atala et al. in 2006.\(^{22}\) Native urothelial and smooth muscle cells were cultured onto ECM scaffolds or a composite scaffold composed of collagen and polyglycolic acid (PGA) in paediatric patients requiring augmentation cystoplasty for myelomeningocele (n = 7). The tissue-engineered scaffolds were implanted with or without an omental wrap and no postoperative complications were noted after 46 months. Furthermore, postoperative cystograms and urodynamic studies demonstrated an increase in bladder capacity and compliance values that were 1.58-fold–2.79-fold improved compared to baseline values. Mean bladder leak point pressure at capacity decreased postoperatively by 56% (67–37.5 cm H\(_2\)O).\(^{22}\)

Notably, one other recent phase 2 clinical trial by Joseph et al. was unable to replicate these encouraging results when an autologous cell seeded polyglycolide/polylactide (PGA/PLA) composite scaffold was utilised for augmentation cystoplasty in patients with spina bifida (n = 10).\(^{42}\) There was no improvement in bladder capacity on urodynamics after 1-year or 3-years and serious adverse events occurred in 4 patients with 5 patients requiring re-operation in the form of a conventional ileocystoplasty.\(^{42}\) Such findings demonstrate that further
prospective studies are needed to demonstrate the clinical effectiveness of tissue-engineering for reconstructing the urinary bladder.

Most recently, urologists have developed a PGA urinary conduit scaffold as an alternative to a conventional ileal conduit for urine drainage after cystectomy. The ‘neo-conduit’ was seeded with autologous smooth muscle cells (SMCs), grown from adipose derived mesenchymal stem cells, for patients undergoing radical cystectomy for bladder cancer.\textsuperscript{43,44} Eight patients have been enrolled in this phase 2 clinical trial to date and early findings have demonstrated regeneration of urothelium, smooth muscle and neuronal tissue on histopathology.\textsuperscript{44} Long-term functional results are currently awaited.

**Urethra**

The anterior urethra frequently requires reconstruction to treat ischaemic strictures caused by trauma or inflammation and congenital hypospadias.\textsuperscript{45} Substitute urethroplasty, with a skin flap or graft, is most often required in instances of strictures/defects greater \(>2\) cm, recurrent strictures or strictures of the penile urethra. Oral mucosa, derived from buccal or lingual tissue, is the gold standard grafting biomaterial in cases where there is insufficient penile skin to utilise as a flap.\textsuperscript{46} Limitations with this graft harvesting technique are donor site morbidity, necessity for \(>1\) harvesting site in cases of longer strictures/defects and the absence of sufficient donor tissue in the cases of repeat urethroplasty.\textsuperscript{47}

Presently, urethral reconstruction using tissue-engineered ECMs is at the forefront of regenerative medicine in urology.\textsuperscript{48} Urethral reconstruction utilising tissue engineering techniques, have been used in hundreds of human patients to date (Table 2). A tissue engineered urethral graft alternative has the potential to eliminate
donor site morbidity, decrease operative time and provide graft material in cases of repeat urethroplasty. Table 1 summarises 21 clinical studies that implant a range of tissue engineered scaffolds to treat urethral stricture and hypospadias. Decellularised urethral scaffolds are derived from porcine SIS, cadaveric bladder acellular matrix graft (BAMG), and human acellular dermal matrix (ADM). A naturally autologous collagen based matrix (Mukocell©) has also been utilised in addition to the synthetic polymer polyglycolic acid (PGA). Five studies have investigated the effect of seeding autologous cells onto tissue engineered urethral scaffolds. These cells are expanded from bladder urothelial cells (BUC), bladder smooth muscle cells (BSMC), oral keratinocytes (OK) and oral fibroblasts (OF).

Table 2 also demonstrates that tissue engineered urethral scaffolds demonstrate favourable clinical outcomes under certain conditions. Specifically, the success of the graft is dependent on the length of the stricture and condition of the urethral bed whereby shorter strictures and healthier urethral beds are more conducive to clinical success. Furthermore, outcomes with bulbar grafts are superior to penile urethral grafts due to the reduced urethral bed present in the penile urethra whereby the scaffold is sutured to surrounding skin rather than within the more abundant corpus spongiosum present in the bulbar urethra. This decreases the vascularity of the scaffold and therefore limits tissue regeneration. Furthermore, although cell seeded scaffolds have shown promise in initial clinical studies, the cost of producing such grafts is estimated to be 6 times that of acellular scaffolds therefore reducing the attractiveness of this approach. Future studies should focus on reducing the cost of cellularised scaffold production or, alternatively, encourage the use of acellular tissue engineered scaffolds under clinical conditions known to be conducive to successful outcomes.
**Future perspectives and conclusions**

Significant clinical progress has been made with tissue engineered ECMs in urology in recent years. Acellular ECMs are useful for repairing small urethral defects and cell-seeded ECMs can augment or replace defective tissue segments in the ureter and bladder. Currently, composite scaffolds composed of cell seeded ECMs and synthetic degradable biomaterials are being investigated as regenerative scaffolds to replace solid organs like the urinary bladder and kidney. Soon, it is envisaged that three-dimensional (3D) bioprinting technology may ultimately lead to whole organ development in tissue-engineering. 3D bioprinting technology is garnering attention as it has enabled 3D printing of ECMs, cell lines and supportive growth factors into living tissues in a 3D format. Limitations with 3D bioprinting in its present format are decreased mechanical strength and poor tissue integration of constructs when implanted in vivo. Concerns over in vivo mechanical durability and biocompatibility need to be addressed to ensure that the progression of tissue engineered ECMs into urological practise continue. Furthermore, differences in the long-term clinical outcomes associated with implanting tissue-engineered constructs in the human urinary tract indicate that the intricacies of the relationships between cell lines, ECM scaffolds and the host's physiological environment are not yet fully understood among researchers and clinicians. These relationships need to be fully clarified and defined before tissue-engineering in urology progresses from bench to bedside in mainstream reconstructive urology.
Acknowledgement

This work was funded in part by the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 708867 awarded to EC.

Figure and Table Captions

Figure 1: Preparation of unseeded tubularised ECM for repair of short urethral defect. During the preparation process decellularisation of porcine tissue is performed by physical, chemical and enzymatic treatments. After the ECM is decellularised it is sterilized by exposure to radiation and/or ethylene oxide and implanted in vivo to repair short urinary tract defects.

Figure 2: Construction of cell-seeded ECM. In the cell-seeded approach, donor cells are harvested and expanded in vitro in large quantities. Exposing a mesenchymal stem cell line to a physiological urinary tract environment allows stem cells to differentiate into urothelial and/or smooth muscle cell lines. Differentiated cells are then cultured onto a decellualrised ECM and expanded again for a period of weeks. The differentiated cell-seed ECM can then be implanted in vivo to repair larger urinary tract defects.

Figure 3: Cell-seeded ECMS over time. Viability/cytotoxicity fluorescence assays with three-dimensional confocal microscopy illustrating viable and proliferating urothelial (UCs) and smooth muscle (SMCs) cell lines on urinary bladder matrix (UBM) after 6, 10, and 14 days of dynamic culture (A, B, and C, respectively).12 The cell-seeded scaffolds are stained with calcein and ethidiumhomodimer-1 (Ethd-1; Molecular Probes, Eugene, OR, USA). Calcein is actively converted to calcein-AM in viable
cells, which appear green under a fluorescence microscope. Ethd-1 accumulates in dead cells and on the ECM scaffold, which appears red.12

Table 1: Summary and clinical outcomes of studies that describe tissue-engineering methods for reconstructing the urinary bladder in human patients.

Table 2: Summary and clinical outcomes of studies that describe tissue-engineering methods for treating urethral stricture disease in human patients.

References

1. H.D. Flood, S.J. Malhotra, H.E. O’Connell, M.J. Ritchey, D.A. Bloom, E.J. McGuire Long-term results and complications using augmentation cystoplasty in reconstructive urology Neurourol Urodyn, 14 (4) (1995), pp. 297-309

2. G. Orlando, K.J. Wood, R.J. Stratta, J.J. Yoo, A. Atala, S. Soker Regenerative medicine and organ transplantation: past, present, and future Transplantation, 91 (12) (2011), pp. 1310-1317

3. M. Garriboli, A. Radford, J. Southgate Regenerative medicine in urology Eur J Pediatr Surg, 24 (3) (2014), pp. 227-236

4. N.F. Davis, B.B. McGuire, A. Callanan, H.D. Flood, T.M. McGloughlin Xenogenic extracellular matrices as potential biomaterials for interposition grafting in urological surgery J Urol, 184 (6) (2010), pp. 2246-2253

5. T. Moore An artificial bladder Lancet, 261 (6772) (1953 Jun), pp. 1176-1178

6. A. Kaleli, J.S. Ansell The artificial bladder: a historical review Urology, 24 (5) (1984 Nov), pp. 423-428

7. P.M. Crapo, T.W. Gilbert, S.F. Badylak An overview of tissue and whole organ decellularization processes Biomaterials (2011), pp. 3233-3243

8. T.W. Gilbert, T.L. Sellaro, S.F. Badylak Decellularization of tissues and organs Biomaterials (2006), pp. 3675-3683

9. A. Callanan, N.F. Davis, M.T. Walsh, T.M. McGloughlin Mechanical characterisation of unidirectional and cross-directional multilayered urinary bladder matrix (UBM) scaffolds Med Eng Phys Inst Phys Eng Med, 34 (9) (2012), pp. 1368-1374

10. M. Pokrywczynska, I. Gubanska, G. Drewa, T. Drewa Application of bladder acellular matrix in urinary bladder regeneration: the state of the art and future directions BioMed Res Intl, 2015 (2015), p. 11, 10.1155/2015/613439

11. M. Horst, S. Madduri, R. Gobet, T. Sulser, V. Milleret, H. Hall, et al. Engineering functional bladder tissues J Tissue Eng Regen Med (2013), pp. 515-522
12. N.F. Davis, R. Mooney, A.V. Piterina, A. Callanan, H.D. Flood, T.M. McGloughlin Cell-seeded extracellular matrices for bladder reconstruction: an ex vivo comparative study of their biomechanical properties Int J Artif Organs, 36 (4) (2013), pp. 251-258

13. B. Brown, K. Lindberg, J. Reing, D.B. Stolz, S.F. Badylak The basement membrane component of biologic scaffolds derived from extracellular matrix Tissue Eng, 12 (3) (2006), pp. 519-526

14. A. Atala, M. Danilevskiy, A. Lyundup, P. Glybochko, D. Butnaru, A. Vinarov, et al. The potential role of tissue-engineered urethral substitution: clinical and preclinical studies J Tissue Eng Regen Med, 2015 (December 2015), pp. 3-19

15. N.F. Davis, A. Callanan, B.B. McGuire, H.D. Flood, T.M. McGloughlin Evaluation of viability and proliferative activity of human urothelial cells cultured onto xenogenic tissue-engineered extracellular matrices Urology, 77 (4) (2011), pp. 1007.e1-1007.e7

16. I.H. Park, P.H. Lerou, R. Zhao, H. Huo, G.Q. Daley Generation of human-induced pluripotent stem cells Nat Protoc, 3 (7) (2008), pp. 1180-1186

17. N.F. Davis, R. Mooney, A.V. Piterina, A. Callanan, B.B. McGuire, H.D. Flood, et al. Construction and evaluation of urinary bladder bioreactor for urologic tissue-engineering purposes Urology, 78 (4) (2011), pp. 954-960

18. M.C. Wallis, H. Yeger, L. Cartwright, Z. Shou, M. Radisic, J. Haig, et al. Feasibility study of a novel urinary bladder bioreactor Tissue Eng Part A, 14 (3) (2008), pp. 339-348

19. N.F. Davis, R. Mooney, A. Callanan, H.D. Flood, T.M. McGloughlin Augmentation cystoplasty and extracellular matrix scaffolds: an ex vivo comparative study with autogenous detubularised ileum PLoS One, 6 (5) (2011), pp. 1-7

20. F. Mantovani, A. Trinchieri, C. Castelnuovo, A.L. Romanò, E. Pisani Reconstructive urethroplasty using porcine acellular matrix Eur Urol, 44 (5) (2003), pp. 600-602

21. N.F. Davis, A. Callanan, B.B. McGuire, R. Mooney, H.D. Flood, T.M. McGloughlin Porcine extracellular matrix scaffolds in reconstructive urology: an ex vivo comparative study of their biomechanical properties J Mech Behav Biomed Mater, 4 (3) (2011), pp. 375-382

22. A. Atala, S.B. Bauer, S. Soker, J.J. Yoo, A.B. Retik Tissue-engineered autologous bladders for patients needing cystoplasty Lancet, 367 (9518) (2006), pp. 1241-1246

23. S.C. Baker, J. Southgate Bladder tissue regeneration Electrospinn Tissue Regen (2011), pp. 225-241

24. P. Caione, R. Boldrinic, A. Salerno, S.G. Nappo Bladder augmentation using acellular collagen biomatrix: a pilot experience in extrophic patients Pediatr Surg Int, 28 (4) (2012), pp. 421-428

25. S. Hariharan, C.P. Johnson, B.A. Bresnahan, S.E. Taranto, M.J. McIntosh, D. Stablein Improved graft survival after renal transplantation in the United States, 1988 to 1996 N Engl J Med, 342 (9) (2000), pp. 605-612
26. N.R. Brook, M.L. Nicholson An audit 2 years' practice of open and laparoscopic live-donor nephrectomy at renal transplant centres in the UK and Ireland BJU Int, 93 (7) (2004), pp. 1027-1031

27. K.H. Moon, I.K. Ko, J.J. Yoo, A. Atala Kidney diseases and tissue engineering Methods, 99 (2016), pp. 112-119

28. D.C. Sullivan, S.H. Mirmalek-Sani, D.B. Deegan, P.M. Baptista, T. Aboushwareb, A. Atala, et al. Decellularization methods of porcine kidneys for whole organ engineering using a high-throughput system Biomaterials, 33 (31) (2012), pp. 7756-7764

29. J.J. Song, J.P. Guyette, S.E. Gilpin, G. Gonzalez, J.P. Vacanti, H.C. Ott Regeneration and experimental orthotopic transplantation of a bioengineered kidney Nat Med, 19 (5) (2013), pp. 646-651

30. H.C. Ott, T.S. Matthiesen, S.-K. Goh, L.D. Black, S.M. Kren, T.I. Netoff, et al. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart Nat Med, 14 (2) (2008), pp. 213-221

31. P.M. Baptista, M.M. Siddiqui, G. Lozier, S.R. Rodriguez, A. Atala, S. Soker The use of whole organ decellularization for the generation of a vascularized liver organoid Hepatology, 53 (2) (2011), pp. 604-617

32. I.K. Ko, M. Abolbashari, J. Huling, C. Kim, S.-H. Mirmalek-Sani, M. Moradi, et al. Enhanced re-endothelialization of acellular kidney scaffolds for whole organ engineering via antibody conjugation of vasculatures Technology, 2 (3) (2014), pp. 243-253

33. T.A.N.T. Ability, M. Abolbashari, S.M. Agcaoili, M.-K. Lee, I.K. Ko, T. Aboushwareb, et al. Discarded human kidneys as a source of ECM scaffold for kidney regeneration technologies Biomaterials, 32 (1) (2014), pp. 52-61

34. B. Song, J.C. Niclis, M.A. Alikhan, S. Sakkal, A. Sylvain, P.G. Kerr, et al. Generation of induced pluripotent stem cells from human kidney mesangial cells J Am Soc Nephrol, 22 (7) (2011), pp. 1213-1220

35. T. Zhou, C. Benda, S. Dunzinger, Y. Huang, J.C. Ho, J. Yang, et al. Generation of human induced pluripotent stem cells from urine samples Nat Protoc, 7 (12) (2012), pp. 2080-2089

36. P.K.J.D. de Jonge, V. Simaioforidis, P.J. Geutjes, O. Oosterwijk, W.F.J. Feitz Recent advances in ureteral tissue engineering Curr Urol Rep, 16 (1) (2015), pp. 1-7

37. E.N. Liatsikos, C.Z. Dinlenc, R. Kapoor, M. Alexianu, P. Yohannes, A.E. Anderson, et al. Laparoscopic ureteral reconstruction with small intestinal submucosa J Endourol, 15 (2) (2001), pp. 217-220

38. T.G. Smith, M. Gettman, G. Lindberg, C. Napper, M.S. Pearle, J.A. Cadeddu Ureteral replacement using porcine small intestine submucosa in a porcine model Urology, 60 (5) (2002), pp. 931-934

39. A.L. Shalhav, A.M. Elbahnasy, E. Bercowsky, G. Kovacs, A. Brewer, K.L. Maxwell, et al. Laparoscopic replacement of urinary tract segments using biodegradable materials in a large-animal model J Endourol, 13 (4) (1999), pp. 241-244
40. Z. Zhao, H. Yu, F. Xiao, X. Wang, S. Yang, S. Li Differentiation of adipose-derived stem cells promotes regeneration of smooth muscle for ureteral tissue engineering J Surg Res, 178 (1) (2012), pp. 55-62

41. W. Liao, S. Yang, C. Song, X. Li, Y. Li, Y. Xiong Construction of ureteral grafts by seeding bone marrow mesenchymal stem cells and smooth muscle cells into bladder acellular matrix Transpl Proc, 45 (2) (2013), pp. 730-734

42. D.B. Joseph, J.G. Borer, R.E. De Filippo, S.J. Hodges, G.A. McLorie Autologous cell seeded biodegradable scaffold for augmentation cystoplasty: phase II study in children and adolescents with spina bifida J Urol, 191 (5) (2014), pp. 1389-1394

43. N.A. Sopko, M. Kates, T.J. Bivalacqua Use of regenerative tissue for urinary diversion Curr Opin Urol, 25 (6) (2015), pp. 578-585

44. M. Kates, A. Singh, H. Matsui, G.D. Steinberg, N.D. Smith, M.P. Schoenberg, et al. Tissue-engineered urinary conduits Curr Urol Rep, 16 (3) (2015 Mar), p. 8, 10.1007/s11934-015-0480-3

45. H. Orabi, A.S. Safwat, A. Shahat, H.M. Hammouda The use of small intestinal submucosa graft for hypospadias repair: pilot study Arab J Urol, 11 (4) (2013), pp. 415-420

46. A. Mangera, J.M. Patterson, C.R. Chapple A systematic review of graft augmentation urethroplasty techniques for the treatment of anterior urethral strictures Eur Urol (2011), pp. 797-814

47. B.M. Browne, A.J. Vanni Use of alternative techniques and grafts in urethroplasty Urol Clin North Am, 44 (1) (2017), pp. 127-140

48. A. Mangera, C.R. Chapple Tissue engineering in urethral reconstruction—an update Asian J Androl, 2012 (October 2012), pp. 89-92

49. A. Atala, L. Guzman, A.B. Retik A novel inert collagen matrix for hypospadias repair J Urol (1999), pp. 1148-1151

50. A.W. El-kassaby, A.B. Retik, J.J. Yoo, A. Atala Urethral stricture repair with an off-the-shelf collagen matrix J Urol, 169 (1) (2003), pp. 170-173

51. K.D. Sievert, U. Nagele, C. Wuelfing, M. Praetorius, J. Seibold, A. Stenzl, et al. ASPEC – Reconstr, 4 (3) (2005), p. 2005

52. P.J. le Roux Endoscopic urethroplasty with unseeded small intestinal submucosa collagen matrix grafts: a pilot study J Urol, 173 (1) (2005), pp. 140-143

53. J. Lin, J. Hao, J. Jin, S. Deng, J. Hu, Y. Na Homologous dermal acellular matrix graft for urethral reconstruction in man (report of 16 cases) Zhonghua Yi Xue Za Zhi, 85 (2005), pp. 1057-1059

54. Donkov II, A. Bashir, C.H.G. Elenkov, P.K. Panchev Dorsal onlay augmentation urethroplasty with small intestinal submucosa: modified Barbagli technique for strictures of the bulbar urethra Int J Urol, 13 (11) (2006), pp. 1415-1417

55. S. Hauser, P.J. Bastian, G. Fechner, S.C. Müller Small intestine submucosa in urethral stricture repair in a consecutive series Urology, 68 (2) (2006), pp. 263-266
56. R. Fiala, A. Vidlar, R. Vrtal, K. Belej, V. Student Porcine small intestinal submucosa graft for repair of anterior urethral strictures Eur Urol, 51 (6) (2007), pp. 1702-1708

57. A. El Kassaby, T. AbouShwareb, A. Atala Randomized comparative study between buccal mucosal and acellular bladder matrix grafts in complex anterior urethral strictures J Urol, 179 (4) (2008), pp. 1432-1436

58. E. Palminteri, E. Berdondini, F. Colombo, E. Austoni Small intestinal submucosa (SIS) graft urethroplasty: short-term results Eur Urol, 51 (6) (2007), pp. 1695-1701

59. M. Fossum, J. Svensson, G. Kratz, A. Nordenskjöld Autologous in vitro cultured urothelium in hypospadias repair{star, open} J Pediatr Urol, 3 (1) (2007), pp. 10-18

60. A. El Kassaby, T. AbouShwareb, A. Atala Randomized comparative study between buccal mucosal and acellular bladder matrix grafts in complex anterior urethral strictures J Urol, 179 (4) (2008), pp. 1432-1436

61. Y.A. Farahat, A.M. Elbahnasy, O.M. El-Gamal, A.R. Ramadan, S.A. El-Abd, M.R. Taha Endoscopic urethroplasty using small intestinal submucosal patch in cases of recurrent urethral stricture: a preliminary study J Endourol, 23 (12) (2009), pp. 2001-2005

62. F. Mantovani, E. Tondelli, G. Cozzi, D. Abed El Rahman, M.G. Spinelli, I. Oliva, et al. Reconstructive urethroplasty using porcine acellular matrix (SIS): evolution of the grafting technique and results of 10-year experience Urologia, 78 (2) (2011), pp. 92-97

63. A. Raya-Rivera, D.R. Esquillano, J.J. Yoo, E. Lopez-Bayghen, S. Soker, A. Atala Tissue-engineered autologous urethras for patients who need reconstruction: an observational study Lancet, 377 (9772) (2011), pp. 1175-1182

64. E. Palminteri, E. Berdondini, F. Fusco, C. De Nunzio, A. Salonia Long-term results of small intestinal submucosa graft in bulbar urethral reconstruction Urology, 79 (3) (2012), pp. 695-701

65. O. Engel, G. Ram-Liebig, P. Reiβ, B. Schwaiger, D. Pfalzgraf, R. Dahlem, et al. 15 tissue – engineered buccal mucosa urethroplasty. Outcome of our first 10 patients J Urol, 187 (4) (2012), p. e6

66. L. Ribeiro-Filho, A. Fazoli, M.A. Arap, A. Mitre, R. Falci, J.L. Chambo, et al. Pd3-02 cadaveric organ-specific acellular matrix for urethral reconstruction in humans: long term results J Urol, 191 (4) (2014), p. e20

67. G. Ram-Liebig, J. Bednarz, B. Stuerzebecher, D. Fahlenkamp, G. Barbagli, G. Romano, et al. Regulatory challenges for autologous tissue engineered products on their way from bench to bedside in Europe Adv Drug Deliv Rev (2015), pp. 181-191
Figure 1: Preparation of unseeded tubularised ECM for repair of short urethral defect. During the preparation process decellularisation of porcine tissue is performed by physical, chemical and enzymatic treatments. After the ECM is decellularised it is sterilized by exposure to radiation and/or ethylene oxide and implanted in vivo to repair short urinary tract defects.
Figure 2: Construction of cell-seeded ECM. In the cell-seeded approach, donor cells are harvested and expanded in vitro in large quantities. Exposing a mesenchymal stem cell line to a physiological urinary tract environment allows stem cells to differentiate into urothelial and/or smooth muscle cell lines. Differentiated cells are then cultured onto a decellularised ECM and expanded again for a period of weeks. The differentiated cell-seed ECM can then be implanted in vivo to repair larger urinary tract defects.
Figure 3: Cell-seeded ECMs over time. Viability/cytotoxicity fluorescence assays with three-dimensional confocal microscopy illustrating viable and proliferating urothelial (UCs) and smooth muscle (SMCs) cell lines on urinary bladder matrix (UBM) after 6, 10, and 14 days of dynamic culture (A, B, and C, respectively). The cell-seeded scaffolds are stained with calcein and ethidiumhomodimer-1 (Ethd-1; Molecular Probes, Eugene, OR, USA). Calcein is actively converted to calcein-AM in viable cells, which appear green under a fluorescence microscope. Ethd-1 accumulates in dead cells and on the ECM scaffold, which appears red.12