Differentiation of a Contractile, Ureter-Like Tissue, from Embryonic Stem Cell–Derived Ureteric Bud and Ex Fetu Mesenchyme

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ABSTRACT

Background There is intense interest in replacing kidneys from stem cells. It is now possible to produce, from embryonic or induced pluripotent stem cells, kidney organoids that represent immature kidneys and display some physiologic functions. However, current techniques have not yet resulted in renal tissue with a ureter, which would be needed for engineered kidneys to be clinically useful.

Methods We used a published sequence of growth factors and drugs to induce mouse embryonic stem cells to differentiate into ureteric bud tissue. We characterized isolated engineered ureteric buds differentiated from embryonic stem cells in three-dimensional culture and grafted them into ex fetu mouse kidney rudiments.

Results Engineered ureteric buds branched in three-dimensional culture and expressed Hoxb7, a transcription factor that is part of a developmental regulatory system and a ureteric bud marker. When grafted into the cortex of ex fetu kidney rudiments, engineered ureteric buds branched and induced nephron formation; when grafted into peri-Wolfian mesenchyme, still attached to a kidney rudiment or in isolation, they did not branch but instead differentiated into multilayer ureter-like epithelia displaying robust expression of the urothelial marker uroplakin. This engineered ureteric bud tissue also organized the mesenchyme into smooth muscle that spontaneously contracted, with a period a little slower than that of natural ureteric peristalsis.

Conclusions Mouse embryonic stem cells can be differentiated into ureteric bud cells. Grafting those UB-like structures into peri-Wolfian mesenchyme of cultured kidney rudiments can induce production of urothelium and organize the mesenchyme to produce rhythmically contracting smooth muscle layers. This development may represent a significant step toward the goal of renal regeneration.

Received October 18, 2019. Accepted June 11, 2020.
Published online ahead of print. Publication date available at www.jasn.org.

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The past decade has seen significant advances toward the goal of generating kidneys from various types of stem cell. It is now possible to produce renal organoids, representing immature kidneys and showing some physiologic functions, from embryonic and induced pluripotent cells of mouse and human.1–6 So far, these organoids have not featured a ureter. This report describes a technique for differentiating mouse embryonic stem (ES) cells into urothelium that can organize fetal peri-Wolfian mesenchyme around it to produce contractile muscle layers.

Our experiments rest on two bodies of prior work. One is an effort to explore the self-organizing properties of cells, specifically renogenic stem cells from young fetal mouse kidney rudiments. It was shown in 2010 that these cells, disaggregated then reaggregated, could interact to produce what have since come to be called renal organoids, containing small collecting duct trees and immature nephrons.7 A series of technical advances,8–10 most of which break the symmetry of the system to create large-scale order, have improved the realism of the tissues produced, resulting in organoids with nephrons arranged around a single collecting duct tree with a single urothelial end.10
The other body of work aimed to produce kidneys from human induced pluripotent stem (iPS) cells. From early application of sequences of signaling molecules to differentiate mouse ES cells into renal epithelia that could integrate into developing kidneys, methods were established to produce complete renal organoids from human iPS and ES cells that are able to connect with host blood systems. Like the 2010 ex fetus mouse organoids, they lack proper large-scale anatomic organization. This issue has been partly addressed by Taguchi and Nishinakamura in 2017, and methods listed in Schedule One of the UK Animals (Scientific Procedures) Act. The kidneys and grafts were cultured in 20% Matrigel in DMEM/F12 medium containing 10% FBS, 0.1 μM RA, 100 ng/ml human Rspordin1 (4645-RS; R&D Systems), 2 ng/ml human GDNF (212-GD; R&D Systems), and 100 ng/ml mouse FGF1 (450-33A; Pepro Tech) in U-bottomed, low cell-binding plates.

Induction of Ureteric Bud Differentiation from Mesenchymal ES Cells
A Hoxb7-GFP mouse ES cell line was a gift from Professor Ryuichi Nishinakamura’s laboratory (Kumamoto University, Kumamoto, Japan). Cells were maintained in GMEM (G5154; Sigma) supplemented with 10% FBS, GlutaMAX (1X, Gibco), MEM-NEAA (1X, Gibco), sodium pyruvate (1 mM, Gibco), β-mercaptoethanol (0.1 mM), and leukemia inhibitory factor (sc-4989, LIF, 1 U/μL; Santa Cruz Biotechnology). The mouse embryonic stem (mES) cell line was differentiated into ureteric bud (UB) cells using a slight modification of a method previously described by Taguchi and Nishinakamura. Briefly, at 0 hours cells were dissociated with Accutase (Gibco); reggregated at 2000 cells/aggregate in 96-well, U-bottomed, low cell–binding plates (650970; Greiner); and cultured to form embryonic bodies (EBs). At 48 hours, the medium was replaced by “base medium,” comprising 75% Iscove modified Dulbecco medium (12440-046; Gibco) and 25% Ham F12 (11765–054; Gibco), with 0.5× N2 (17502–048; Gibco), 0.5× B27 (12587–010; Gibco), 0.5× penicillin/streptomycin, 0.05% BSA (Sigma), 2 mM L-glutamine (Life Technologies), 0.5 mM ascorbic acid (Sigma), 450 μM 1-thioglycerol (Sigma), with the addition of 10 ng/ml human Activin A (338-AC; R&D Systems) as step 1. At 72 hours (all times are from 0 hours), the medium was changed for base medium containing 0.3 ng/ml human BMP4 (314-BP; R&D Systems) and 10 μM CHIR90021 (TOCRIS 4423) as step 2. At 108 hours, the medium was changed for base medium containing 0.1 μM retinoic acid (R-2625, RA; Sigma), 100 ng/ml human FGF9 (273-F9; R&D Systems), and 10 μM SB431542 (TOCRIS 1614) as step 3. At 132 hours, the medium was changed for base medium containing 100 ng/ml human FGF9, and 5 μM CHIR90021 as step 4. At 156 hours, the medium was changed for base medium containing 10 μM Y27632 (72302; Stem Cell Technologies), 0.1 μM RA, 1 μM CHIR90021, 5 ng/ml human FGF9, and 10% growth factor–reduced Matrigel (354230; Corning) as step 5. At 180 hours, 3 μM CHIR90021 and 1 ng/ml GDNF (212-GD; R&D Systems) were added to a fresh change of the medium used from 156 hours, as step 6. At 204 hours, this was changed to the same medium with 2 ng/ml GDNF and without FGF9, as step 7. After 24 hours of step 7, the EBs developed numerous ES cell–derived UB-like radiating tubules, which we refer to as engineered uteric buds (eUBs) in this report.

For culture in Matrigel, projecting eUB tubules were isolated by manual dissection from EBs produced using the method described above, and were suspended in 20% Matrigel in DMEM/F12 medium containing 10% FBS, 0.1 μM RA, 100 ng/ml human Rspordin1 (4645-RS; R&D Systems), 2 ng/ml human GDNF (212-GD; R&D Systems), and 100 ng/ml mouse FGF1 (450-33A; Pepro Tech) in U-bottomed, low cell–binding plates.

Grafting of eUBs into Cultured Kidney Rudiments
E11.5 kidneys were isolated from CD1 mouse embryos, and the rudiments were cultured on 24-mm, 0.4-μm-pore membranes (3450, Transwells; Corning) in kidney culture medium (KCM) comprising Minimum Eagle Medium with Earle salts (M5650, MEM; Sigma) with 10% FBS. Hoxb7-GFP eUBs were isolated manually from day 10 (approximately 230 hours) EBs using sharpened tungsten needles, and grafted into either the metanephric mesenchyme (Figure 1A) or the peri-Wolffian mesenchyme (Figure 3A) of E11.5 embryonic kidneys in culture as above. The kidneys and grafts were cultured...
Figure 1. eUB branches and induced nephrogenesis in renal metanephric mesenchyme. (A) Steps of grafting eUB into MM. (B and C) Combined bright-field and GFP fluorescence image of a HoxB7-GFP mES cell–derived eUB grafted into the MM of an E11.5 kidney at (B) 0, and (C) 5 days of culture. (D) Immunofluorescence of (C); the grafted eUB is indicated by the arrow (the sample has been rotated by 90° because of the differences between microscopes); (D’) region of (D) showing the eUB tips surrounded by SIX2⁺ nephron progenitor cells.
for 5 or 9 days in KCM, with the medium being changed every 2 days.

**Combination of eUB with Peri-Wolffian Mesenchyme or Metanephric Mesenchyme**

Peri-Wolffian mesenchyme was isolated by manual dissection from E11.5 nephrogenic areas using sharp tungsten needles, and dispersed by incubation in 1× trypsin/EDTA (T4174; Sigma) at 37°C for 2 minutes. Around 150,000 cells were suspended in 150 μl KCM, and centrifugation (3 minutes at 3000 g) was used to obtain a cell pellet. Pellets were allowed to warm to room temperature, suspended in 1 ml KCM, and the immersed samples were fixed in methanol, and the immersed samples were incubated at 37°C and 5% CO₂. After 24 hours, the combination had formed a compact spheroid, which was transferred to a 24-mm, 0.4-μm-pore Transwell membrane (3450) in a well containing 1.5 ml KCM (Figure 4A). The same method was used to combine eUB with isolated metanephric mesenchyme (Figure 2A), with the spheroids being cultured in KCM for 5 days, with the addition of 50 μl KCM containing 10% Matrigel on top.

**Immunofluorescence**

Samples were fixed by immersion in cold methanol, and the immersed samples were allowed to warm to room temperature (19°C±5°C) over 30 minutes. They were washed with PBS and blocked in staining buffer, comprising 5% BSA in PBS, overnight at 4°C. For Krt15 and NP63 staining, samples were fixed in 4% PFA in PBS and blocked using 5% BSA and 0.2% Triton X-100 (Sigma) in PBS. After blocking, primary antibodies (Supplemental Table 1) diluted in staining buffer were applied to samples at 4°C for 24 hours. Unbound primary antibody was washed off in PBS (3×5 minutes), and secondary antibodies (Supplemental Table 1) in staining buffer were applied overnight at 4°C. Samples were washed (3×15 minutes PBS) and mounted onto a slide using Vectashield (H-1000; Vector Laboratories).

**Paraffin Wax–Embedded Tissue Sectioning**

Samples were fixed in methanol as above, and were then placed in an automatic wax processing machine (Sakura VIP E300; Sakura). Wax-infused samples were embedded in paraffin wax blocks, and 6-μm sections were cut using a Leica RM2245 microtome. Sections were floated out before mounting on slides (Superfrost plus; Thermo Fisher Scientific) and dried in a 37°C oven. Samples were dewaxed in xylene (30 minutes), rehydrated in an ethanol series (100%, 90%, 70%, for 5 minutes each), and then placed under running water. Antigen retrieval was carried out by microwaving the dewaxed slides in sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0) for 3×15 minutes. Immunohistochemical staining was carried out as described above.

**RESULTS AND DISCUSSION**

**Engineering of GFP-Labeled UBs from mES Cells**

We differentiated HoxB7-GFP mESC⁴,¹⁶ to UB cells using the method of Taguchi and Nishinakamura.⁴ In agreement with their findings, by day 2, mES cells formed Hoxb7-GFP+ EBs (Supplemental Figure 1, A and B). By day 10, they developed numerous epithelial projections (Supplemental Figure 1C) expressing the UB marker HoxB7-GFP (Supplemental Figure 1D; six runs, at least six EBs in each, all showing these features). When isolated and cultured in three-dimensional Matrigel supplemented with GDNF, R-Spondin1, FGF1, and retinoic acid,⁴ the epithelial projections branched in a manner similar to natural UBs in gel culture (Supplemental Figure 1, F and G; three runs, four samples in each, all branching).¹⁷ Expression of HoxB7-GFP was maintained (Supplemental Figure 1H), suggesting retention of UB character, and the UB markers Calbindin D₂₈k, pan-cytokeratin, Krt8, E-cadherin (Cdh1), Gata3, and Pax2 were present in all three samples tested (Supplemental Figure 1, I–L). Furthermore, the epithelia expressed the GDNF receptor c-Ret (Supplemental Figure 2, A–D; all five samples) and the “tip” marker Sox9 (Supplemental Figure 2, E and F; all six samples). They did not, however, bind the “stalk” maker Dolichos biflorus agglutinin¹⁸ (Supplemental Figure 2, G and H; zero out of three samples). Therefore, the whole structure had the character of ureteric tip, with no evidence of differentiation to stalk. We refer to tubular structures as eUBs.
Importantly for our study, they found that ureteric tips grafted into the peri-Wolffian mesenchyme expressed the urothelial marker, Uroplakin (UPK). We tested whether eUBs showed the same plasticity, beginning with grafting to the metanephric mesenchyme (Figure 1A). Grafted eUBs (Figure 1B) grew and branched to produce a tree (Figure 1C: all ten branched; 100%, 95% CI, 95% to 100%). As well as expressing Hoxb7-GFP (Figure 1B) and KRT8 (Figure 1D), they organized a nephrogenic response in the host metanephric mesenchyme. Their tips became surrounded by SIX2+ cap mesenchyme cells (Figure 1D; all three samples tested, 100%, 95% CI, 83% to 100%). Early-stage nephrons, with WT1+ glomerular poles and JAG1+ proximal tubules, formed near the grafted eUBs and eventually connected to them (Figure 1, E and E'; all eight samples tested 100%, 95% CI, 94% to 100%). All but one graft into metanephric mesenchyme were UPK+, with the UPK− host ureter acting as a positive staining control (Figure 1D; five out of six tested). The exception was in a damaged host kidney that had lost its own ureter and had a torn mesenchyme. Across these experiments, UPK expression rate was therefore 17% (95% CI, 0% to 56%).

The host UB is not necessary for this response. When eUBs were grafted into isolated metanephric mesenchyme (Figure 2A), they still branched (Figure 2B) and induced the differentiation of nephrons with the UPK− host ureter acting as a positive staining control (Figure 1D; five out of six tested). The exception was in a damaged host kidney that had lost its own ureter and had a torn mesenchyme. Across these experiments, UPK expression rate was therefore 17% (95% CI, 0% to 56%).

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Figure 3. mES cell–derived eUBs differentiate into ureter tissue when grafted into peri-Wolffian mesenchyme. (A) Steps of grafting eUB into peri-Wolffian mesenchyme (PWM). (B) A combined bright-field (BF) and GFP image of a Hoxb7-GFP eUB grafted into PWM cells at the time of grafting, and (C) 7 days later. (D) Immunofluorescence stain of an eUB grafted into PWM showing expression of UPK in the...
WT1+ glomerular poles and Jagged-1+ proximal tubules (Figure 2C; all three samples tested, 100%, 95% CI, 83% to 100%). Again, this observation confirms the prior work of Taguchi and colleagues.

eUBs Differentiate into Ureter-Like Epithelia in a Peri-Wolfian Mesenchyme Environment

When grafted instead into the peri-Wolfian mesenchyme of ex fetu kidney rudiments (Figure 3A), eUBs did not branch and did not induce nephrons, although they retained Hoxb7-GFP expression (Figure 3, B and C). They now showed robust expression of UPK (Figure 3D; all 12 samples examined; 100%; 95% CI, 96% to 100%, a range that does not overlap the 95% CI of grafts into metanephric mesenchyme described in the previous paragraph). In addition to expressing UPK, they acquired a smooth muscle layer expressing α-smooth muscle actin (Figure 3, D and E).

It is known that the ureteric stalk epithelium and the mesenchyme that surrounds it collaborate to produce a ureter via reciprocal inductive signaling. There is strong evidence that epithelium-derived SHH signaling to the mesenchyme is necessary for the mesenchyme to express BMP4 as a result of an internal FOXF1-dependent pathway and to become competent to differentiate into muscle.22 BMP4 from the mesenchyme signals to the epithelium to drive urothelial differentiation,16 whereas the epithelium signals to the mesenchyme to drive smooth muscle differentiation. This urothelium-to-mesenchyme communication involves β-catenin-mediated WNT signaling, probably by WNT7B and/or WNT9B, both present in the epithelium.23 In addition, retinoic acid signaling is required for a correct balance of differentiation in both compartments.24 Are local paracrine signals such as these sufficient to drive urothelial eUB differentiation, or are influences from the kidney or natural ureter needed? Testing this by combination of eUBs with isolated ex fetu peri-Wolfian mesenchyme (Figure 4A) again resulted in the eUBs remaining unbranched (Figure 4B), activating UPK expression and gaining a smooth muscle layer (Figure 4C; all three cases examined, 100%, 95% CI, 83% to 100%). This argues that local interactions between peri-Wolfian mesenchyme and the eUB are sufficient to induce differentiation.

When in the peri-Wolfian mesenchyme, either in the kidney or isolated, the form of these grafts was fully or obliquely spherical, with no evidence of elongation into a tube. Within the structures, the eUB-derived urothelium differentiated to form the layered structure similar to a natural ureter. At the core were cells showing strong expression of UPK (Figure 4, C and D), a classic superficial (“S”) cell marker (UPKIII).25 In some samples, there was evidence of a lumen, albeit somewhat collapsed rather than inflated (Figure 4D). Between the superficial cells and the basement membrane were cells showing strong expression of KRT5 (Figure 4D; all three samples), a classic basal (“B”) cell marker.26,27 Within the B cell zone were occasional cells expressing KRT15 (Figure 4E; all five samples), as in natural ureter.28 In some places along the least-basal parts of the zone dominated by B cells were cells expressing no KRT5 and only very weak UPK (Figure 4D), and expressing strong NP63 (Figure 4F; all four samples), a pattern characteristic of intermediate (“I”) cells.25

Ureter-Like Tissues Made by Combination of eUBs with Peri-Wolfian Mesenchyme Show Spontaneous Contractions

By 7 days after combination, the ureter-like tissues formed by grafting eUBs into peri-Wolfian mesenchyme of host kidneys started to show rhythmic contractions. These became stronger and more frequent by day 9 and were detectable in all three of the samples filmed under time lapse (100%, 95% CI, 83% to 100%; Supplemental Video 1). To assess whether contractions of the graft were synchronized with those of the host ureter, this video was analyzed frame by frame and the times of peak contraction (minimum diameter) of the graft and host were recorded separately. Times of individual contractions are shown in Supplemental Figure 3. The period of contraction of the natural ureter, averaged over eight intervals between nine contractions in the recording, was 12 seconds (SEM 0.8 seconds), comparable with that in vivo.29 The period of the graft, averaged over seven intervals between its eight contractions, was slightly slower at 15 seconds (SEM 0.6 seconds). There was no obvious relationship between the timings, the graft sometimes leading and sometimes lagging the host (Supplemental Figure 3). No contractility was detected in any eUB grafted in the metanephric mesenchyme.

The asynchronous contraction of graft and host implies that the contractions of the muscles formed around the graft are spontaneous and independent of activity in the nearby natural ureter. To verify this, we filmed combinations of eUBs and pure peri-Wolfian mesenchyme with no host ureter present. These still showed large spontaneous rhythmic contractions (Supplemental Figure 4, Supplemental Video 2), of period 11 seconds (SEM 0.8 seconds) in one video and 20 seconds (SEM 0.7 seconds) in another. This indicates not only that the muscles are functional, but that at least some have the “pacemaker” activity usually ascribed to atypical muscle cells normally found at the proximal end of the ureter or renal pelvis.30 Careful observation showed that, between these large contractions, there were very small
contractions that, with the large contractions, formed a steady sequence with periods 6.4 seconds (SEM 0.4 seconds) and 7.5 seconds (SEM 0.5 seconds) in the same two videos. It is already known from electrical measurements that pacemaker activity in ureter smooth muscle cells runs at two to four times the frequency of gross peristaltic

**Figure 4.** Urothelial differentiation in pure peri-Wolffian mesenchyme. (A) Steps of recombination of Hoxb7-GFP-eUB with PWM cells. (B) A combined bright-field (BF) and GFP image of a Hoxb7-GFP eUB recombined with PWM cells. (C) Immunofluorescence stain of an eUB recombined with PWM cells showing expression of UPK in the adluminal epithelium, KRT8 in the urothelium as a whole, and smooth muscle actin (ASMA) around the epithelium. (D) A 6-μm section of an eUB combined with PWM shows UPKIII expression in superficial ("S") cells, KRT5 in basal ("B") cells, and also the presence of KRT5 intermediate ("I") cells in the less basal zone of the area otherwise dominated by B cells. (E) Krt15 is expressed by occasional cells in the B cell layer; the counterstain E-cadherin (Cdh1) marks all epithelial cells of the eUB graft. (F) Cells expressing the intermediate cell marker NP63 (arrows) and others expressing UPK; the insert (F') shows the UPK channel alone, for clarity. MM, metanephric mesenchyme; PWM, peri-Wolffian mesenchyme.
contraction because of the mechanism of muscle contraction having a refractory period\textsuperscript{29}; the small contractions we observed between large ones may reflect this underlying clock. We did not observe small contractions in either host or grafted UBs in the whole-kidney samples described in the previous paragraph, perhaps because the more closely packed stroma in these prevented visible small movements.

This is not the first report of differentiation of ES and iPSC cells into urothelial cells, but previous examples\textsuperscript{31} lacked three-dimensional structure, and both these and those of Santos et al.\textsuperscript{32} lacked smooth muscle. A recent publication by Mullenders et al.\textsuperscript{33} described ureter organs made from bladder cancers and from adult human tissue. They adopted a cyst-like shape with a lumen, but with no organization of mesenchymal components or muscle and no evidence of contraction. Our approach is distinct in combining ES-derived ureters with ex\textit{fetu} mesenchymal cells to generate multiple epithelial layers and smooth muscle coat that contract spontaneously. Important future goals are to develop techniques for differentiating peri-Wolffian mesenchyme from ES cells, and inducing the engineered tissue to elongate into a proper tube.

DISCLOSURES

All authors have nothing to disclose.

FUNDING

Work in this report was funded by Medical Research Council grant MR/R026483/1 (to J.A. Davies) and Kidney Research UK grants RP_002_20160223 and ST_001_20161116 (to J.A. Davies). M. Sallam is funded by a scholarship from Newton-Mosharafa program between the Egyptian Cultural and Educational Bureau and the British Council in Egypt.

ACKNOWLEDGMENTS

We thank Prof. Tung-Tien Sun (New York University) for the uroplakin antibody and Prof. Ryuichi Nishinakamura (Kumamoto University) for the FoxB7-GFP cell line.

Dr. May Sallam conducted experiments and led manuscript writing. Dr. Anwar Palakkan helped in data analysis and paper editing. Dr. Christopher G. Mills helped in drafting experiments. Ms. Julia Tarnick helped in time-lapse video recording and paper editing. Dr. Mona Elhendawi assisted with thoughtful discussions. Prof. Lorna Marson co-supervised Dr. May Sallam’s work and made suggestions about the strategy for experiment. Prof. Jamie A. Davies is primary supervisor of Dr. May Sallam’s work, participated in overall project design and detailed experimental design, advised about methods of analysis, analyzed timing of contractions, and participated in writing the manuscript. All authors approved the final version of the manuscript.

SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at http://jasn.asnjournals.org/looxSupp/suppl/doi:10.1681/ASN.2019101075/-/DCSupplemental.

Supplemental Table 1. Antibodies used for immunofluorescence analysis.

Supplemental Video 1. A GFP-expressing eUB grafted into the peri-Wolffian mesenchyme of an intact nephrogenic zone, showing regular smooth muscle contractions.

Supplemental Video 2. An eUB combined with peri-Wolffian mesenchyme in the absence of an associated kidney, also showing regular smooth muscle contractions.

Supplemental Figure 1. Production of eUBs from FoxB7-GFP mES cells.

Supplemental Figure 2. ES cell-derived eUBs show tip markers.

Supplemental Figure 3. Contraction in grafted eUB-derived ureter-like tissue and in the natural ureter.

Supplemental Figure 4. Contraction in eUB-derived ureter-like tissue in isolated peri-Wolffian mesenchyme.

REFERENCES


## Supplementary Information

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**Table 1S: antibodies and lectins used in this study.**

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**Figure 1S legend** Production of eUBs from Hoxb7-GFP mESC. (A) Bright field image showing an embryoid body (EB), on day 2 of the HoxB7-GFP mESC differentiation; (B) it expresses no GFP. (C) Bright field image of an EBs 10 days after induction of differentiation, showing UB-like projections, and (D) expressing HoxB7-GFP. (E-H) A single eUB bud isolated on day 10 and cultured in 3D gel with ramogenic mixture at days (E) 0, (F) 4, and (G) 7; it branches and (H) continues to express GFP. (I-L) Immunofluorescence images of day 10 EBs expressing the UB markers Calbindin-D28k, pan-cytokeratin (PCK), E-cadherin (Cdh1), Cytokeratin 8 (Krt8), GATA3 and Pax2; DAPI is used as a nuclear stain. Scale bar = 100µm.
Figure 2S legend: ES-cell derived eUBs express UB tip markers. (A, B) Show both bright field and immunofluorescence images of an eUB stained for RET showing positive expression. (C, D) are the corresponding secondary-only control. (E-H) show day 10 eUBs stained for Sox9 (E), CDH1 (F), and with DBA (G); H is a cultured embryonic kidney stained with DBA as a positive staining control.
Figure 3S: Contraction in grafted eUB-derived ureter-like tissue and in the natural ureter.

Figure 3S legend: Contraction in grafted eUB-derived ureter-like tissue and in the natural ureter. (a) shows a starting frame of the video recording (video 1S); the graft is identifiable by the GFP fluorescence in its ES-derived urothelial tissues, and the arrow indicates the place at which contractions in the nearby natural ureter were timed (they passed this point as waves from kidney to distal ureter). (b) shows the timings at which contractions occurred, depicted as dots on the same time-scale. The time-stamps on the recording incremented at intervals of 1.1s, which sets a lower limit of resolution of the timings shown on the graph. Timings for the host were 6.6, 16.5, 38.5, 50.6, 59.4, 71.5, 81.4, 93.5 and 102.3s, and for the graft, 4.4, 17.6, 29.7, 49.5, 63.8, 79.2, 94.6 and 110s.
Figure 4S: Contraction in eUB-derived ureter-like tissue grafted into isolated peri-Wolffian mesenchyme

Figure 4S legend: Contraction in eUB-derived ureter-like tissue in isolated peri-Wolffian mesenchyme. (a) and (b) show the timing of very small contractions (blue), and of large contractions (red, these large contractions being comparable to those in Fig 3S), in two different examples. In (a), timings of contractions were 2, 8, 14, 21, 26, 33, 41, 47 and 52s and in (b) they were 5, 12, 17, 25, 31, 41, 58, 64, 73, 78, 88s (colour code as in the charts).