Supporting Information

High-Throughput Microfluidic Spheroid Technology for Early Detection of Colistin-Induced Nephrotoxicity with Gradient-Based Analysis

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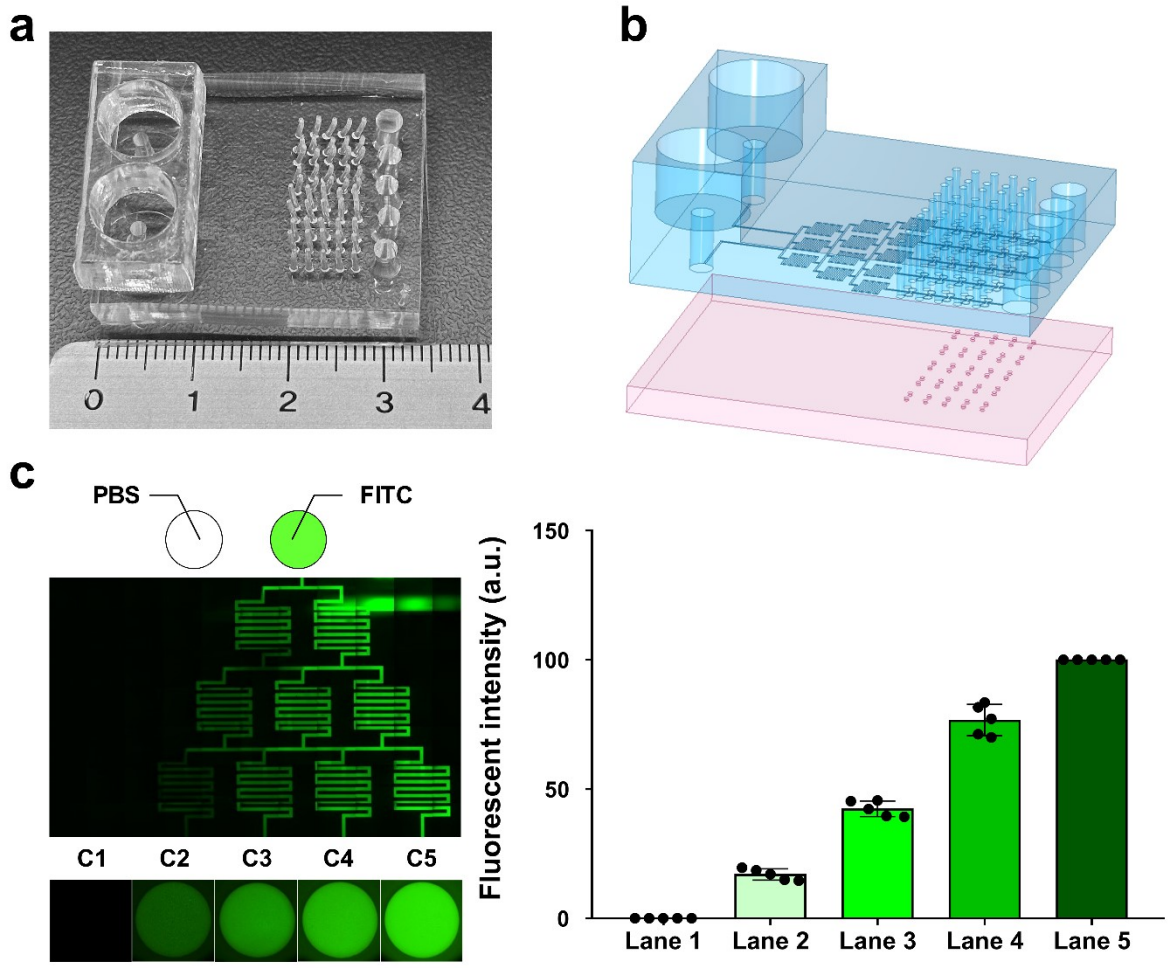

Figure S1. Design of spheroid nephrotoxicity assessing platform (SNAP). (a) View of the SNAP. (b) Schematic showing two layers in the SNAP. (c) Concentration gradient of fluorescein isothiocyanate (FITC) in the SNAP.

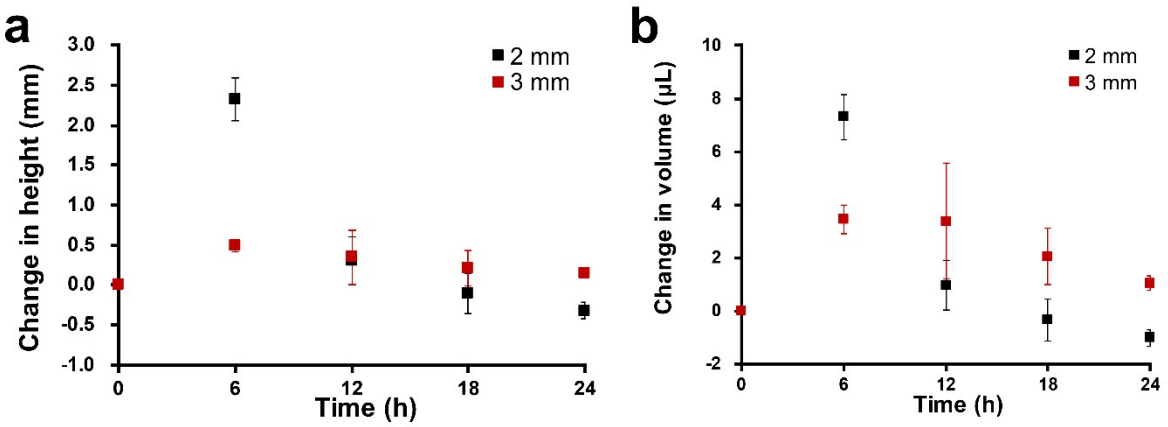

Figure S2. Comparison of flow rate in SNAP depending on the outlet size. Change of (a) liquid height and (b) liquid volume in outlet after introducing liquid into the inlets.

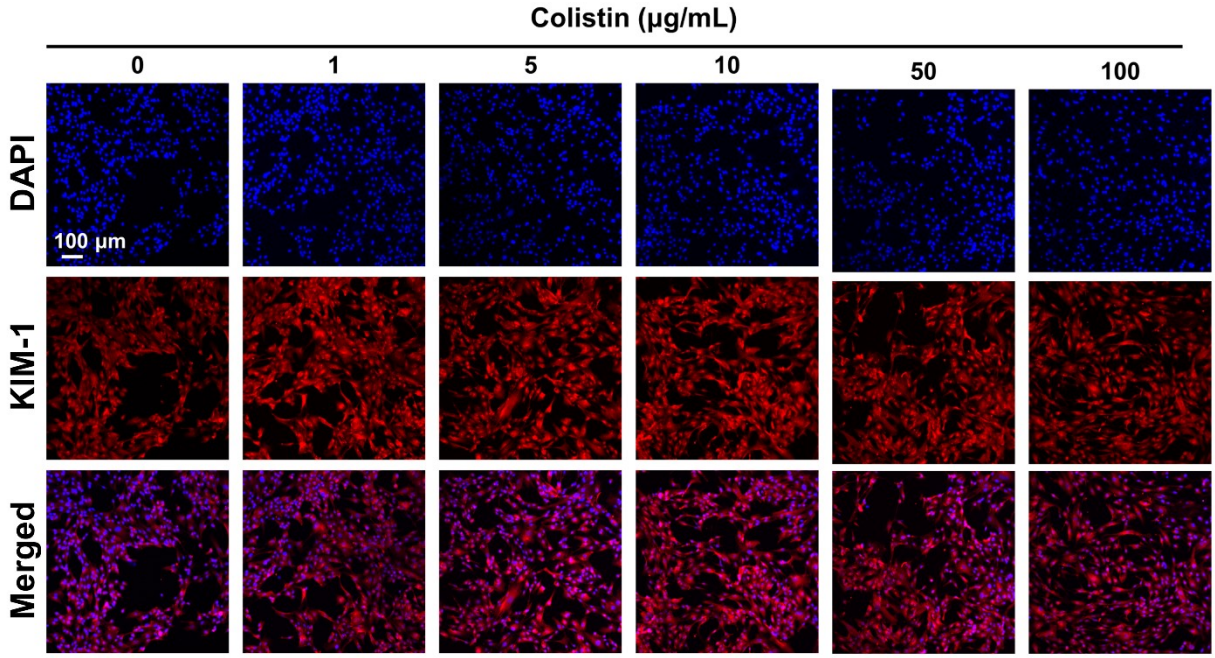

Figure S3. Kidney injury marker -1 (KIM-1) expression after treating colistin on renal proximal tubular epithelial cells (RPTECs) in 2D culture.

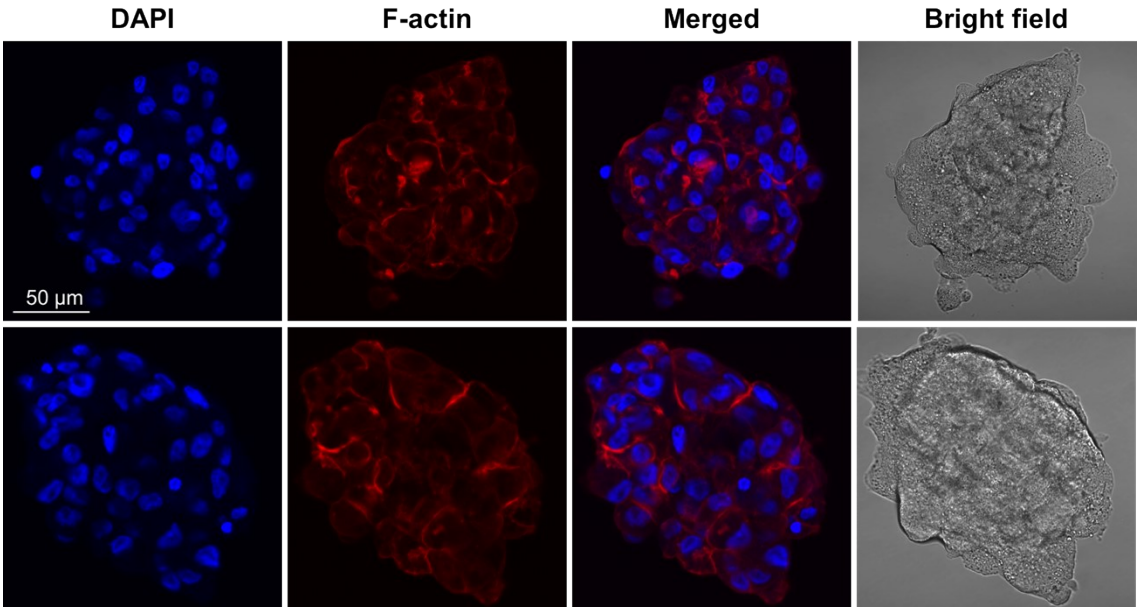

Figure S4. Expression of Filamentous actin (F-actin) from RPTEC spheroid. The spheroids were collected, fixed, and permeabilized following the procedures described in the main text. Acti-stain™ 555 fluorescent phalloidin (Cytoskeleton, Inc.) was used to stain F-actin according to the manufacturer's instructions. DNA was stained using 4’,6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich).

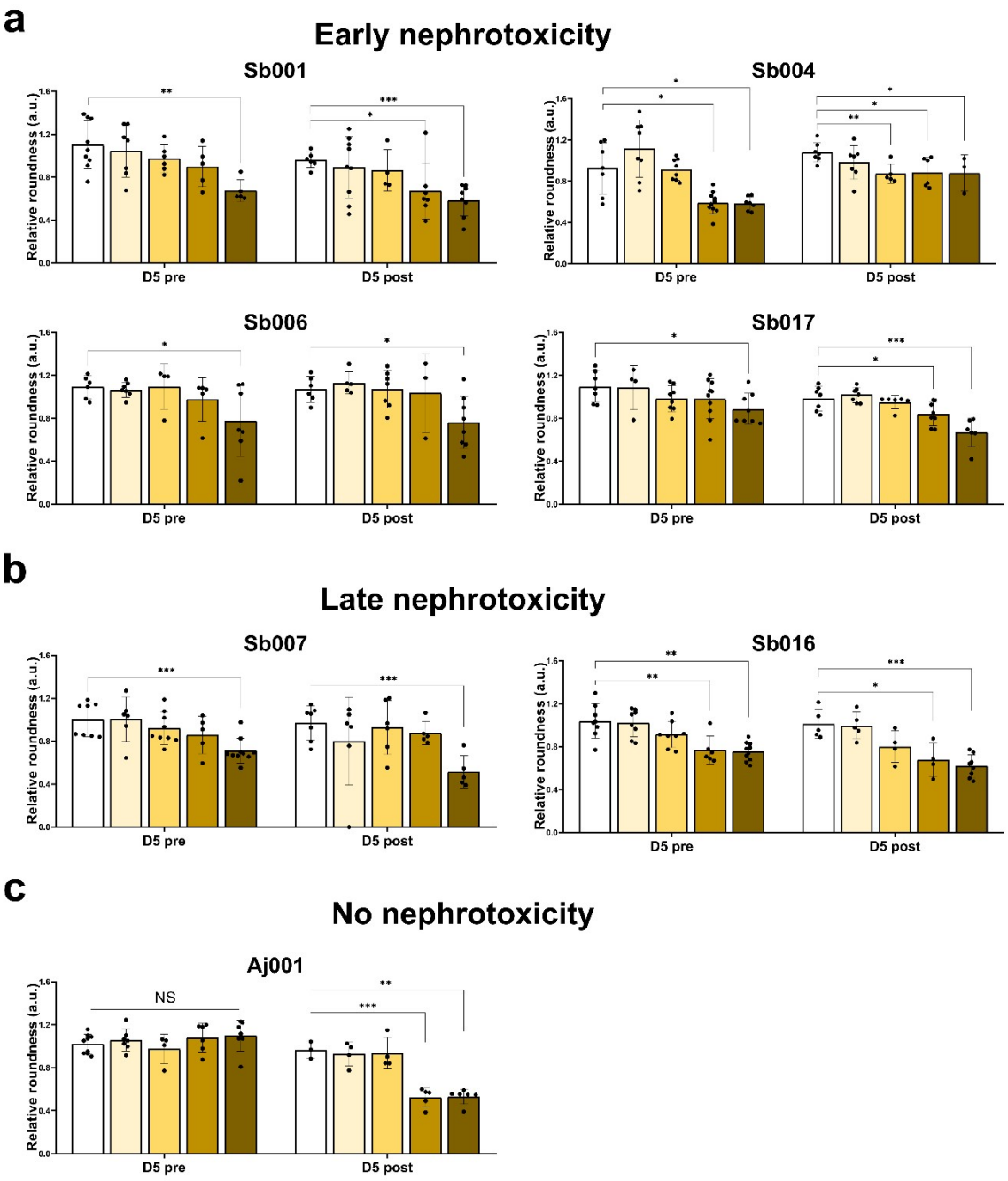

Figure S5. Nephrotoxicity in microfluidic after treating plasma from colistin-administered patients. Change in roundness of RPTEC spheroid after treating plasma from (a) patient showed early nephrotoxicity, (b) patient showed late nephrotoxicity, or (c) patient did not show nephrotoxicity (n>3, Student's t-test: NS $P > 0.05$, *$P < 0.05$, * $P < 0.01$, ***$P < 0.001$).

Patient	Age / Sex	Underlying disease	Primary site of infection	Causative microorganism	Duration of colistin treatment (Day)	In-hospital mortality	Colistin level (μg/mL)		AKI Observed point (Day of colistin administration)		Additional nephrotoxic drug
							pre	Post			
Sb001	78/M	Hypertension, solid cancer	Respiratory tract	CRAB	11	Yes	4.81	6.57	D5	Plasma collection day	Diuretics
Sb004	92/M	Hematologic disease	Respiratory tract	CRAB	5	Yes	5.04	9.17	D5		(-)
Sb005	86/F	Hypertension, cardiovascular disease	CRBSI	CRE	14	No	5.51	15.36	D5		(-)
Sb006	58/F	Diabetes mellitus, solid cancer	Hepatobiliary tract	CRPA, CRE	17	No	1.35	6.13	D5		Diuretics
Sb017	45/M	Lung disease, cardiovascular disease, hematologic disease	Primary bacteremia	CRPA	15	No	4.13	10.79	D6		Tacrolimus
Sb007	76/M	Solid cancer, hematologic disease	Complicated intra-abdominal infection	CRE	9	No	1.16	7.78	D11	**5 day** after plasma collection	(-)
Sb008	73/M	Hypertension, diabetes mellitus, cardiovascular disease	Respiratory tract	CRPA	14	No	3.73	10.23	D9	**4 day** after plasma collection	(-)
Sb016	51/M	Diabetes mellitus, kidney disease, solid cancer, hematologic disease	Genitourinary tract, CRBSI	CRPA	8	No	3.93	7.39	D12	**6 day** after plasma collection	Acyclovir
Sb015	71/M	Diabetes mellitus, liver disease, solid cancer	Hepatobiliary tract	CRE	14	No	3.95	6.23	(-)		(-)
Aj001	47/F	Lung disease, cardiovascular disease	Respiratory tract	CRAB	9	No	3.08	3.36	(-)		Diuretics

Table S1. Patient characteristics from which plasma used in SNAP analysis was obtained

Abbreviations: CRBSI, catheter related bloodstream infection; CRAB, carbapenem-resistant *Acinetobacter baumannii*; CRE, carbapenem-resistant Enterobacteriaceae; CRPA, carbapenem-resistant *Pseudomonas aeruginosa;* AKI, acute kidney injury.