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Human Urine-Derived Stem Cells Originate from Renal Glomerular Parietal Epithelial Cells and Generate Nephrogenic Organoids for Translational Kidney Applications

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Abstract

Background: Although specialized stem/progenitor cells can be non-invasively isolated from human urine, their precise anatomical origin within the genitourinary tract has remained controversial. Elucidating the cell lineage and tissue niche of human urine-derived stem cells (USCs) is essential to validate and optimize their translational potential. This study aimed to identify the physiological origin of USCs and evaluate their nephrogenic multi-lineage differentiation capacity.

Methods: Urine specimens were collected from 16 donors, including healthy males, patients with ureteropelvic junction obstruction, and female recipients of sex-mismatched (male donor) kidney transplants. USCs isolated from female recipients were analyzed using fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), and cytogenetic karyotyping to trace their tissue origin. Surface immunophenotyping was performed via flow cytometry. USCs were evaluated for native marker expression, subjected to directed podocyte differentiation, and cultured in 3D kidney extracellular matrix (k-ECM) hydrogels to assess organoid formation.

Results: Y-chromosome detection via FISH and PCR in female recipients of male kidney transplants confirmed that USCs originate directly from the donor kidney/upper urinary tract. Flow cytometry and gene expression profiling demonstrated that USCs natively express mesenchymal stem cell and renal parietal epithelial cell (PEC) markers (CD24, CD133, CD44, CD146, ABCG2, PAX2, PAX8, and Claudin-1) while lacking mature podocyte markers (Podocin and Synaptopodin [SYNP]). Directed podocyte induction downregulated PEC markers and induced binucleated podocyte morphology with high SYNP and Podocin expression. In 3D k-ECM hydrogel cultures, USCs organized into tubular structures expressing podocyte (SYNP, E-Cadherin) and proximal/distal tubular epithelial markers (AQP1, AQP3).

Conclusions: USCs originate directly from renal glomerular PECs and possess robust multipotent capacity to differentiate into both podocytes and renal tubular epithelial cells. These findings establish

USCs as a non-invasive, patient-specific renal progenitor source for disease modeling, nephrotoxicity screening, precision diagnostics, and regenerative nephrology applications.

(312 words)

Keywords: Urine-derived stem cells, Parietal epithelial cells, Kidney donor origin, Nephrogenic differentiation, Podocytes, Renal tubular epithelial cells, 3D organoid culture, Renal extracellular matrix, Regenerative medicine.

Introduction

Somatic stem cells derived from bodily fluids—collectively designated as body fluid-derived stem cells—represent an accessible, non-invasive cell source capable of self-renewal and multi-lineage differentiation^{1,2}. Among these, human urine-derived stem cells (USCs) have gained substantial attention in regenerative medicine owing to their non-invasive collection, rapid expansion kinetics, multipotent differentiation, secretome-mediated paracrine effects, and immunomodulatory properties³⁻⁸. Furthermore, USCs can be reprogrammed into integration-free induced pluripotent stem cells (iPSCs) with high efficiency⁹⁻¹¹. These traits have driven extensive preclinical research into USCs for urological tissue repair, ischemic injury recovery¹², diabetic nephropathy treatment¹³⁻¹⁷, biomarker discovery¹⁸, and drug-induced nephrotoxicity testing¹⁹⁻²¹.

Despite these developments, the precise anatomical niche and biological lineage of USCs have remained a subject of ongoing debate. Early hypotheses suggested that USCs shed from the basal layer of the bladder urothelium²². Subsequent studies indicated an upper urinary tract origin⁵—possibly from the renal papilla, capsule or convoluted tubules of the nephron (including proximal and distal tubule epithelium),—yet the specific cellular precursor was not conclusively identified^{23, 24}. Identifying whether USCs derive from lower urinary tract urothelium or renal parenchymal structures is critical to establishing standardized isolation protocols, understanding their physiological role, and directing their differentiation toward functional renal cell types.

Chronic kidney disease affects more than 850 million people worldwide and remains a major cause of morbidity and mortality²⁵. Progress in kidney regenerative medicine has been hindered by limited access to autologous renal progenitor cells and the absence of scalable patient-specific cell sources for disease modeling and drug development²⁶. Although kidney biopsies provide direct access to renal tissue, they are invasive and unsuitable for repeated sampling²⁷. Therefore, identifying a non-invasive, renewable human renal progenitor source represents a critical unmet need. In the mammalian kidney, glomerular

parietal epithelial cells (PECs) lining Bowman's capsule serve as a resident progenitor population for both visceral podocytes at the vascular pole and renal tubular epithelial cells at the tubular pole²⁸. Determining whether USCs represent shed glomerular PECs could resolve their lineage origin and validate their utility in kidney tissue engineering.

In this study, we investigated the origin of human USCs using sex-mismatched kidney transplant recipient models, detailed immunophenotyping, and gene/protein profiling against native human renal tissues. In addition, we evaluated the nephrogenic plasticity of USCs to differentiate into podocytes and renal tubular epithelial cells within monolayer cultures and 3D kidney extracellular matrix (k-ECM) organoid systems.

Materials and Methods

Ethics Statement and Sample Collection

This study was approved by the Institutional Review Board of Wake Forest University Health Sciences (IRB00014033). Written informed consent was obtained from all human donors prior to sample collection. A total of 37 urine samples were collected from 16 donors (aged 1.5–67 years) categorized into three groups (G): G1: Healthy male donors (n=5): 15 voided urine samples. G2. Patients with ureteropelvic junction obstruction (UJO) (n=5; 2 males, 3 females): 7 upper urinary tract urine samples and 3 bladder wash specimens obtained during pyeloplasty surgery. G3. Sex-mismatched kidney transplant recipients (n = 6 females with male donor kidneys): 15 voided urine samples. Kidney tissue was collected from a 43-year-old female diagnosed with a benign renal tumor (renal oncocytoma) following surgical resection. The tissue was collected from a grossly normal region of the kidney located distant from the tumor and free of tumor involvement. The donor had no history of other kidney diseases or significant underlying medical conditions.

Primary Isolation and Cell Culture

Urine samples were centrifuged at 500 x g for 5 minutes at room temperature. Cell pellets were resuspended in complete primary culture medium comprising Keratinocyte Serum-Free Medium (KSFM) and Embryonic Fibroblast Medium (EFM) at a 1:1 ratio, supplemented with 5% fetal bovine serum (FBS), and seeded onto 24-well tissue culture plates. Individual cell colonies ("rice-grain" morphology) appeared within 4–6 days and were expanded routinely.

Flow Cytometry

USCs at passage 2 (P2) were trypsinized, washed, and resuspended in ice-cold phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA). Cells (5×10^5) were incubated in the dark at

4°C for 30 minutes with fluorophore-conjugated primary antibodies targeting CD14, CD24, CD29, CD31, CD34, CD44, CD45, CD73, CD90, CD105, CD117, CD133, CD146, SSEA-4, and STRO-1 (**Table 1**). Matched isotype controls were used to assess background fluorescence. Antigen expression was quantified using a FACS Calibur flow cytometer and analyzed using FlowJo VX software.

Sex Chromosome Analysis (FISH, PCR, and Karyotyping)

To trace the tissue origin in female recipients of male donor kidneys: A). FISH: Monolayer USCs were fixed and hybridized with α -satellite DNA probes specific for the X and Yq12 chromosome regions according to the manufacturer's protocol. Interphase nuclei (n=500) were assessed under fluorescence microscopy. B). Genotyping PCR: Genomic DNA was extracted using a DNeasy Kit (Qiagen). Amelogenin gene fragments were amplified using primers (5'-CCCTGGGCTCTGTAAAGAATAGTG-3' and 5'-ATCAGAGCTTAACTGGGAAGCTG-3') to differentiate X (102 bp) and Y (106 bp) alleles (**Table 2**). C). Karyotyping: USCs were treated with 0.075 M KCl hypotonic solution, fixed in 3:1 methanol:glacial acetic acid, dropped onto chilled slides, stained with Giemsa, and analyzed metaphase-by-metaphase.

Multilineage Induction Monolayer Assays

Osteogenic Differentiation: USCs at 4,000 cells/cm² were cultured in DMEM low-glucose supplemented with 100 nM dexamethasone, 10 mM β -glycerophosphate, and 50 μ M ascorbic acid-2-phosphate for 28 days. Mineralization was visualized by 2% Alizarin Red S staining. Adipogenic Differentiation: USCs at 21,000 cells/cm² were cultured in DMEM low-glucose containing 1 μ M dexamethasone, 500 μ M IBMX, 10 μ g/mL insulin, and 100 μ M indomethacin for 28 days. Intracellular lipid droplets were detected by 0.3% Oil Red O staining. Podocyte Differentiation: USCs (P3–P5) were cultured on kidney matrix-coated plates in high-glucose DMEM containing 10% FBS for 14 days.

3D Kidney Organoid Formation

Decellularized porcine kidney extracellular matrix (k-ECM) was prepared as previously described²¹. Trypsinized USCs at 1,000 cells/well were seeded into ultra-low attachment round-bottom 96-well plates with or without 10% solubilized k-ECM (1 μ g/mL) and cultured for 14 to 28 days to promote 3D renal structure formation. Primary renal tubular epithelial cell (RTEC) organoids served as positive controls.

Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted using a 5 PRIME RNA Isolation Kit and cDNA synthesized using a High-Capacity cDNA Reverse Transcription Kit. Quantitative PCR was performed using TaqMan Universal PCR Master Mix and specific probes for *SYNP*, *PAX8*, *NR3C2*, and *L1CAM* on an Applied Biosystems 7300 Real-Time PCR System. Relative gene expression was calculated using the $2^{-\Delta\Delta C_T}$ method with β -actin as internal normalization (**Tables 3-4**).

Western Blotting

Proteins were extracted using RIPA lysis buffer with protease inhibitors. Protein lysates (15--20 μ g) were resolved by SDS-PAGE and transferred to PVDF membranes. Membranes were blocked with 5% nonfat milk, probed overnight at 4°C with primary antibodies against PAX2, PAX8, Claudin-1, Podocin, Synaptopodin, PEDF, BMP7, and Six1, followed by HRP-conjugated secondary antibodies. Signal detection was carried out using chemiluminescence (**Tables 3 and 4**).

Immunofluorescence and Immunohistochemistry

To investigate the renal origin of USCs, human kidney tissue and corresponding USCs were analyzed. The donor was a 43-year-old female diagnosed with a benign renal tumor (renal oncocytoma) who had no history of other renal diseases or significant comorbidities. Following surgical resection, kidney tissue was obtained from a grossly normal area of the kidney located remote from the tumor and free of tumor involvement. This normal renal tissue was used for comparative analyses with USCs. Paraffin-embedded

human kidney tissue sections (5 μ m) and cultured cells/organoids were fixed in 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, blocked, and incubated with primary antibodies overnight at 4°C. Fluorescent secondary antibodies (FITC/Texas Red) or streptavidin-HRP DAB detection systems were applied. Nuclei were counterstained with DAPI or propidium iodide (PI) and visualized under upright fluorescence/confocal microscopy (**Tables 3 and 4**).

Results

Isolation, Morphology, and Phenotypic Marker Profile of USCs

Primary cells isolated from bladder wash specimens displayed a "cobblestone" epithelial morphology typical of terminally differentiated urothelial cells (UCs). These cells failed to adhere efficiently or expand past passage 1. Conversely, urine samples from voided urine, upper urinary tract aspirates, and sex-mismatched transplant patients consistently yielded single cell clones with a "rice-grain" spindle morphology 4–6 days post-plating. These cells expanded rapidly with strong clonogenicity (**Figure 1**).

Flow cytometry revealed that USCs strongly expressed mesenchymal/stem cell markers, including CD24 (100%), CD29 (99.6%), CD44 (99.3%), CD73 (99.8%), CD90 (99.0%), CD146 (99.6%), and SSEA-4 (99.0%). They exhibited moderate expression of CD133 (21.0%) and STRO-1 (27.1%), low expression of CD105 (9.01%), and negligible expression of hematopoietic/endothelial markers CD14 (2.57%), CD31 (3.51%), CD34 (1.92%), and CD45 (1.63%) (**Table 1, Figure 2A**). Confocal microscopy confirmed that USCs co-expressed CD24 and CD133, matching the marker profile of renal progenitor cells situated

along Bowman's capsule in human kidney sections (**Figure 2B**). Furthermore, primary USCs expressed robust membrane and cytoplasmic levels of the stem cell efflux transporter ABCG2 (**Figure 3**).

Table 1. Flow Cytometry Characterization of Human Urine-Derived Stem Cells

Marker Category	Surface Markers (% Positive Cells)
<i>MSC/Progenitor Markers (>99%)</i>	CD24, CD29, CD44, CD73, CD90, CD146, SSEA-4
<i>Subpopulation Progenitor Markers</i>	CD133 (~21%), STRO-1 (~27%), CD105 (~9%)
<i>Hematopoietic/Endothelial Markers</i>	CD14 (<3%), CD31 (<4%), CD34 (<2%), CD45 (<2%)

Multilineage Multipotency:

Upon induction, USCs underwent robust osteogenic differentiation (intracellular calcium deposition confirmed by Alizarin Red S). Adipogenic induction produced modest lipid accumulation (Oil Red O) compared to adipose stem cell controls (**Figure 4**).

Confirmation of Renal Origin via Sex-Mismatched Models

To establish whether USCs originate from the upper or lower urinary tract, USCs isolated from female recipients of male donor kidneys were analyzed: i) FISH Analysis: 99% of USC interphase nuclei from female recipients exhibited distinct Y-chromosome signals (bright blue/pink) (**Figure 5A**), identical to male controls. ii) PCR Genotyping: Amelogenin PCR amplified both 102 bp (X) and 106 bp (Y) bands across all 15 USC clone samples from female recipients (**Figure 5B**), matching the male genomic profile. iii) Karyotype Analysis: Metaphase spreads confirmed a standard male 46,XY chromosomal complement in USCs isolated from female recipients (**Figure 5C**). Because the transplanted organ was the only male tissue in these female recipients, these results demonstrate that USCs originate from the donor kidney/upper urinary tract rather than the recipient's lower urinary tract.

Concordant results from FISH, amelogenin PCR, and karyotype analyses demonstrated that the isolated USCs carried male donor-specific genetic markers, confirming their renal (donor kidney) origin rather than origin from the female recipient's urinary tract (**Table 2**).

Table2. Genetic Lineage Tracing Confirms Renal Origin of Urine-Derived Stem Cells		
Method	Experimental Result	Lineage Conclusion
Fluorescence In Situ Hybridization (FISH)	Y chromosome-positive cells detected in USCs isolated from female recipients of male donor kidneys	Donor kidney origin
Amelogenin PCR	Presence of the 106-bp Y chromosome-specific amelogenin band	Donor kidney origin
Karyotyping	46, XY chromosomal complement	Donor kidney origin
Study Model: Female kidney transplant recipients receiving male donor kidneys		

Localization of USCs to Glomerular Parietal Epithelial Cells (PECs)

Gene analysis showed that uninduced USCs expressed key PEC and renal lineage markers, including PAX2, PAX8, Claudin-1, NR3C2 (mineralocorticoid receptor), and L1CAM, similar to native human kidney tissue (**Table 3**). In contrast, cultured ureteral urothelial cells (UCs) and smooth muscle cells (SMCs) lacked these markers. Uninduced USCs showed absent or minimal baseline expression of mature visceral podocyte markers (Podocin and Synaptopodin) (**Figure 6**).

Table 3: Gene and Protein Expression Profiles in nephrogenic differentiated USCs				
Gene Symbol	Full Gene Name	Encoded Protein (Full Name)	Renal Expression & Localization	Functional Role / Clinical Significance
NR3C2	Nuclear Receptor Subfamily 3 Group C Member 2	Mineralocorticoid Receptor (MR)	Epithelial cells of the renal distal nephron (particularly the cortical collecting duct)	Regulates sodium reabsorption and potassium secretion to maintain fluid and blood pressure homeostasis.

Table 3: Gene and Protein Expression Profiles in nephrogenic differentiated USCs

Gene Symbol	Full Gene Name	Encoded Protein (Full Name)	Renal Expression & Localization	Functional Role / Clinical Significance
L1CAM	L1 Cell Adhesion Molecule	L1 Cell Adhesion Molecule	Basolateral membrane of principal cells in the renal collecting system and distal tubules	Mediates cell adhesion, migration, and signaling; critical for normal kidney development.
PAX2	Paired Box 2	Paired Box Protein Pax-2	Collecting ducts of the adult kidney	Plays a key role in kidney development; re-expressed in certain adult renal structures.
PAX8	Paired Box 8	Paired Box Protein Pax-8	Proximal tubules, distal tubules, collecting ducts, and Bowman's capsule	Plays a key role in kidney development; serves as a key biomarker for renal cell carcinomas (RCC).

Monolayer and 3D Nephrogenic Differentiation Potential

Podocyte Differentiation: When cultured under podocyte-inducing conditions for 14 days, USCs changed morphologically into enlarged, binucleated cells with intricate cytoplasmic processes. Induced USCs significantly downregulated PEC markers (PAX2, PAX8, Claudin-1) while upregulating mature podocyte proteins Synaptopodin and Podocin at both mRNA and protein levels (**Figure 7**). 3D Organoid Tubulogenesis: In 3D k-ECM organoid culture, USCs organized into tubular structures over 2 to 4 weeks. Induced USC organoids strongly expressed podocyte markers (SYNP, E-Cadherin) and proximal/distal renal tubular epithelial markers Aquaporin-1 (AQP1) and Aquaporin-3 (AQP3) (**Figures 8 and 9**), approaching levels observed in native primary renal cell organoids and human kidney tissue sections.

Discussion

The biological origin of body fluid-derived stem cells remains a fundamental question that directly influences their interpretation, standardization, and clinical translation. Although human USCs have been extensively investigated for regenerative medicine, disease modeling, and drug screening applications, their precise anatomical source has remained controversial for more than a decade. Previous studies proposed that USCs may originate from bladder urothelium²², renal tubules, renal papilla, or other components of the nephron⁵, largely based on indirect marker expression and morphological observations. In the present study, we combined genetic lineage tracing, tissue immunophenotyping, molecular profiling, and functional differentiation analyses to provide compelling evidence that human USCs originate from glomerular parietal epithelial cells (PECs) located along Bowman's capsule for potential applications of patient specific USCs (**Figure 10**).

A major strength of this study is the use of sex-mismatched kidney transplantation as an *in vivo* human lineage-tracing model. Female recipients carrying male donor kidneys provide a unique opportunity to determine whether urine-derived cells originate from the transplanted kidney or from the recipient's lower urinary tract. The presence of Y chromosomes in USC clones isolated from female recipients, confirmed independently by FISH, amelogenin PCR, and conventional cytogenetic karyotyping, demonstrates that these cells originate from donor renal tissue. Because the transplanted kidney represented the only source of male cells in these recipients, the findings effectively exclude the possibility that USCs arise from recipient bladder or urethral epithelium. This genetic evidence provides perhaps the strongest support to date for an upper urinary tract and renal parenchymal origin of USCs.

Following establishment of renal origin, our histological and molecular analyses further localized the USC niche to Bowman's capsule. Among all renal and urinary tract structures examined, PECs demonstrated the distinctive CD14+/CD31- immunophenotype observed in primary USCs. In contrast, urothelial cells lacked CD146 expression, while vascular endothelial cells co-expressed CD146 and CD31. Moreover, freshly isolated USCs exhibited a molecular signature highly consistent with renal PEC progenitors,

including expression of CD24, CD133, PAX2, PAX8, Claudin-1, NR3C2, L1CAM, and ABCG2. These markers have previously been associated with renal progenitor populations residing within Bowman's capsule and have been implicated in renal homeostasis, tissue repair, and glomerular regeneration. Collectively, these phenotypic findings strongly support the conclusion that USCs represent exfoliated PEC-derived progenitor cells rather than mature epithelial cells passively shed from the urinary tract.

The identification of ABCG2 expression in USCs is particularly noteworthy. ABCG2 is a member of the ATP-binding cassette transporter family and functions as a key determinant of the "side population" phenotype associated with adult tissue stem cells. Beyond its established role in xenobiotic transport and cellular protection from toxic injury, ABCG2 has been linked to stem cell self-renewal, tissue regeneration, and resistance to oxidative stress. Its robust expression in USCs suggests that these cells possess intrinsic progenitor characteristics that may contribute to their extraordinary clonogenicity, proliferative capacity, and long-term expansion observed in culture. Because the kidney is continuously exposed to metabolic waste products and circulating toxins, expression of ABCG2 may also represent a protective adaptation that allows PEC-derived progenitors to withstand exposure to urinary metabolites during physiological shedding into urine.

Recent advances in renal developmental biology have substantially changed the understanding of PECs. Historically considered a passive structural component of Bowman's capsule, PECs are now recognized as a heterogeneous population containing resident progenitor cells capable of participating in glomerular and tubular regeneration²⁹⁻³¹. Studies in both human and animal systems have demonstrated that progenitor-like PECs located near the glomerular vascular pole can migrate and differentiate into mature podocytes³²⁻³⁵, whereas PEC populations situated closer to the tubular pole contribute to tubular epithelial renewal following injury³⁶. Therefore, PECs occupy a unique position at the interface between glomerular and tubular compartments and may serve as a reservoir for renal repair throughout life³⁷. Our functional experiments support this emerging paradigm. Under podocyte-inducing conditions, USCs underwent characteristic morphological transformation into enlarged, often binucleated cells with complex

cytoplasmic extensions resembling mature podocytes. This transition was accompanied by downregulation of progenitor-associated PEC markers and concurrent upregulation of podocyte-specific proteins, including Synaptopodin and Podocin. These findings indicate that USC_s retain developmental plasticity characteristic of authentic PEC progenitors (**Table 3**). Importantly, podocyte loss is a central pathological feature in many forms of chronic kidney disease, including focal segmental glomerulosclerosis, diabetic nephropathy, and hypertensive nephrosclerosis. Consequently, the ability of USC_s to generate podocyte-like cells may have significant implications for disease modeling and future regenerative approaches targeting glomerular injury.

In addition to podocyte differentiation, USC_s demonstrated robust nephrogenic potential within three-dimensional kidney extracellular matrix (k-ECM) organoid cultures. The use of decellularized kidney matrix provides tissue-specific biochemical and structural cues that more closely mimic the native renal microenvironment than conventional monolayer culture. Within this environment, USC-derived organoids developed organized tubular structures expressing the proximal tubular marker AQP1 and distal tubular marker AQP3, together with epithelial markers associated with functional renal tissue. These observations further support the concept that PEC-derived USC_s possess dual glomerular-tubular differentiation potential and highlight the importance of microenvironmental signals in directing renal lineage specification. The translational implications of these findings are substantial. Access to human renal progenitor cells has long been restricted by the invasive nature of kidney biopsy procedures and the limited proliferative capacity of primary renal cells. In contrast, USC_s can be obtained repeatedly from routine urine samples without discomfort, risk, anesthesia, or specialized clinical procedures. This accessibility enables longitudinal sampling from the same patient over time, creating opportunities to study disease progression, treatment responses, and dynamic molecular changes during renal injury. Such serial collection is particularly attractive for precision nephrology, where individualized cellular models may improve patient stratification and therapeutic decision-making.

The confirmation that USCs originate from renal PEC progenitors further strengthens their relevance for personalized kidney disease modeling. Patient-specific USCs may preserve donor genetic backgrounds and disease-associated molecular signatures, enabling the generation of individualized renal organoids for studying inherited nephropathies, diabetic kidney disease, glomerular disorders, and chronic kidney disease. Furthermore, because these cells are obtained directly from the urinary system, they may capture kidney-specific pathological alterations more accurately than non-renal stem cell sources. This feature could facilitate development of personalized in vitro platforms for evaluating disease mechanisms and predicting therapeutic responses.

Compared with induced pluripotent stem cell (iPSC)-derived kidney organoids, USC-derived systems possess several practical advantages. iPSC technologies have revolutionized kidney developmental modeling but require cellular reprogramming, prolonged differentiation protocols, extensive quality control, and substantial manufacturing costs. Moreover, residual pluripotent cells may introduce safety concerns related to genomic instability and tumorigenicity. In contrast, USCs are readily isolated without genetic manipulation and can be expanded rapidly while retaining renal lineage characteristics. Although USC-derived organoids do not entirely replicate the complexity of nephrogenesis achieved by pluripotent stem cell-derived organoids, their simplicity, scalability, and patient specificity make them highly attractive for translational applications where clinical feasibility is essential.

Another promising application involves drug safety assessment. Drug-induced nephrotoxicity remains one of the leading causes of compound failure during pharmaceutical development and continues to contribute substantially to adverse clinical outcomes. Existing in vitro platforms frequently fail to capture patient-specific susceptibility because they rely on immortalized cell lines or non-human experimental systems. The capacity of USCs to generate both podocyte-like and tubular epithelial-like phenotypes within nephrogenic organoids suggests that USC-based models could provide a scalable and physiologically relevant platform for assessing nephrotoxic injury, identifying protective interventions, and supporting individualized therapeutic optimization. Because USCs can be generated directly from

patients with specific genetic backgrounds and disease states, they may enable more accurate prediction of personalized drug responses than currently available models.

Collectively, our observations support a revised biological framework for USC biology that may be described as the "floating renal progenitor cell" hypothesis. Under physiological conditions, a small population of PEC-derived progenitor cells may naturally detach from Bowman's capsule and enter the urinary filtrate. These viable progenitor cells subsequently traverse the urinary tract and can be recovered from voided urine. Rather than representing cellular debris or terminally differentiated urinary epithelial cells, USCs appear to constitute a naturally occurring population of living renal progenitors that retain self-renewal capacity, clonogenicity, and multilineage differentiation potential. This concept offers a mechanistic explanation for both the renal identity and regenerative properties of USCs. Several limitations should be acknowledged. First, the study cohort was relatively small and recruited from a single institution, which may limit generalizability. Second, although the lineage-tracing results strongly support a PEC origin, single-cell transcriptomic profiling was not performed to resolve potential cellular heterogeneity within USC populations or to identify distinct progenitor subpopulations. Third, while nephrogenic differentiation was demonstrated in vitro and in organoid systems, the long-term regenerative function of USC-derived renal cells was not evaluated in vivo. Future studies incorporating single-cell RNA sequencing, spatial transcriptomics, lineage trajectory analyses, and preclinical kidney injury models will be important for further defining USC biology and validating their regenerative potential. Such investigations may also clarify whether specific USC subpopulations exhibit enhanced podocyte, tubular, or reparative capacities that could be exploited therapeutically.

Overall, the present findings provide a biologically coherent explanation for the origin, phenotype, and regenerative behavior of human urine-derived stem cells and establish glomerular PECs as their primary cellular source. This knowledge provides an important foundation for future development of USC-based technologies in precision nephrology, disease modeling, nephrotoxicity testing, and regenerative renal medicine.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) / Ethics Committee of Wake Forst University School of Medicine (Approval/Protocol Number: IRB-00033014). Written informed consent was obtained from all study participants, including healthy volunteers and kidney transplant donors/recipients, prior to urine specimen collection.

Consent for publication

Not applicable. No individual patient data or identifiable clinical details were included in this manuscript.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YZ conceived and designed the study. RW, GL, ND, YF, HL, and AF performed the experiments, including cell isolation, FISH/PCR analyses, cytogenetics, and 3D organoid cultures. YZ, RW, GL, ND, YF, HL, and AF collected, analyzed, and interpreted the data. YZ, RW, and HL wrote the initial draft of the manuscript. YZ, RW, AF, HL, DB and AA critically revised the draft for important intellectual content. All authors read and approved of the final manuscript.

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Supplementary Information

Table 4. Primary Antibodies Used for Flow Cytometry, Immunofluorescence, Western Blot, and Immunohistochemistry

Antibody Antigen	Host Species	Clonality / Fluorophore	Vendor	Catalog Number	Dilution	Validation / Application
CD14	Mouse	APC	BD Biosciences	561708	1:100	Flow Cytometry (Hematopoietic)
CD24	Mouse	FITC	BD Biosciences	555427	1:100	Flow Cytometry (USC / PEC)
CD29	Mouse	PE	BD Biosciences	555443	1:100	Flow Cytometry (MSC Marker)
CD31	Mouse	FITC	BD Biosciences	560984	1:100	Flow Cytometry (Endothelial)
CD34	Mouse	581	BD Biosciences	574093	1:100	Flow Cytometry (HSC Marker)
CD44	Mouse	PE	BD Biosciences	555479	1:100	Flow Cytometry (MSC Marker)
CD45	Mouse	PE	BD Biosciences	555483	1:100	Flow Cytometry (Hematopoietic)
CD73	Mouse	APC	BioLegend	344006	1:100	Flow Cytometry (MSC Marker)
CD90	Mouse	APC	BioLegend	328114	1:100	Flow Cytometry (MSC Marker)
CD105	Mouse	SN6h	BD Biosciences	573572	1:100	Flow Cytometry (MSC Marker)
CD117	Mouse	PE	BD Biosciences	340867	1:100	Flow Cytometry (Progenitor)
CD133	Mouse	PE	Miltenyi Biotec	130-090- 826	1:50	Flow Cytometry (PEC / EPC)
CD146	Mouse	PE	BD Biosciences	550315	1:100	Flow Cytometry (Pericyte/PEC)

Table 4. Primary Antibodies Used for Flow Cytometry, Immunofluorescence, Western Blot, and Immunohistochemistry

Antibody Antigen	Host Species	Clonality / Fluorophore	Vendor	Catalog Number	Dilution	Validation / Application
SSEA-4	Mouse	Monoclonal	R&D Systems	MAB1435 -100	1:200	Flow Cytometry (Stem Cell)
STRO-1	Mouse	Monoclonal	R&D Systems	NBP1- 48356SS	1:200	Flow Cytometry (MSC Marker)
CD24	Rabbit	Monoclonal	Abcam	ab20207 3	1:200	Immunofluorescenc e (PEC)
CD133	Rabbit	Polyclonal	Abcam	ab19898	1:200	Immunofluorescenc e (PEC)
PAX2	Rabbit	Monoclonal	Abcam	ab79389	1:200	Western Blot / IF (PEC)
PAX8	Rabbit	Polyclonal	Proteintech	10336-1- AP	1:200	Western Blot / IF (Renal)
Claudin-1	Rabbit	Polyclonal	Abcam	ab15098	1:200	Western Blot / IF (Tight Junc.)
Podocin	Rabbit	Polyclonal	Abcam	ab50339	1:200	Western Blot / IF (Podocyte)
Synapto podin	Mouse	Monoclonal	Progen	61094	1:200	Western Blot / IF / IHC
β-Actin	Mouse	Monoclonal	Sigma- Aldrich	A5441	1:500	Western Blot (Loading Control)

Abbreviations: β -Actin: Beta-actin, APC: Allophycocyanin, CD: Cluster of Differentiation, EPC: Endothelial Progenitor Cell, FITC: Fluorescein Isothiocyanate, HSC: Hematopoietic Stem Cell, IF: Immunofluorescence, IHC: Immunohistochemistry, MSC: Mesenchymal Stem Cell, PAX2 / PAX8: Paired Box Gene 2 / 8, PE: Phycoerythrin, PEC: Parietal Epithelial Cell, SSEA-4: Stage-Specific Embryonic Antigen-4, STRO-1: Stromal Precursor Cell Surface Antigen-1, USC: Urine-derived Stem Cell

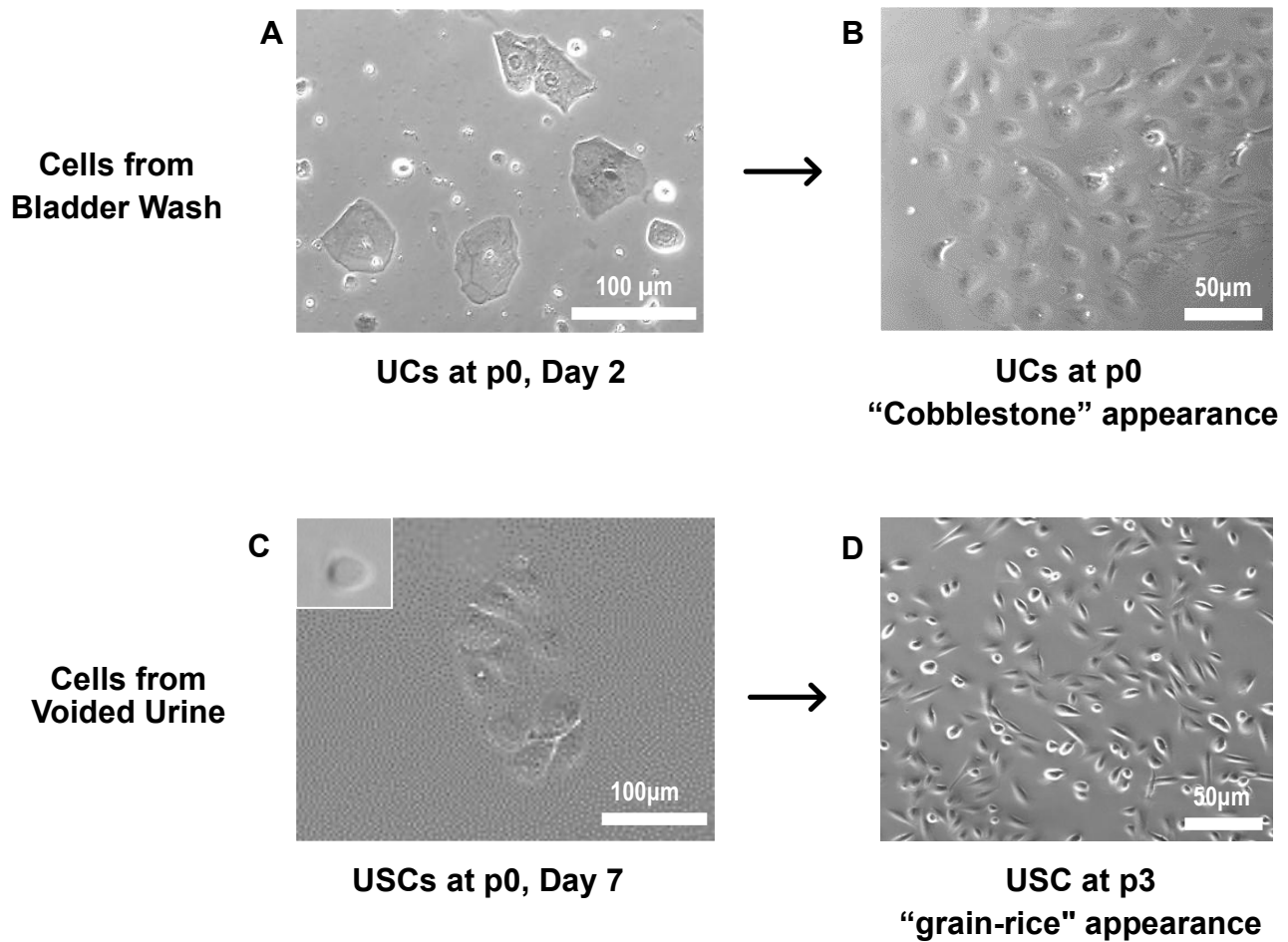
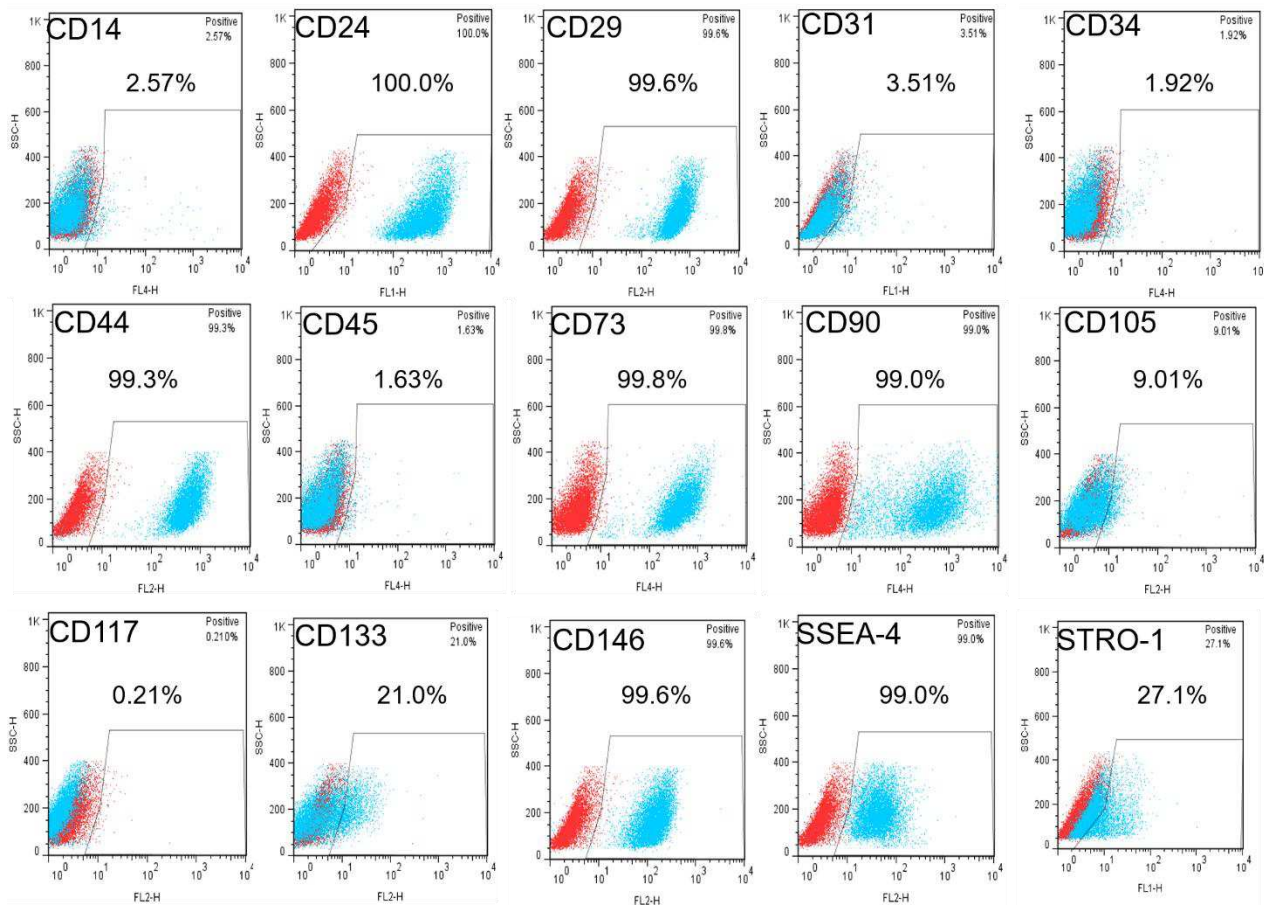


Figure 1. Morphological characterization of human urine-derived stem cells (USCs) in primary culture. Representative phase-contrast light microscopy images displaying distinct cell populations isolated from voided urine and bladder wash. (A) Urothelial cells (UCs) at passage 0 (P0) displaying a characteristic "cobblestone" epithelial morphology. (B) USC as a single cell at P0 on Day 2 post-plating. (C) USCs at P0 on Day 7, forming distinct colonies. (D) Proliferating USCs at passage 3 (P3) demonstrating a uniform "rice-grain" or spindle-like mesenchymal stem cell morphology. Differences in morphology between cells obtained from bladder wash and those from voided urine or renal pelvic aspirates suggest distinct anatomical origins. Scale bars: 100 μ m/50 μ m as indicated in individual panels.

A.



B.

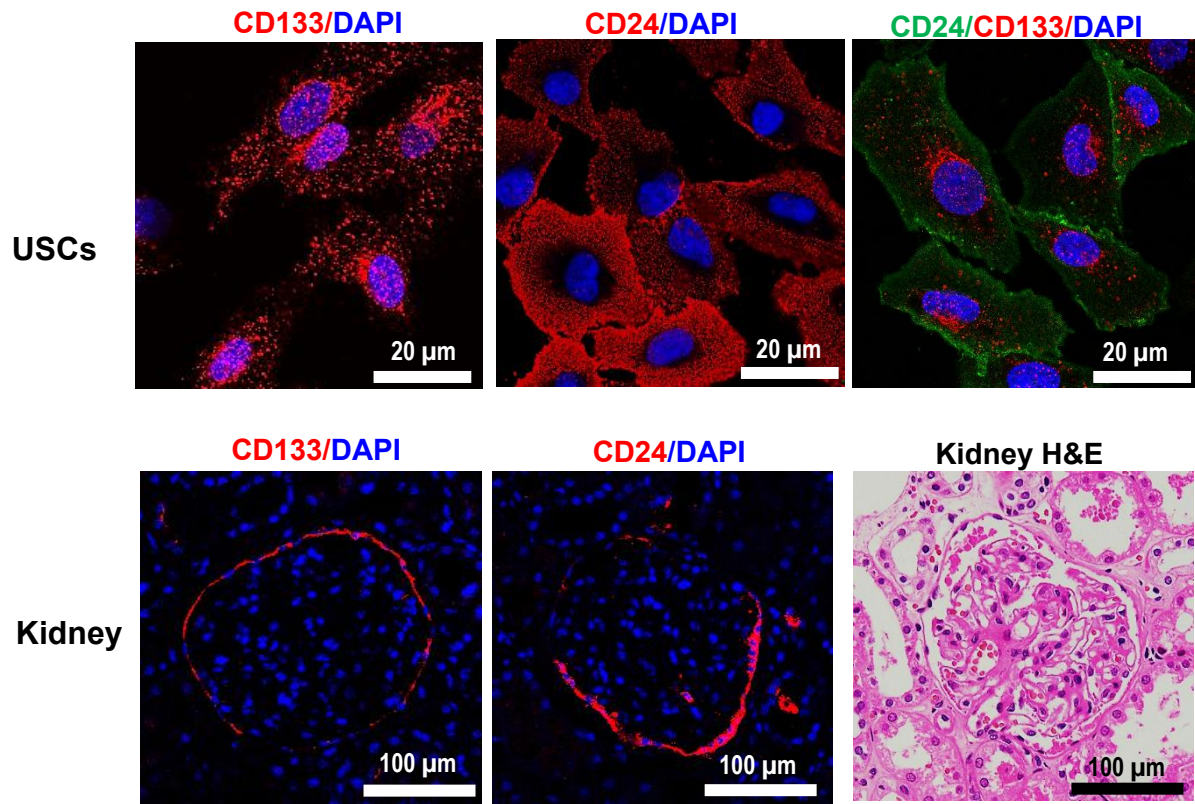


Figure 2. Immunophenotypic characterization and renal progenitor marker expression of USCs.
(A) Flow cytometric analysis (FACS) of cell surface antigen expression on USCs. USCs exhibit strong positive expression (>99%) for mesenchymal stem cell markers CD24, CD29, CD44, CD73, CD90, and SSEA-4; moderate expression (20%--30%) for progenitor markers CD133 and STRO-1; low expression (<10%) for CD105; and negative/lack of expression (<5%) for hematopoietic and endothelial lineage markers CD14, CD31, CD34, and CD45. (B) Confocal immunofluorescence double staining showing co-expression of renal progenitor cell surface markers CD24 and CD133 in cultured USCs, mirroring the expression profile of renal progenitor cells located along Bowman's capsule in human kidney tissue sections. Nuclei counterstained with DAPI (blue).

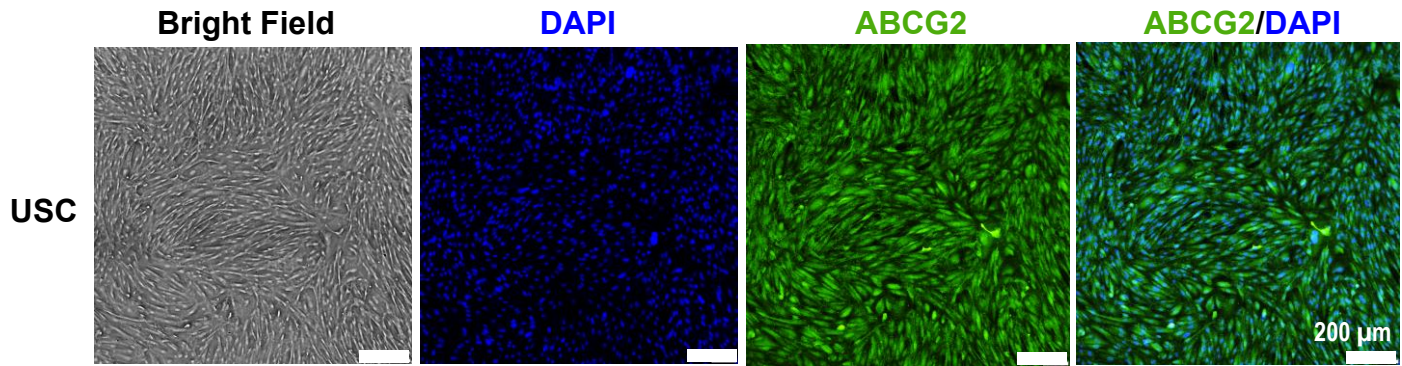


Figure 3. Expression of ABCG2 stem cell marker in USCs. Characterization of ATP-binding cassette sub-family G member 2 (ABCG2) progenitor marker expression in primary USCs at passage 3 (P3) following 10 days in culture. Immunofluorescent staining demonstrates robust expression of ABCG2 (green) localized in the cell membrane and cytoplasm. Cell nuclei are counterstained with DAPI (blue). Scale bar = 100 μm .

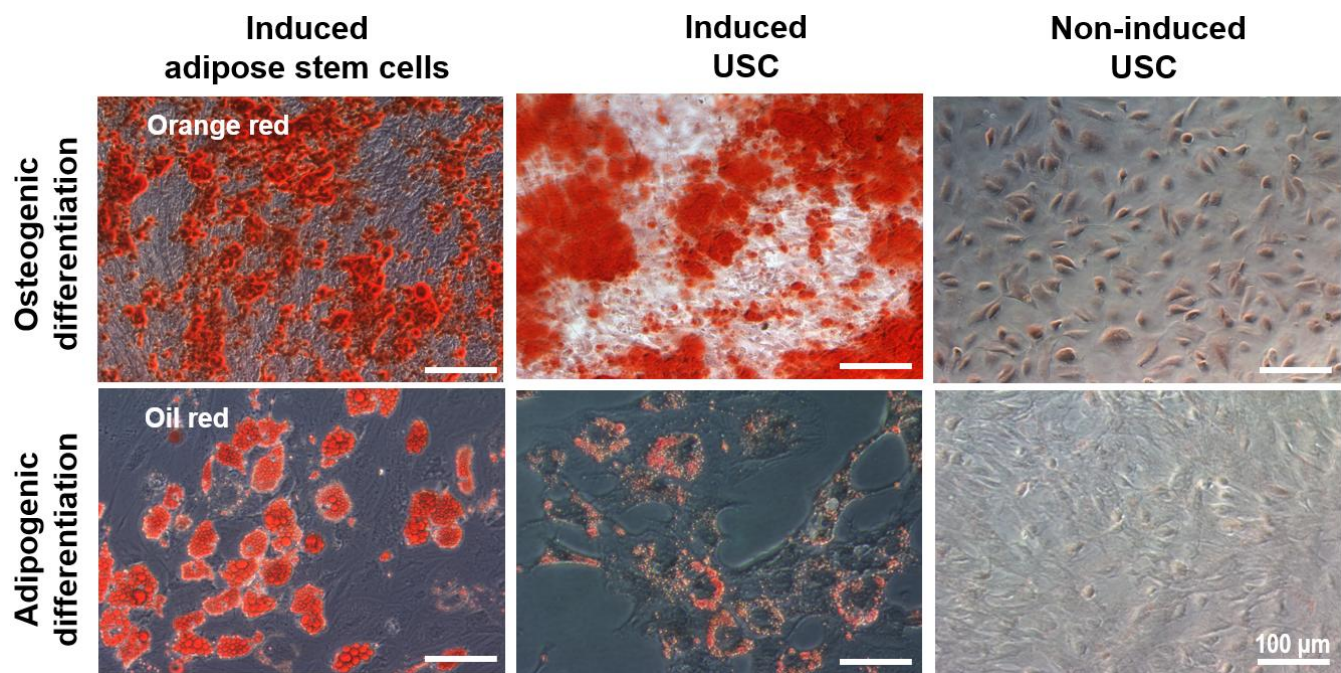
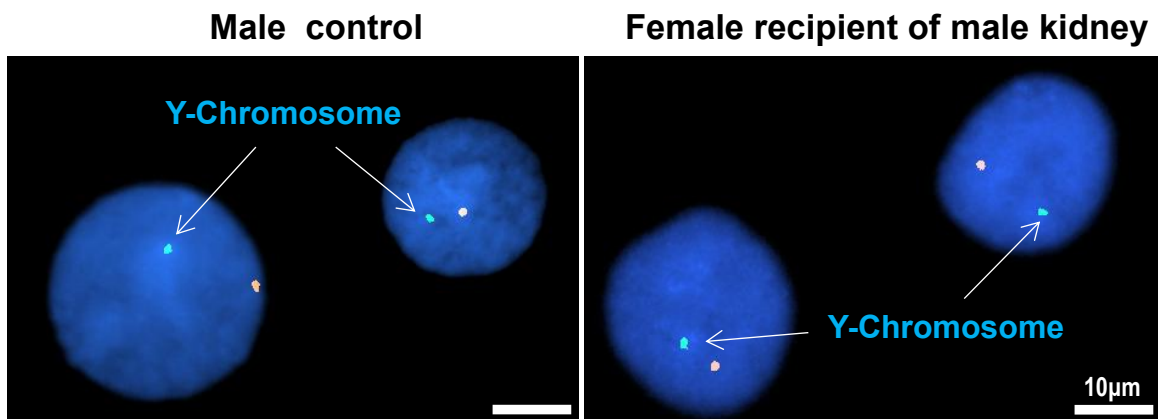


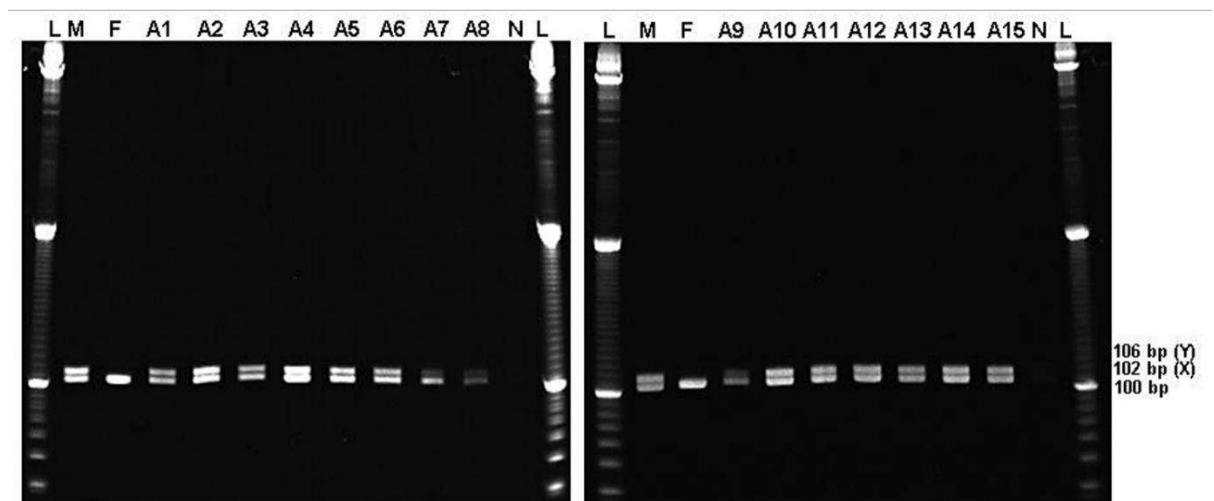
Figure 4. Multipotency and multilineage differentiation potential of USCs. Assessment of osteogenic and adipogenic differentiation potential in induced versus non-induced USCs. (Top Panels) Phase-contrast microscopy showing cell morphology under bright-field culture. (Middle Panels) Alizarin Red S staining demonstrating calcium phosphate mineralization in osteogenic-induced USCs compared to non-induced controls. (Bottom Panels) Oil Red O staining illustrating intracellular neutral lipid droplet accumulation in adipogenic-induced USCs compared to non-induced controls. Scale bar = 100 μ m.

A.



B.

X/Y Chromosome of USC for 15 kidney transplanted female patients with male denoted kidneys



C.

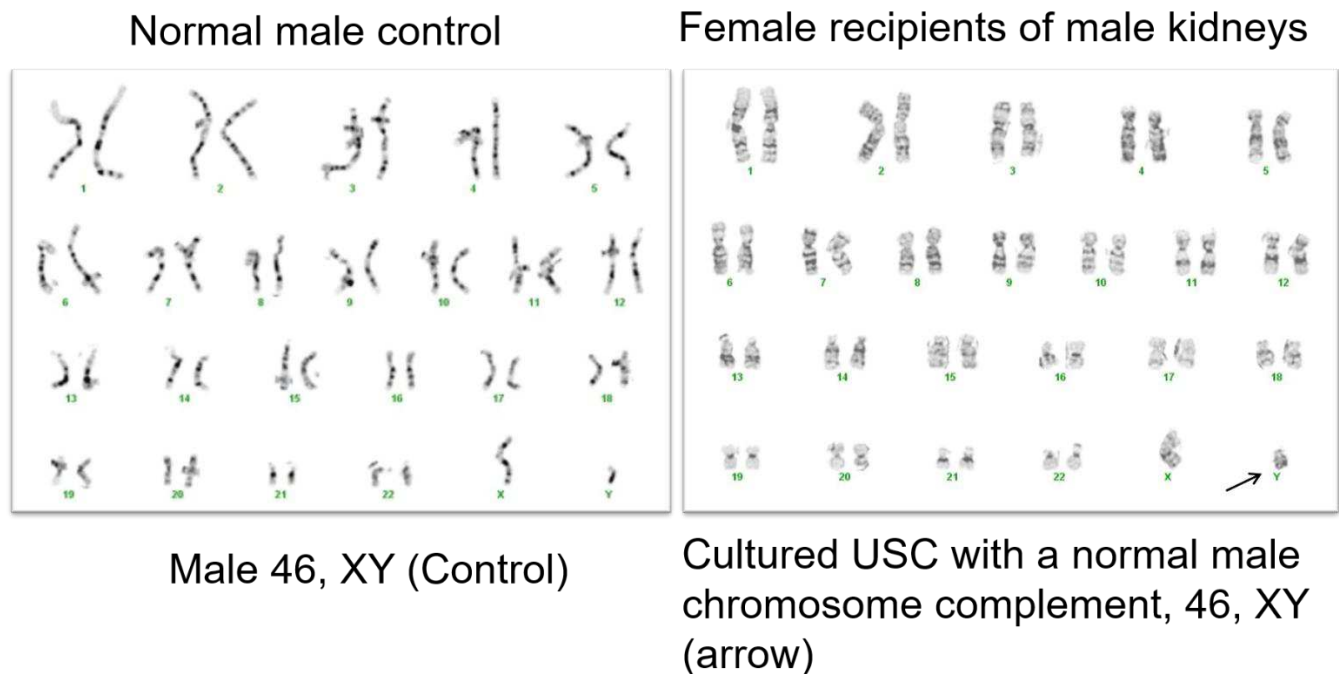


Figure 5. Determination of the anatomical origin of USCs via sex-mismatched renal transplant models. Genetic and cytogenetic origin identification of USCs collected from female recipients of male donor kidneys. (A) Fluorescence in situ hybridization (FISH) for X (pink) and Y (bright blue) chromosomes in USCs isolated from female recipients with male donor kidneys, alongside normal male and female controls. (B) Polymerase chain reaction (PCR) amplification of Y-chromosome-specific sequences confirming the donor (male) origin of USCs isolated from female transplant recipients. (C) Cytogenetic karyotype analysis of cultured USCs showing a normal male chromosome complement (46 XY).

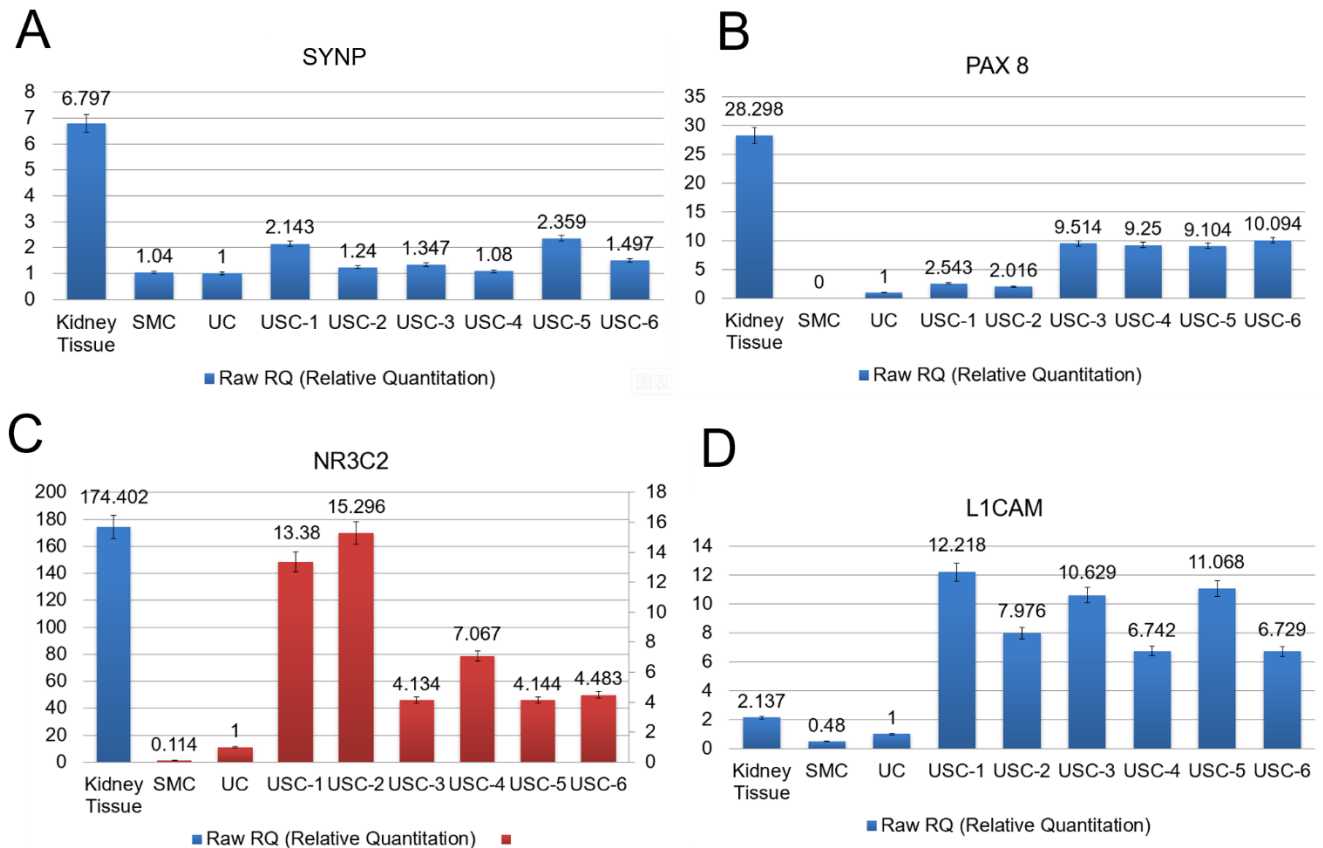
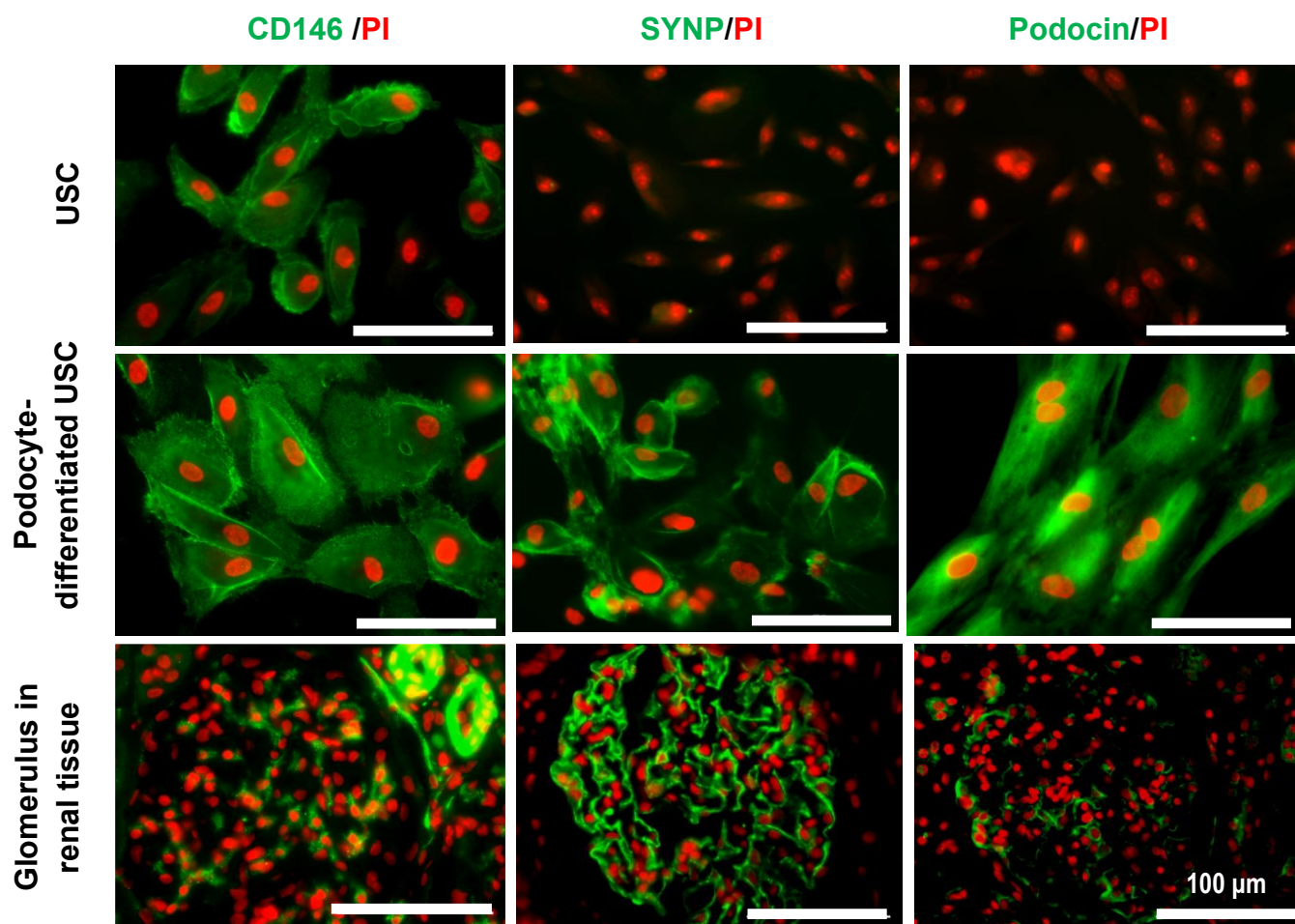
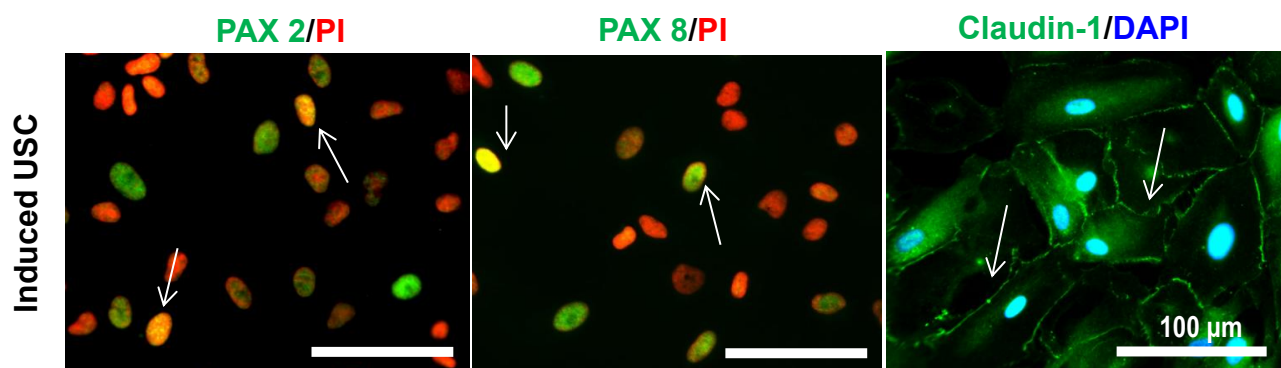


Figure 6. Expression of parietal epithelial cell (PEC) and renal-specific markers in USCs. (A) Quantitative real-time PCR (qRT-PCR) measuring mRNA fold-changes of parietal epithelial cell genes SYN (A), PAX8 (B), NR3C2 (C), and L1CAM (D) between non-induced USCs and USCs induced toward nephrogenic lineages. (B) Western blot analysis comparing protein expression levels of renal transcription factors and podocyte markers (PAX2, PAX8, Podocin, Synaptopodin [SYNP], and Claudin-1) in human kidney tissue (H-Kidney), smooth muscle cells (SMC), urothelial cells (UC), HEK293 cells, non-induced USCs, and induced USCs. β -Actin was used as a loading control. (C) Immunofluorescent staining of PAX2, PAX8, and Claudin-1 in induced USCs.

A.



B.

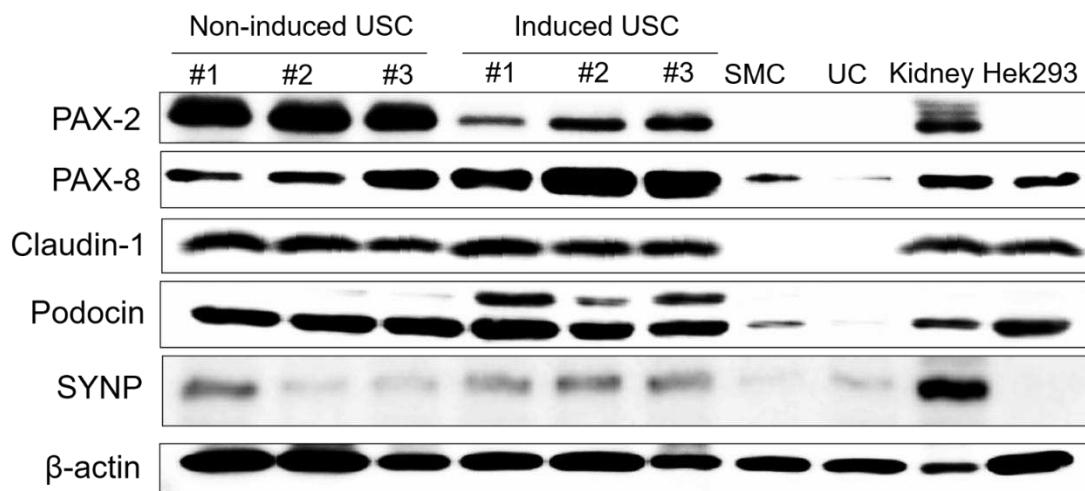


Figure 7. Expression profile of renal progenitor transcription factors and podocyte differentiation markers in USCs. (A) Confocal immunofluorescence microscopy of podocyte-differentiated USCs versus native human renal glomeruli. Differentiated USCs express key mature podocyte proteins, including PAX2, PAX8, Claudin-1, Synaptopodin (SYNP), CD146, and Podocin. Cell nuclei are counterstained with propidium iodide (PI, red) or DAPI (blue). (B) Representative Western blot analysis demonstrating the expression of renal epithelial markers in USCs, differentiated USCs, and primary renal cells. Protein expression patterns indicate enhanced renal differentiation in induced USC organoids, with expression levels approaching those observed in primary renal cell-derived cells. The bottom panel serves as the loading control.

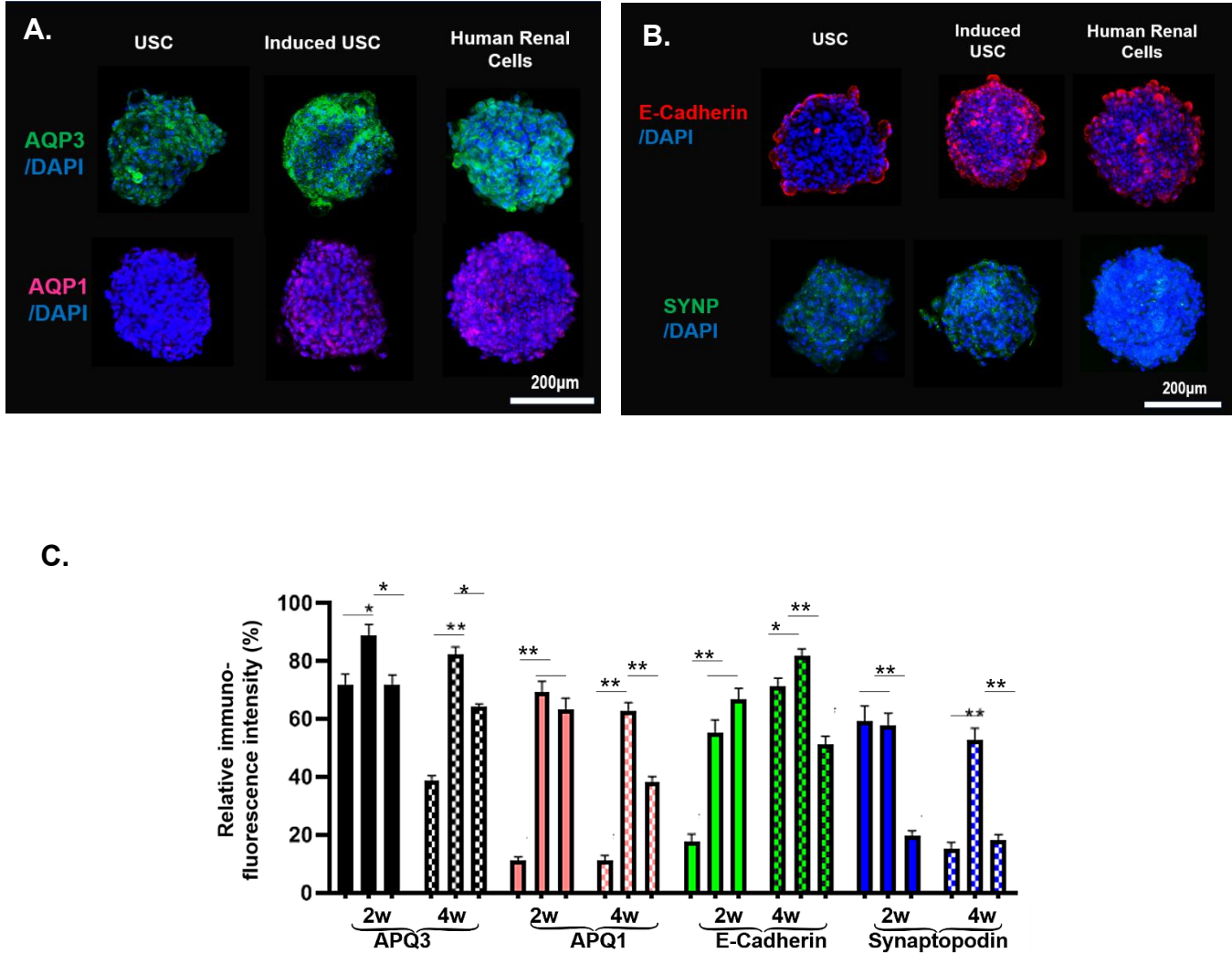


Figure 8. 3D organoid formation and reno-differentiation of USCs into tubular epithelial and podocyte lineages. (A) Immunofluorescence staining of 3D USC organoids (4×10^3 cells/organoid", P3) at 2 weeks post-induction with triple growth factors, evaluating kidney tubular epithelial markers Aquaporin-1 (AQP1) and Aquaporin-3 (AQP3) compared to native human renal cells and uninduced USCs. Scale bar = 100 µm. (B) Expression of podocyte-specific biomarkers E-Cadherin and Synaptopodin (SYNP) in 3D USC organoids following 2 weeks of renal induction. Scale bar = 100 µm. (C) Semi-quantitative analysis of the fluorescence intensity of AQP3, AQP1, E-cadherin, and Synaptopodin in organoids derived from non-induced USCs (1st bar), differentiated USCs (2nd bar), and primary renal cells (3rd bar). Each 3-bar set represents the three cell sources within a given time point (2 or 4 weeks) and antibody group.

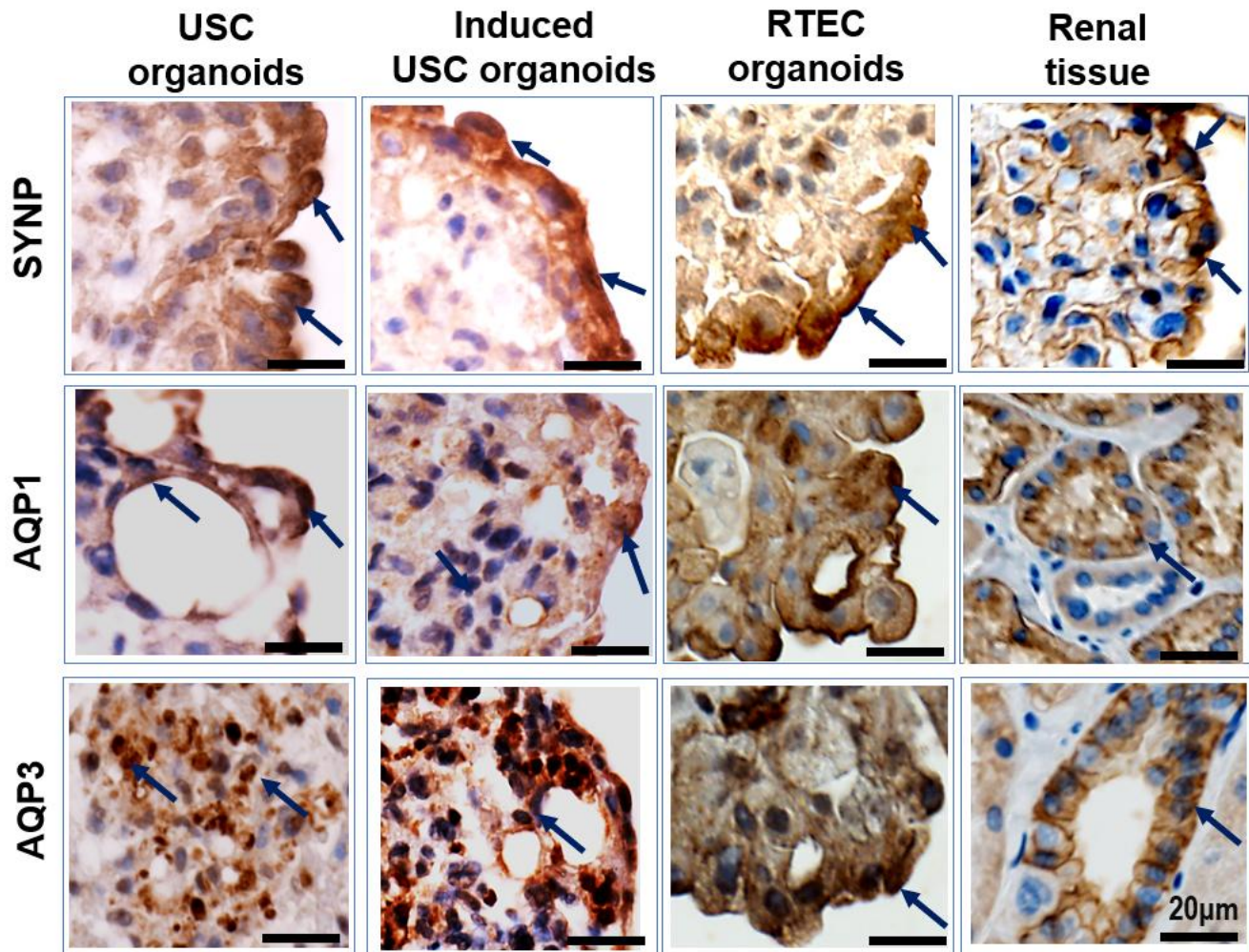


Figure 9. Immunohistochemical expression of renal epithelial markers in 3D tissue organoids. Comparative immunohistochemical (IHC) staining of Synaptopodin (SYNP), Aquaporin-1 (AQP1), and Aquaporin-3 (AQP3) in non-induced USC organoids, reno-induced USC organoids, renal tubular epithelial cell (RTEC) organoids, and native human renal tissue sections. Induced USC organoids exhibit spatial expression profiles comparable to native kidney tissue. Scale bar = 20 μ m.

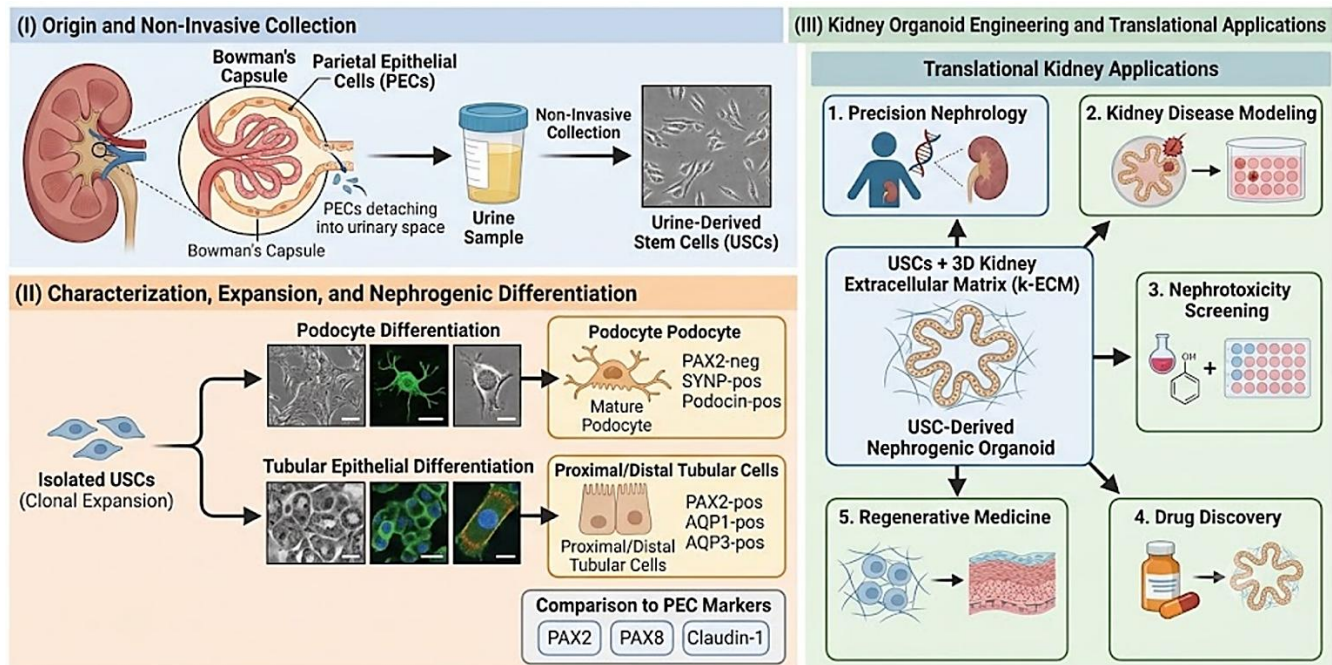


Figure 10. Proposed cellular origin, renal differentiation hierarchy, and potential applications of human USC. Parietal epithelial cells (PECs) located along Bowman's capsule detach physiologically into the urinary filtrate and are excreted in urine. Following non-invasive urine collection, USCs can be isolated, clonally expanded, and differentiated into renal lineages including podocytes and tubular epithelial cells. In three-dimensional kidney extracellular matrix (k-ECM) environments, USCs generate nephrogenic organoids that recapitulate key renal cellular phenotypes. The accessibility, patient specificity, and nephrogenic potential of USCs support their applications in precision nephrology, kidney disease modeling, nephrotoxicity screening, drug discovery, and regenerative medicine.

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