Development of a microphysiological model of human kidney proximal tubule function

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The kidney proximal tubule is the primary site in the nephron for excretion of waste products through a combination of active uptake and secretory processes and is also a primary target of drug-induced nephrotoxicity. Here, we describe the development and functional characterization of a 3-dimensional flow-directed human kidney proximal tubule microphysiological system. The system replicates the polarity of the proximal tubule, expresses appropriate marker proteins, exhibits biochemical and synthetic activities, as well as secretory and reabsorptive processes associated with proximal tubule function *in vivo*. This microphysiological system can serve as an ideal platform for *ex vivo* modeling of renal drug clearance and drug-induced nephrotoxicity. Additionally, this novel system can be used for preclinical screening of new chemical compounds prior to initiating human clinical trials.

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Several publically funded initiatives now seek to drive the development of innovative, human cell-derived preclinical models that would accelerate the drug development process, shorten the time it takes to move a new molecular entity into early clinical trials, and reduce the excessively high failure rate of clinical trials. Our goal is to apply flow-directed microphysiological technologies to model the physiological functions of the human kidney proximal tubule, as it plays a vital role in active secretory and reabsorptive transport of drug molecules and is a primary site of drug-induced nephrotoxicity due to these concentrative processes.[1] Whereas existing cell culture and animal models of proximal tubular function have utility, there are serious limitations stemming from functional deficits of conventional cell culture systems and differing physiology between animal models and humans.

In this report, we describe the development of a 3-dimensional (3D) microphysiological system (MPS) of the human proximal tubule. The kidney tubule MPS exhibits long-term viability, retains polarized expression and function of proteins essential for reabsorptive and secretory transport, responds to physiological stimuli, and performs critical biochemical synthetic activities.

RESULTS

Structural recapitulation of the proximal tubule microenvironment

The stepwise construction of a microphysiological model of human proximal tubule is presented in Figure 1a*i* to *iv*. Human primary proximal tubular epithelial cells (PTECs) were grown in monolayer cultures following isolation from renal cortical tissue. After about 7 days in culture, the cell monolayers displayed a uniform cobblestone-like appearance with dome formation that is characteristic of PTECs (Figure 1a*ii*).[2,3] Following seeding into the MPS, PTECs adhered to the channel surface and grew to form a tubule-like structure (Figure 1a*iv*); its physical dimension is close to that reported for the proximal portion of the renal tubule in the human kidney (i.e., 6 mm long × 120 μm thick in the MPS compared with 14 mm long × 40 μm thick *in vivo*).[4] In its present format, the MPS holds approximately 5000 PTECs. Microfluidic technology allowed media perfusion, which

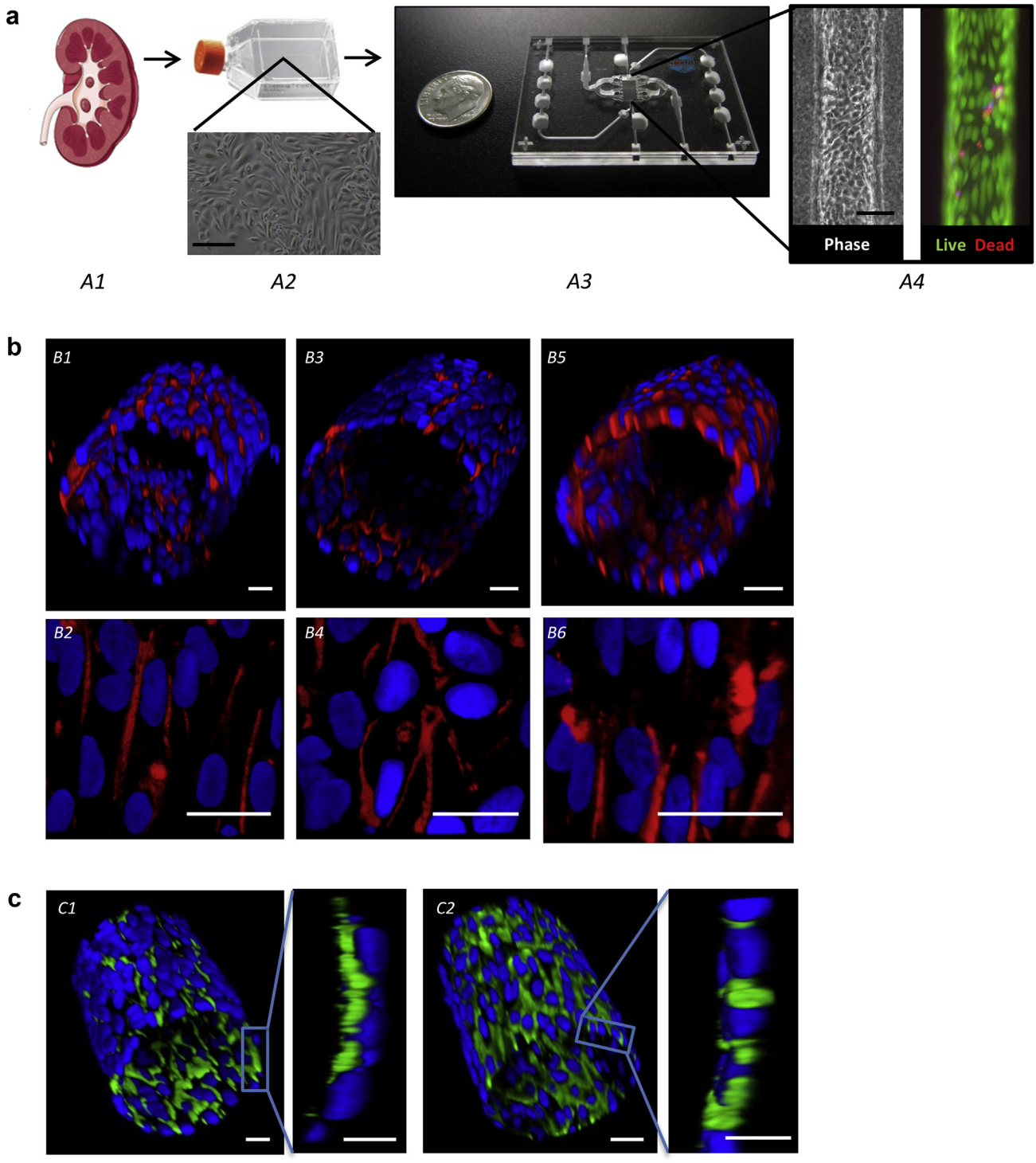

Figure 1 | PTEC viability and basic functionality in human kidney 3D MPS. (**a**) Scheme depicting construction of human proximal tubular epithelial cells (PTECs) in a microphysiological system (MPS). (*A1*) Cell isolation from human kidney cortex. (*A2*) Cell culture in 2 dimensions. (*A3*) Cell seeding and culture in 3-dimensional (3D) MPS. (*A4*) Phase contrast and viability of PTECs in MPS at day 28. (**b**) A 3D projection of MPS matrix shows PTEC tubule structure: (*B1*, *2*) surface expression of epithelial cell marker CD13 (red) (original magnification ×400); (*B3*, *4*) cell self-assembly confirmed by E-cadherin expression (red) (original magnification ×400); (*B5*, *6*) proximal tubule origin confirmed by expression of aquaporin 1 (red) (original magnification ×400). (**c**) Polarization confirmed by (*C1*) tight junction formation via apical localization of ZO-1 (green) and (*C2*) basolateral expression of Na+/K+ adenosine triphosphatase (green). Tubule diameter is ~120 μm. Bars = (**a***ii*) 200 μM; (**a***iv*) 50 μM; (**b**,**c**) 20 μM.

exposed PTECs to fluid shear force at the apical surface and delivered nutrients continuously under normoxic conditions (Figure 1a*iii*).[5,6] PTEC in 3D culture exhibited excellent viability (>95%) for up to 4 weeks, as demonstrated by extensive green fluorescent calcein acetoxymethyl signal in live cells with minimal red fluorescent signal indicative of