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Supplementary information

Biomimetic models of the glomerulus

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Supplementary table 1: Main characteristics of the glomerular biomimicry approaches

	Source	Type	Signature phenotypic markers/ staining	Morphology, apicobasal polarity, slit diaphragm/size-selectivity	GBM components	Mimicked	Strong points	Use and convenience
GLOMERULUS ON-A-CHIP								
Petrosyan *et al.* [1][1]	Human primary	Podocytes	- NPHS-1 - F-actin		- COL4A4, COL4A12 - Laminin α5 (LAMA5)	- GFB	- Natural GBM synthesis for achieving size-selectivity - Determination of most suitable podocyte source - Mimicry of toxicity and proteinuria	- Disease modeling - Toxicity screening - Selection of most suitable cell source for human mature podocytes
	Human immortalized	Podocytes						
	Human amniotic fluid-derived	Podocytes						
	Primary human	Glomerular endothelial cells	- CD31					
Musah *et al.* [2][2]	hPSC-derived	Podocytes	- NPHS-1, NPHS-2 - WT1 - PAX2			- GFB - Synthetic (PDMS) membrane	- Improvement of differentiation/maturation hPSCs to podocytes with flow - Mimicry of toxicity and proteinuria	-Disease modeling -Toxicity screening -Avoidance of de-differentiation
	Primary human	Glomerular endothelial cells ACBRI-128	- VE-cadherin					
Wang *et al.* [3][3]	Mice whole glomeruli	Podocytes	- SYNPO			- GFB	- Synthesis of natural GBM - Mimicry of diabetic glomerulus	- Disease modeling
		Glomerular endothelial cells	- CD31					
Xie *et al*. [4][4]	Mice	Podocytes E11	- NPHS-1, NPHS-2 - F-actin			- GBF (early stages of kidney development)	- Proof of the importance of spatial cues in glomerular filtration - Mimicry of 3D architecture in a microfluidic system for relevant native characteristics	- Combination of 3D characteristics and microfluidics
	Lewis rat primary vein	Endothelial cells	- VE-cadherin					
Zhou *et al*. [5][5]	Mice	Podocytes MPC-5	- NPHS-1, NPHS-2 - F-actin - SYNPO			- GFB - Synthetic (PC) membrane	- *In vitro* GFB model - Mimicry of healthy and diseased stages (hypertension)	- Disease modeling - Toxicity screening
	Mice	Glomerular endothelial cells	- CD31 - von Willebrand Factor (vWF)					
MEMBRANES AND 3D SCAFFOLDS FOR GLOMERULAR MODELING								
Slater *et al.* [6][6]	Human conditioallly immortalized	Podocytes	- NPHS-2				- Constructs that mimic the GBM with electrospun collagen	- Research on biomaterials and manufacturing techniques for GMB membranes

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	Human conditionally immortalized	Glomerular endothelial cells	- PECAM-1					
Li *et al.* [7][7]	Human conditionally immortalized	Podocytes	- NPHS-2		- Collagen IV - Fibronectin (cell-ECM atttachment)		- Constructs that mimic the GBM with dECM from renal tissue - Presence of VE-cadherin supports GFB in co-culture	
	Human conditionally immortalized	Glomerular endothelial cells	- VE-cadherin					
Tuffin *et al.* [8][8]	Human conditionally immortalized	Podocytes	- PODOXL - WT-1		- Collagen IV		- Assembly of glomerular structures within a hydrogel	
	Human conditionally immortalized	Glomerular endothelial cells	- PECAM-1					
Waters *et al*. [9][9]	Human primary	Podocytes	- NPHS-1, NPHS-2 - WT1 - SYNPO		- COL1α1, COL4α1		- Tri-culture of the three main glomerular cell-types within a hydrogel	- Research on biomaterials for glomerular scaffolds
	Human immortalized	Glomerular endothelial cells	- CD31 - vWF					
	Human immortalized	Mesangial	- Actin					
Lu *et al.* [10][10]	Nenonatal rat	Renal cells	- Flk-1				- Assembly of glomerular structures within a hydrogel	
Wang *et al.* [11][11]	Mice	Glomerular endothelial cells			- α-actin - Integrin ß-1 - Fibronectin		- Alternative manufacturing process for GBM	
	Mice	Mesangial	- Thy-1					
Pullela *et al*. [12][12]	No cells used						- Membrane with size selectivity	- Research on biomaterials for GMB membranes
Du *et al.* [13][13]	hPSCs	Renal progenitors	- PAX2 - CD31 - NPHS2				- Scaffolds made of kidney dECM	- Research on biomaterials for glomerular scaffolds
ORGANOIDS								
Hale *et al.* [14][14]	hPSCs	Podocytes	- NPHS-1, NPHS-2 -PODXL - SYNPO - F-actin	- Primary and secondary foot processes - Bowman's capsule	- LAMα5, ß2, γ1 - Collagen IV α1, α2 in the GBM, α3, α4 appeared in low amounts, α5α6 in	- Podocyte organoids - *In vitro* CNS models - Toxicity screening	- Exclusive inclusion of podocytes in glomerular tissue organoids (although other cell markers were found) - Detailed characterization of the GBM - CNS models	- Disease modeling - Glomeruli toxicity screening

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			Other non-podocyte genes were found, including - Endothelial cell markers (PECAM) - Mesangial markers (PDGF-ß, MMP2)	- Nephrin in the apical side of the cells (as in slit diaphragms)	the Bowman's capsule, COL4A3 and COL4A4 genes were also found - Nidogen-1/2 - Heparan sulphate			
Higgins *et al.* [15][15]	hPSCs	Podocytes	- MAFB - NPHS-1 - PODXL		- Laminin	- Kidney organoids	- Increased repeatability and throughput to create organoids by 3D bioprinting	- Toxicity screening
		Endothelial cells	- CD31					
Homan *et al*. [16][16]	hPSCs	Podocytes	- NPHS-1 - PODXL - SYNPO	- Foot processes - Capillary loops		-Vascularized glomerulus and proximal tubule	- Achievement of vascularization and maturation without relying on a host	- Potential connection to the host of vascular networks organoids cultured in flow conditions
		Endothelial cells	- PECAM1 - CD31					
		Pericyte precursors	- PDGF-ß					
Bantounas *et al.* [17][17]	hPSCs	Podocytes	- WT1 - NPHS-1 - PODXL - SYNPO	- Slit diaphragsm prominent on basal aspect of podocytes	- Collagen IV (immature)	- Kidney organoids (following Takasato *et al.* (2015) protocol) - Glomerular structures (capillary tufts were not found)	- Feeder-free culture of kidney organoids - Obtention of mature nephron from implanted kidney progenitor cells	- Regenerative purposes of implanted differentiated hPSCs
		Glomerular endothelial cells	- CD31 - PECAM1 (but negative glomerular tuft)					
Sharmin *et al.* [18][18]	hPSCs	Podocytes	- WT1 - NPHS1, NPSH2 - PODOXL - SYNPO	- Apical-to-basal polarity - Primary foot processes connected by immature slit diaphragms - Bowman's capsule surrounding glomeruli	- Collagen IV	- Invading blood vessels arrange themselves into vascularized glomeruli	- First report of glomerular vascularization upon murine host implantation[17] - New technique for organoid implantation (VEGF-soaked agarose rods to accelerate vasculogenesis)	- Proposed new organoid implantation method

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van den Berg *et al.* [19][19]	hPSCs	Podocytes	- WT1 - NPHS-1	- Putative Bowman's capsule - Aligned podocytes with small immature foot processes which become mature upon implantation - Apical-to-basal migration of tight junctions - Capillary loop was not evident - Prolonged culture led to endothelial cell loss (vascular gene expression decreased) - Membrane selectivity up to 2,000 kDa	- Not found in *in vitro* culture. Tri-layer present after implantation	-Vascularized nephron with structurally and mature glomerulus	- Feeder-free culture of kidney organoids - Presence of functional vascularization and more mature characteristics upon transplantation on a murine host	- Inclusion of mature glomerulus in an implantable organoid
		Endothelial cells	- CD31					
		Pericytes	- PDGF-ß					
Low *et al.* [20][20]	hPSCs	Podocytes	- WT1 - NPHS-1 - PODXL	- Localized slit diaphragms - Membrane selectivity up to 70 kDa (dextran uptake assay) - Bowman's capsule space - Perfusable capillary tufts - Developed podocytic processes		- Multi-segmented nephron obtained from hPSCs and patient-derived iPSCs for ARPKD modeling	- Wnt fine-tuning to control the relative proportion between segmented sections - Murine hosts implantation that enhances glomerular maturation and organoid vascularization	- Regenerative purposes - Toxicity screening
		Endothelial cells	- CD31 - PECAM1					
		Mesangial population (after implantation)	- PDGF-ß					

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