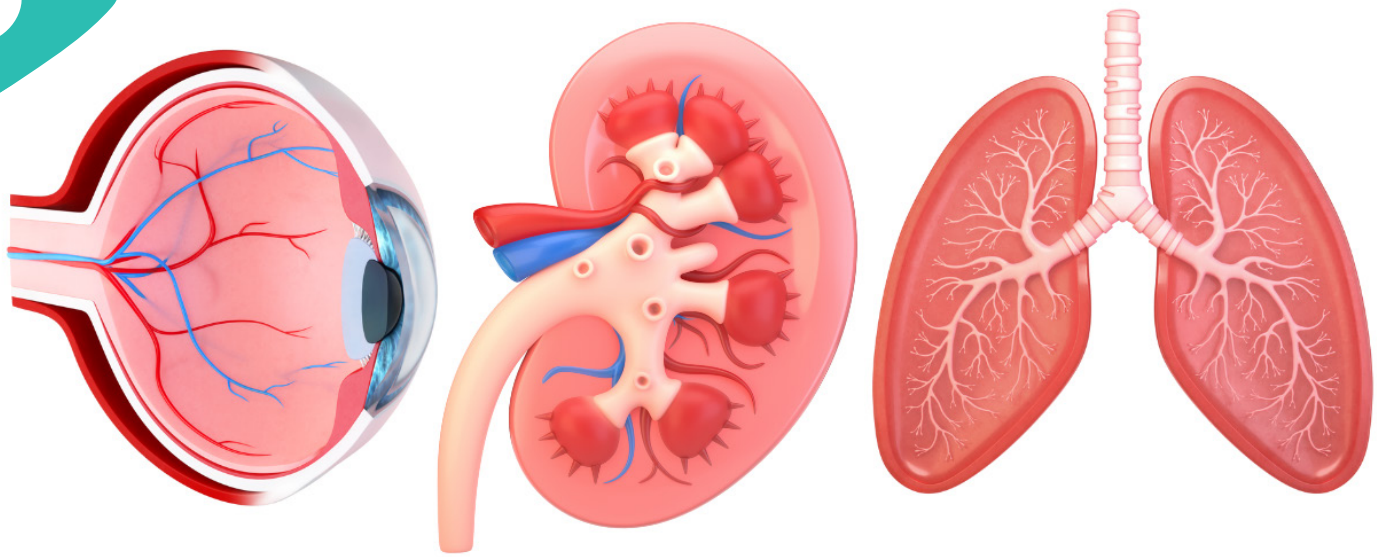
Newcells Products and Services

We build custom, functional *in vitro* models, mimicking *in vivo* physiology, to improve clinical translation and accelerate drug discovery.



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NEWCELLS

IN VITRO MODELS TO PREDICT *IN VIVO* OUTCOMES

Newcells Products and Services

Contents

aProximate™

aProximate™ is a validated, near-physiological, *in vitro*, primary proximal tubule cell (PTC) model for use in drug transport and nephrotoxicity studies.

aProximate™ key features:

- Expression of all key renal transporters
- Expression of FDA approved clinically relevant biomarkers of toxicity
- Cross-species comparison
- High-throughput format

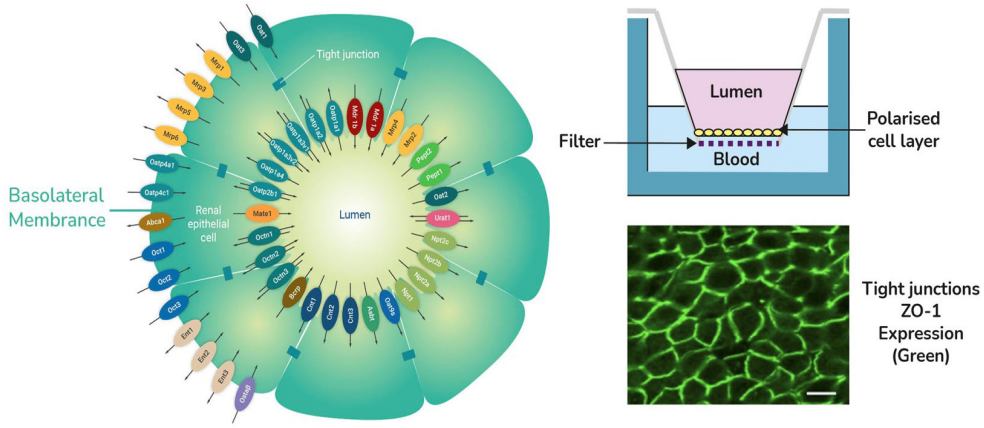


Figure 1: aProximate™ proximal tubule cell (PTC) model. Schematic diagram of aProximate™ PTCs showing the expression of all key renal transporters (left) and the formation of tight junctions as shown by ZO-1 tight junction protein labelling (bottom right). Diagram of Transwell® plates demonstrating the aProximate™ model: PTCs grown on filters remain fully differentiated as a polarised cell layer (top right).

Renal Toxicity Assays

Newcells provides a renal toxicity service quantifying FDA qualified kidney-specific injury biomarkers, KIM-1, NGAL and clusterin. aProximate™ is therefore a reliable tool to assess PTC toxicity and evaluate renal drug safety during drug discovery.

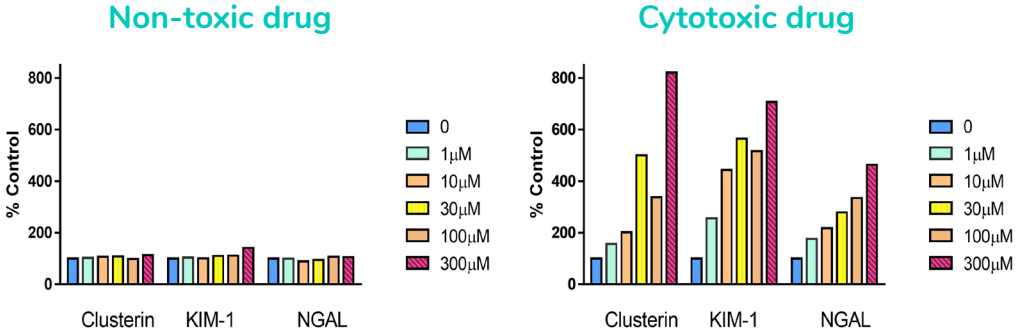
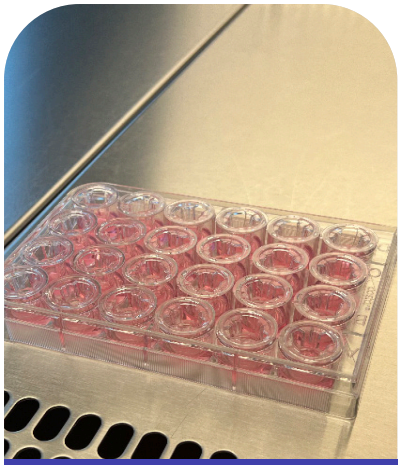


Figure 2: Comparison of two compounds for drug safety prediction using the aProximate™ platform indicating a likely nephrotoxic effect for compound B (right panel) with increased levels of injury bio-markers. Compound A (left panel) is predicted as non-toxic.



aProximate™ Assay-Ready Plates

aProximate™ is available in an Assay-Ready Transwell® plate format.

Plates are shipped with the following included:

- aProximate™ proximal tubule cell cultures on high throughput plates
- Maintenance media
- Comprehensive user guide
- Quality assurance

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Transporter Assays

For new drugs, early understanding of drug handling in kidney PTCs is a common strategy to mitigating the risk of failure during preclinical and clinical development. Drug-drug interactions are complex but can be predicted *in vitro* using aProximate™ as all key renal transporters are expressed in the model.

For example, *in vitro* drug transport studies of diabetic drug Meformin shows that it is handled by OCT and MATE. This was demonstrated by using OCT and MATE inhibitors Cimetidine (a gastric drug) and Pyrimethamine (an infectious disease drug). The inbibitors significantly reduced Metformin's basolateral to apical flux (J_{BA}). The results are comparable to *in vivo* studies.

in vivo

Transporter	Drug	Transporter Inhibitor	AUC fold increased	CL_R decrease (%)
hOCT2, hMATE1, and hMATE2-K	Metformin	Cimetidine	1.5	28
	Metformin	Cimetidine	1.5	45
	Metformin	Pyrimethamine	1.4	35
	Metformin	Dolutegravir	2.5	N.D.

aProximate™

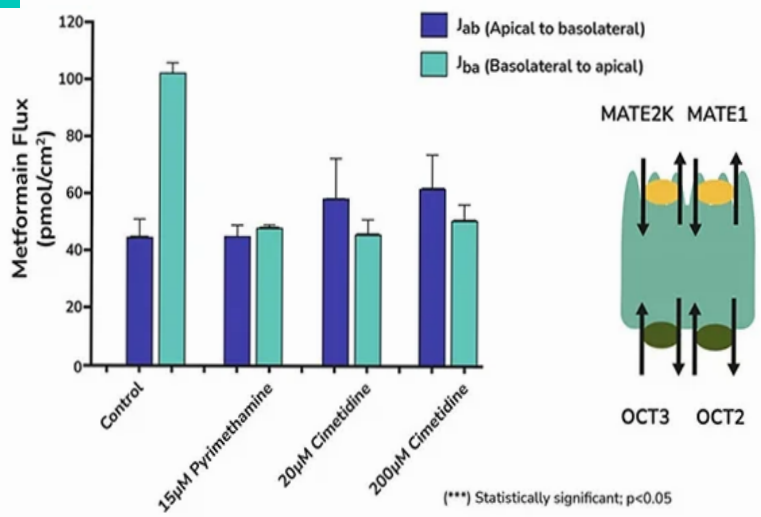

Figure 3: Predictions of drug/transporter interactions in aProximate™ showing a reduction in renal clearance of metformin upon inhibition of OCT and MATE transporters, comparable to that observed *in vivo*.

Glomerular Model

Podocyte Renal Toxicity Assay

Newcells now offers the first, fully differentiated, primary podocyte cell model isolated from fresh kidney tissue for the assessment of drug-induced renal toxicity, specifically the effect on the glomerular filtration barrier as well as assessment of glomerular permeability.

Podocytes express all relevant podocyte markers and are grown in 96-well Transwell® plates into a monolayer with size and charge selectivity comparable to *in vivo*.

The assay evaluates podocyte injury by assessing increased podocyte permability as follows:

- Trans-epithelial electrical resistance (TEER)
- Permeability of 70 kDa FITC-dextran
- ATP assay of cell viability

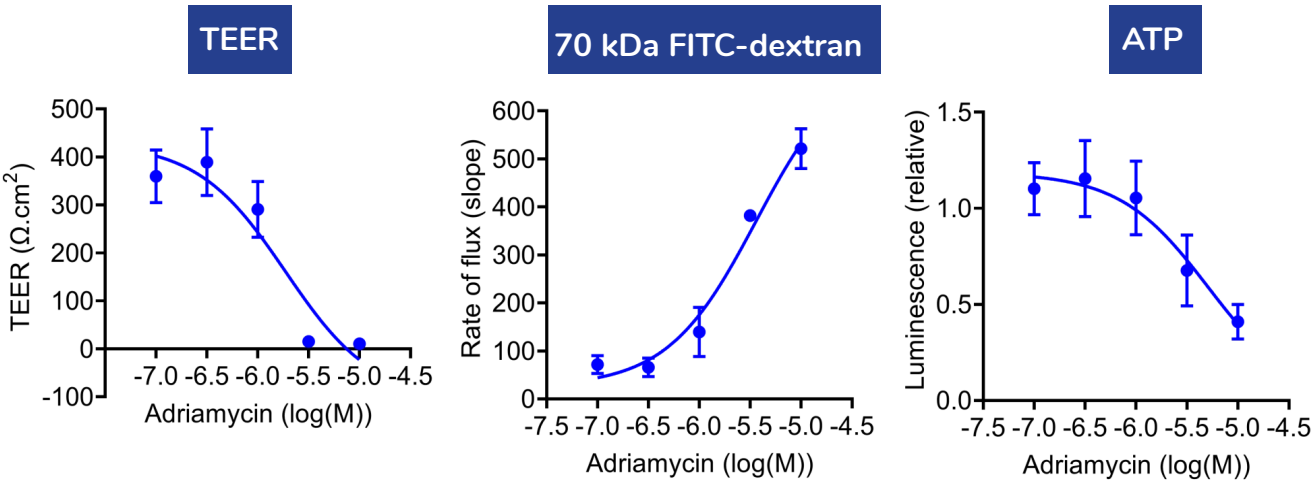

Figure 4: Podocytes treated with increasing concentrations of Adriamycin. TEER decreases as Adriamycin concentration increases, while the permeability of 70kDa FITC dextran increases indicating disruption to the podocyte cell membrane. A decrease in cell viability was also measured using an ATP assay.

Additional assays are also available as part of an enhanced renal toxicity service including quantification of biomarkers such as IL-6, VEGF, Angiopoietin 1 and Osteopontin.

MPS Flow Model

aProximate™ Flow

By the simple adaption of our primary human proximal tubule cells grown in Transwells we have demonstrated that flow induces:

- Higher cillia expression
- Increased expression of a number of key transporters, including large molecule transporters.

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Retinal Models

Product Information

Newcells' iPSC-derived retinal models offer a unique *in vitro* platform for disease modelling and investigating drug safety and efficacy.

Our retinal organoids are fully stratified and present all of the major cell types of the human retina.

Importantly, our retinal organoids:

- Contain functionally active cells and respond to light
- Have been validated on a variety of applications including gene therapy and toxicity testing

Specification

Format	• 10 retinal organoids per 5 ml microfuge tube • 150 ml of optimized cell culture medium per 100 organoids • 2 x 96 well plates per 100 organoids • 3 x Pasteur pipettes
Cell Types	Retinal Organoids • Cone and Rod photoreceptors • Retinal ganglion cells (RGCs) • Bipolar cells • Horizontal cells • Amacrine cells • Müller glial cells RPE • Retinal pigment epithelial cells
Species	• Human

Applications

- Retinopathy modelling
- Drug safety and efficacy testing
- Gene therapy
- Developmental studies

NOTE: Our organoids are typically shipped at day 150 but can be requested at various timepoints throughout the differentiation process e.g. day 60.

Retinal Organoid differentiation timeline

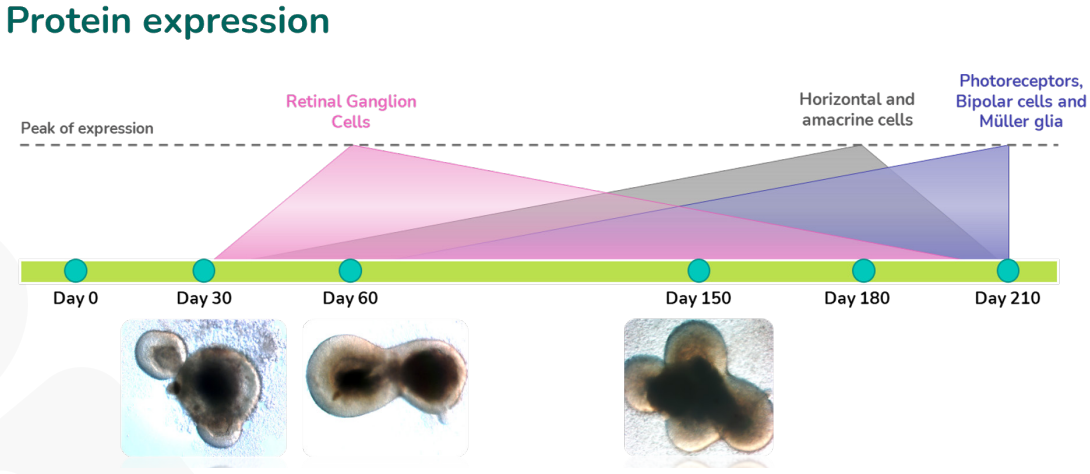

Figure 5: Differentiation of iPSCs follows the developmental timeline of embryonic development of the retina with various cell types arising at different times in a sequential manner.

Retina Services

Retinal Drug Safety and Toxicity

Retinal organoids have been tested for the response of known toxins such as thioridazine and doxorubicin. The intrinsic fluorescence of doxorubicin facilitates the visualisation of the drug penetrating the retinal organoid (Figure 6). Exposure of the iPSC-derived retinal organoids to doxorubicin reduces cell viability in a dose-dependent manner (Figure 7).

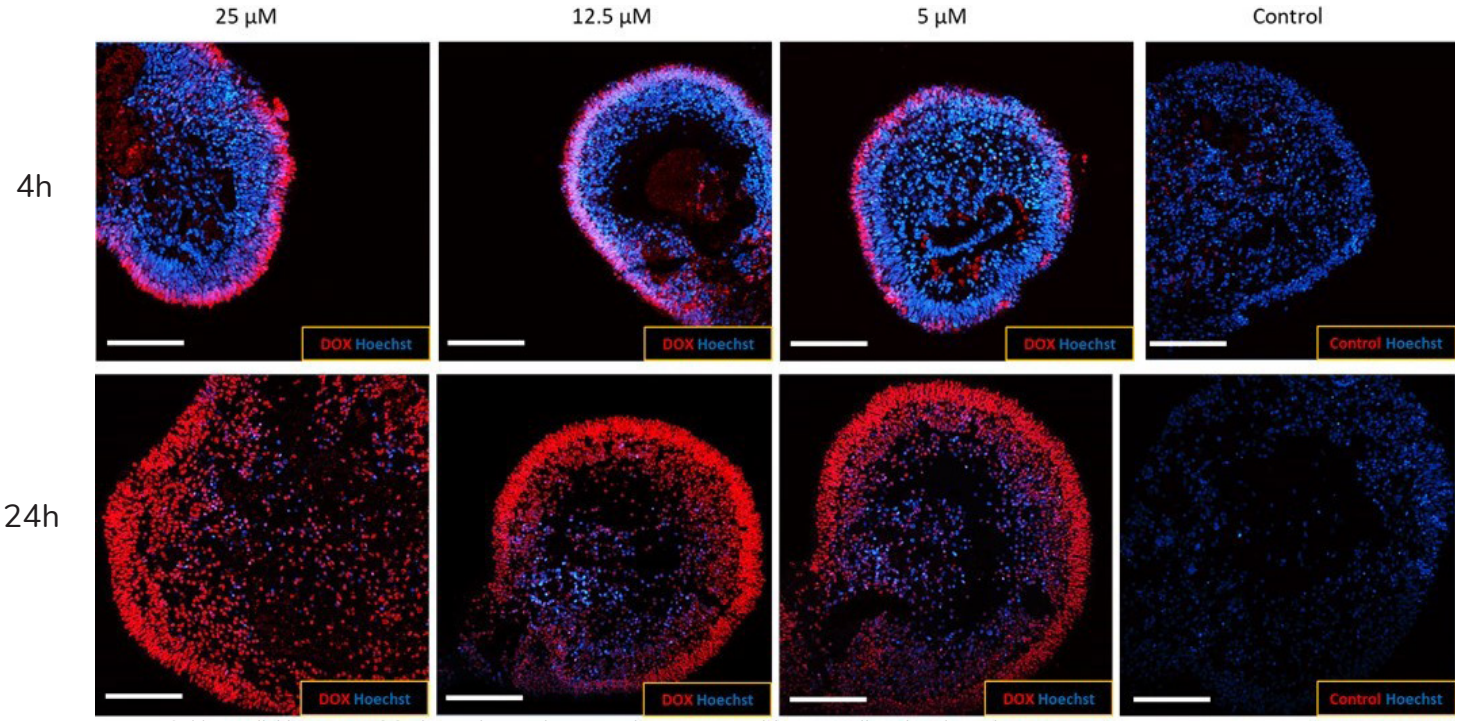

Figure 6: Newcells' human iPSC-derived retinal organoids are permeable to small molecules. The penetration of doxorubicin, a naturally fluorescent small toxic molecule (red), into the retinal organoids increases over time (4h to 24h) demonstrating the permeability of the organoids to drugs.

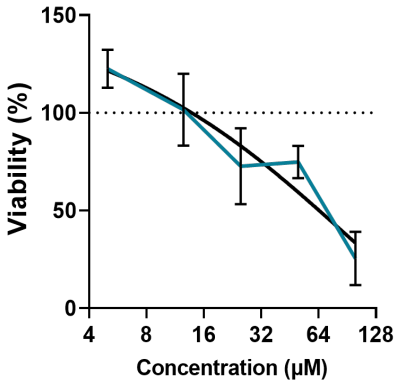

Figure 7: The retinal organoids were treated with increasing dose of doxorubicin over a period of 24h and cell viability was measured using an ATP assay. A dose-dependent decrease in cell viability was observed following increasing exposure to the drug.

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Model for Gene Therapy using AAV

Human photoreceptor-like cells within Newcells' iPSC-derived retinal organoids are efficiently transduced with AAV vectors, demonstrating suitability of the organoids for preclinical testing and assessment of retinal gene therapy treatments.

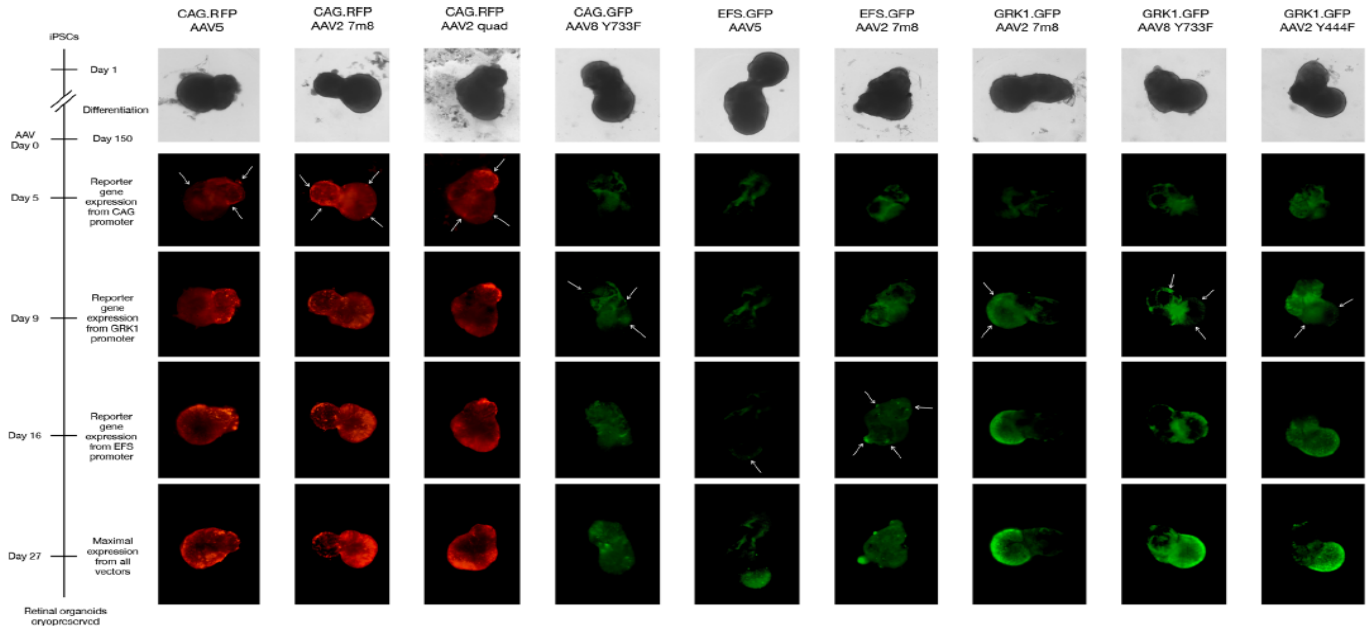


Figure 8: Live cell imaging of reporter gene expression up to 27 days post-transduction. Arrows indicate the areas where the onset of reporter gene expression first appeared. Allowing evaluation of which cell types each AAV vector transduced preferentially.

Retinal Pigment Epithelium (RPE)

The RPE *in vitro* cell model is composed of a monolayer of RPE cells cultured in 24-well Transwell® plates. RPE characterisation includes: morphology assessment, pigmentation, RPE-specific expression at the protein level (BEST1, TYRP1), the analysis of phagocytosis of photoreceptor outer segments, trans-epithelial resistance (TEER), polarity of apical Pigment Epithelium-Derived Factors (PEDF) and basal vascular endothelial growth factor (VEGF) secretion.

Available as a service for the following applications:

- AAV vector evaluation
- Safety
- Disease modelling

Lung Services

Fibroblast-to-Myofibroblast Transition

A high-throughput assay for the screening of anti-fibrotic compounds.

Fibroblast activation and transformation into myofibroblasts is a crucial process in wound healing and tissue repair. Persistent myofibrobast activation is a hallmark of lung fibrosis and is believed to contribute to excessive extracellular matrix deposition and increased lung stiffness.

Following the stimulation of primary,human fibroblasts with a physiologically relevant concentration of TGF-β1 our 384-well, high content imaging assay enables assessment of changes in the expression and deposition of extracellular matrix (ECM) proteins and α-smooth muscle actin (α-SMA), a protein associated with fibroblast activation.

Available service outputs:

- Cell number (nuclei staining)
- Active cell proliferation (EdU incorporation)
- Deposition of Collagen I and Collagen III by immunocytochemistry
- Detection of α-SMA

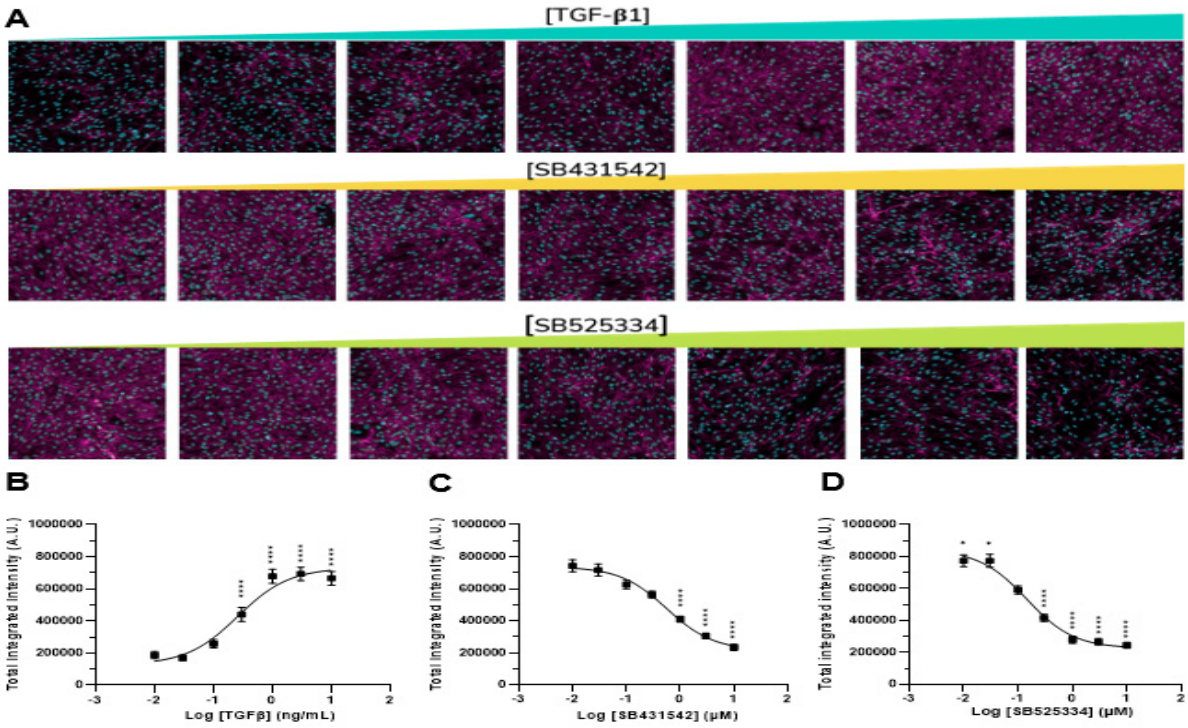


Figure 9: **Collagen I deposition is regulated by the ALK5 signalling pathway.** (A,B) TGF-β1 induces a dose dependent increase in extracellular collagen I deposition. (A,C,D) TGF-β1 induced collaged I deposition is decreased dose-dependently by the ALK5 inhibitors, SB431542 and SB525334.

Lung Services

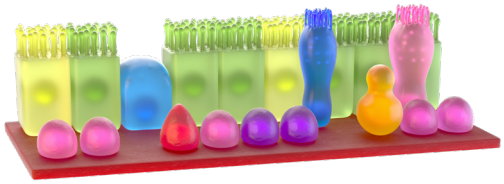
Small Airway Epithelial Cell Model

A robust, physiologically relevant epithelial model to enable scientific research and drug discovery

Newcells' human, small airway epithelial cell (SAEC) model closely recapitulates the epithelial physiology of the lung. Derived from differentiated small airway basal cells, our SAEC model comprises of the key epithelial cell types; basal, club, goblet and ciliated cells. With an established epithelial barrier, active mucus production, and functional cilia, our SAEC model is a valuable tool for both scientific research and drug discovery.

Available service outputs:

- Trans Epithelial Electrical Resistance (TEER)
- Gene expression changes
- Immunocytochemistry
- Cell viability & compound toxicity
- Cytokine release



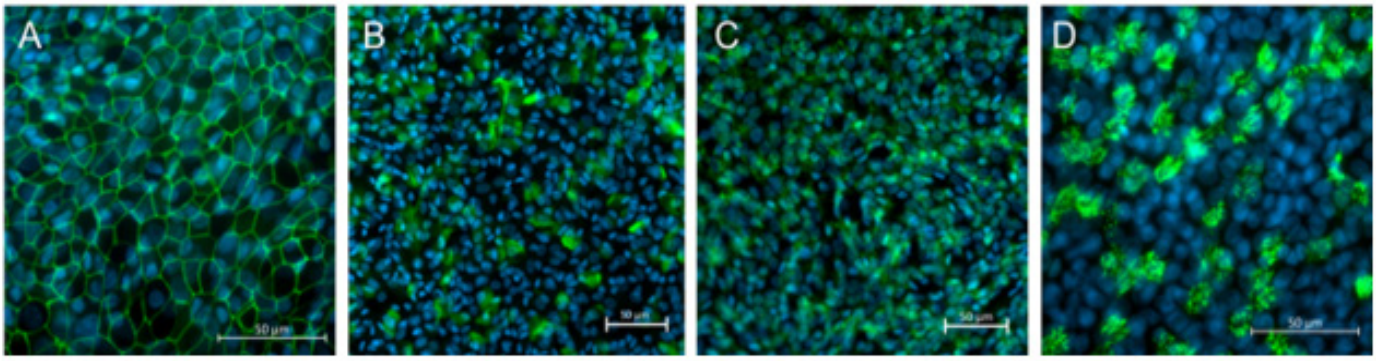


Figure 10: **Newcells' small airway epithelial cell model expresses key cell specific proteins.** (A) ZO1 staining shows presence of epithelial tight junctions. Presence of club cells (B), goblet cells (C) and ciliated epithelial cells (D) as indicated by CC10, MUC5B and acetylated tubulin (AcT) expression.

Cultured on 3D permeable supports, our polarized SAEC model allows assessment of treatment effects on epithelial barrier integrity and cell viability.

Cytotoxicity can be assessed by the quantification of LDH release and cellular ATP activity

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Our recent publications

Generating microglia-like cells from human iPSCs and incorporating them into retinal organoids. We demonstrated the feasibility of generating an immunocompetent in vitro model of functional retina derived from iPSCs

Find out more here

Tropism of AAV Vectors in Photoreceptor-Like Cells of Human iPSC-Derived Retinal Organoids. We have validated our retinal organoids for their use in preclinical testing of AAV vectors for retinal gene therapies

Find out more here

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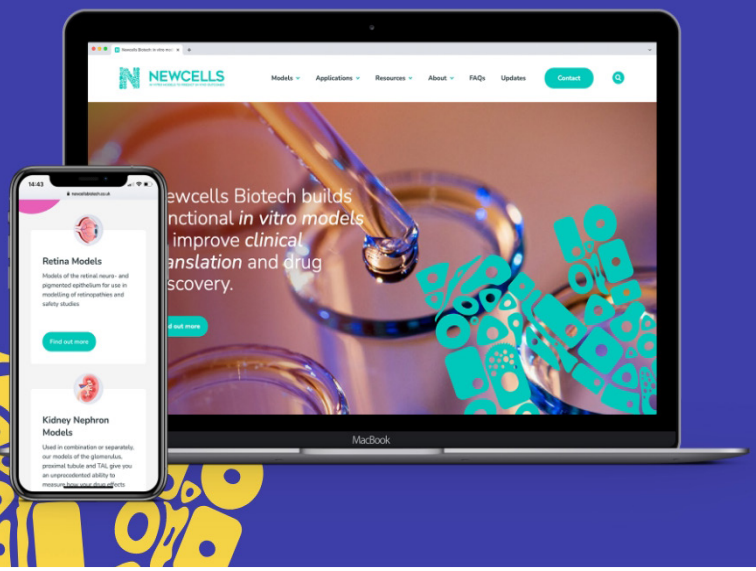

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