

## **Renal Cell Culture**

Most undergraduate courses now have a generic cell culture component that outlines the basics of the technique and highlights some of the fundamental mistakes. This article will not cover every aspect of the procedure but aims to help with the specifics of growing renal cells *ex vivo*. A lot of this information has come from members of the Renal Association Scientist Working Group so if you have any questions please get in touch with any of us, our contact details are on the website.

### **General**

Renal tissue is predominantly obtained from the normal pole of kidneys removed for neoplasia or those deemed unsuitable for transplant. The age of the patient supplying the tissue influences the rate at which the cells grow out and also the amount of time for which the cells can be cultured.

Glomeruli are most usually isolated using a series of sieves which renders the tissue virtually free from tubules. At this point a further step of enzymatic digestion can be performed which encourages cells to outgrow.

Renal cortex is chopped into pieces of about 1-2mm<sup>3</sup> and then pressed through a sieve of 250µm. The glomeruli are then passed through a 150µm sieve, which removes tubules and strips away Bowman's capsule. The filtrate is then passed through a 106µm sieve to capture the glomeruli. A final digestion using collagenase, 1mg/ml 20min 37°C can be performed followed by passing through a 63µm sieve. The filtrate contains epithelial cells whilst the glomerular remnants can be used for mesangial cell culture.

### **Epithelial cells**

Two types of epithelial cells can be isolated from the glomerulus. If the Bowman's capsule is left behind the culture will yield parietal epithelial cells, but if the capsule is removed then visceral epithelial cells (or podocytes) are thought to predominate.

### **Mesangial cells**

If glomeruli are left in culture for extended periods of time then mesangial cells will overgrow all other cell types. From this point cultures can be maintained in MEM-D-valine to inhibit fibroblast cell growth.

### **Endothelial cells**

These are notoriously difficult to isolate as a pure population. These cells grow out rapidly but are quickly overtaken by epithelial cells and require a positive purification step. After the Collagenase digestion glomerular remnants need to be left undisturbed for 2 days and then monitored closely. At the point where there are a predominance of endothelial cells they are trypsinised of and glomerular remnants removed by sieving. The endothelial cells can then be purified using an anti-CD31 and magnetic beads. Cell numbers must be estimated so that approx 3 beads per cell are added – too few and you won't purify the cells, too many and the cells will not reattach. This needs to be performed a couple of times to gain a totally pure population.

### **Tubular Epithelial Cells**

These cells are easily grown by explant culture on dishes coated with gelatine and FCS. They can be grown in a 1:1 mix of DMEM and F12 containing transferrin/insulin/selenite, hydrocortisone, tri-iodothyronine and epidermal growth factor. The cells will vacuolated after 5-6 passages and can not be recovered after this.

Cell type	morphology	staining (+)	Confluence	Outgrowth	Passage
Epithelial	Cobblestone	CALLA, cytokeratin, GLEPPI	Monolayer	7-10 days	3-4
Mesangial	Stellate	vimentin, $\alpha$ -SMA, myosin, VLA-1	Multilayer – form hillocks	Post 14 days	Up to 7
Endothelial	'Fried egg'	CD31, vWF, HLA-DR	Monolayer	First 3 days	3-5
Tubular	cobblestone	cytokeratin, HEA, alk phos	monolayer	5-7 days	5-6

#### Media

Epithelial RPMI 1640  
 10% FCS  
 0.15U/ml insulin  
 20ug/ml transferrin  
 8ng/ml selenium  
 2mM Glutamine  
 100U/ml penicillin  
 100ug/ml streptomycin

Mesangial RPMI 1640  
 10% FCS  
 5ug/ml insulin  
 5ug/ml transferrin  
 5ng/ml selenium  
 2mM Glutamine  
 100U/ml penicillin  
 100ug/ml streptomycin

Tubular DME/F12 1:1  
 5ug/ml insulin  
 5ug/ml transferrin  
 5ng/ml selenium  
 36ng/ml hydrocortisone

4pg/ml tri-iodothyronine  
 10ng/ml EGF  
 100U/ml penicillin  
 100ug/ml streptomycin  
 2mM L-glutamine

Endothelial M199  
 10% NBS  
 10% Human serum  
 2mM L-glutamine  
 5IE/ml heparin  
 0.15mg/ml ECGF  
 100U/ml penicillin  
 100ug/ml streptomycin

Collagenase

Type II is often used for kidneys but this can vary between groups

Species	Species Detail	Cells	Enzyme(s)	Medium	Reference
Human	Human, adult	Renal proximal tubule and cortical fibroblasts	Collagenase Type 2: 383 u/ml	DMEM/F-12	Johnson, D., Saunders, H., Johnson, F., Huq, S., Field, M., and Pollock, C.: Cyclosporin exerts a direct fibrogenic effect on human tubulointerstitial cells: roles of insulin-like growth factor I, transforming growth factor beta1, and platelet-derived growth factor, <i>J Pharmacol Exp Ther</i> 289, 535-42, 1999
Human	Human	Renal cortex	Trypsin: 0.1%	Tissue Culture Grade Water	McAteer, J, Kempson, S., and Evan, A: Culture of Human Renal Cortex Epithelial Cells, <i>J Tiss Cul Meth</i> 13, 143, 1991
Human	Human	Papillary duct	Collagenase: 400 u/ml	Eagle's MEM-HEPES buffer w/L-glutamine	Trifillis, A. and Kahng, M.: Characterization of an In Vitro System of Human Renal Papillary Collecting Duct Cells, <i>In Vitro Cell Dev Biol</i> 26, 441, 1990
Human	Human	Mesangial	Trypsin: 0.25%	DMEM/Ham's F-12	Heieren, M., van der Woude, F., and Balfour Jr., H.: Cytomegalovirus Replicates Efficiently in Human Kidney Mesangial Cells, <i>Proc Natl Acad Sci U S A</i> 85, 1642, 1988
Human	Human	Tubular	Collagenase: 250 u/ml	PBS	Yang, A., Gould-Kostka, J., and Oberley, T.: In Vitro Growth and Differentiation of Human Kidney Tubular Cells on a Basement Membrane Substrate, <i>In Vitro Cell Dev</i>

					<a href="#">Biol 23 (1), 34, 1987</a>
<b>Human</b>	Human, adult, 14-66 years	Tubular	Collagenase: 100 u/ml	Joklik's MEM	<a href="#">Trifillis, A., Regec, A., and Trump, B.: Isolation, Culture, and Characterization of Human Renal Tubular Cells, J Urol 133, 324, 1985</a>
<b>Human</b>	Human	Malignant Stromal	Papain: 0.009%	Sacks solution	<a href="#">Hemstreet, G., Enoch, P., and Pretlow, T.: Tissue Disaggregation of Human Renal Cell Carcinoma with Further Isopyknic and Isokinetic Gradient Purification, Cancer Res 40, 1043, 1980</a>
<b>Human</b>	Human, 24 newborn and stillborn (also rabbit)	Renal	Trypsin: 0.25%	See reference	<a href="#">Montes De Oca, H., and Malinin, T.: Dispersion and Cultivation of Renal Cells After Short-Term Storage of Kidneys, J Clin Microbiol 2 (3), 243, 1975</a>

#### Refs

*Glomerular epithelial and mesangial cell culture and characterization. Wilson HM, Stewart KN. Methods Mol Med. 2005;107:269-82.*

Happy culturing

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