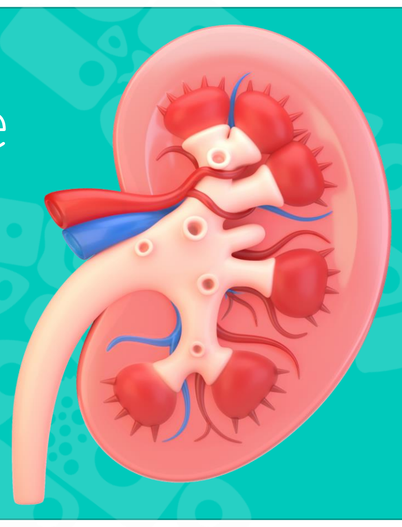
aProximate™: A predictive in vitro primary proximal tubule platform for de-risking of antibiotic induced kidney injury



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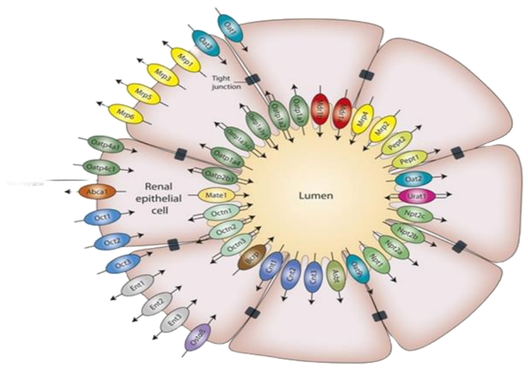
Introduction

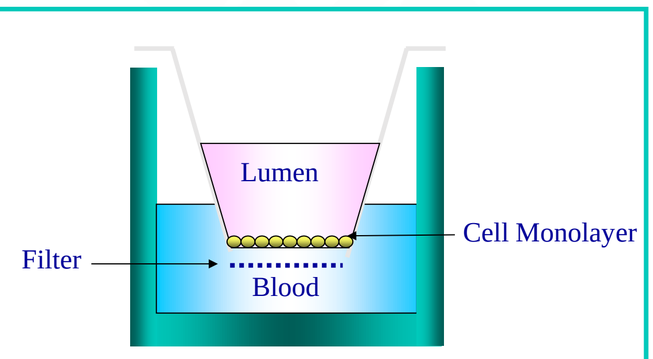
Nephrotoxicity is a major reason for drugs failing during clinical development.

Currently there is no in vitro platform that enables cross-species comparisons of drug transport or nephrotoxicity.

Our innovative solution is to develop highly differentiated assay platforms using primary renal proximal tubule cells(PTCs) derived from key animal species to measure both drug transport and drug induced kidney injury a range of biomarkers across species

Here we showcase data from our highly differentiated Human proximal tubule **model**





aProximate Proximal Tubule cell monolayers express a full range of transport proteins and form tight monolayers when grown on Transwell filter supports

Methods

PTCs were isolated from fresh Human kidneys and cultured onto Transwell inserts

Test toolkit was made up of a compounds with known nephrotoxicity liability and clinical data were screened using the model Toolkit was made up of 19 Primary PT-toxic and 17 Secondary PT-toxic (3) or non-nephrotoxic (14) compounds

Aminoglycoside test toolkit included Neomycin, Tobramycin, Gentamicin, Amikacin and Streptomycin

Monolayer were exposed for 72 hours to a range of concentrations (0-300µM) of test compound

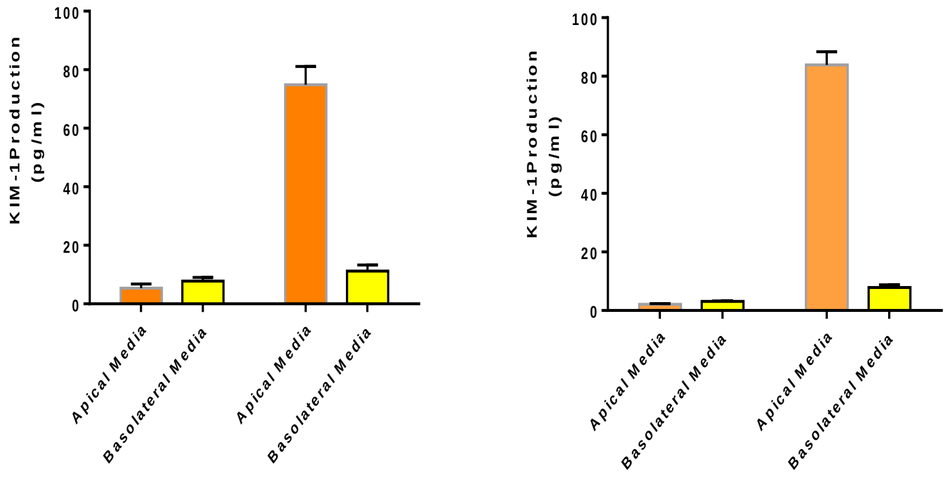
Toxicity was measured used 6 parameters of cell health: Transepithelial Resistance (TEER), Intracellular ATP concentration, LDH release , KIM-1 release, Clusterin release NGAL release

Biomarkers were measured on an MSD platform

ATP was measured using a Promega CellTiter Glo2 assay

LDH was measured using a Promega LDH-glo assay

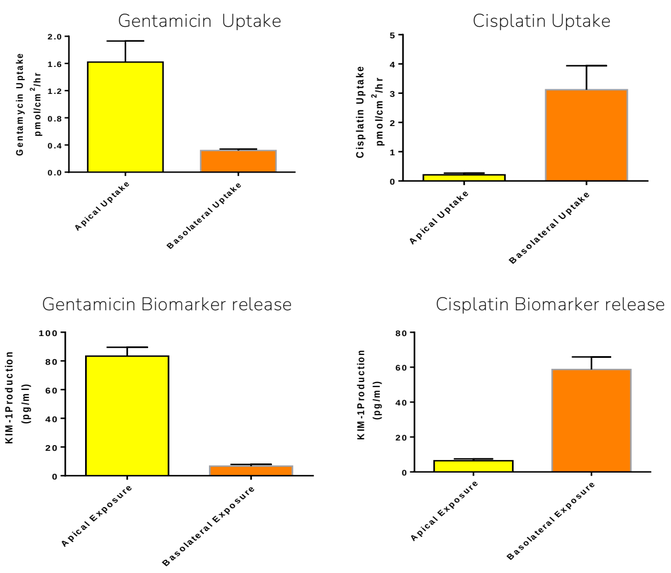
Results



Cells were challenged with either gentamicin (200**µ**g/ml) or cisplatin (10**µ**M) for 72 hours.
Biomarkers are were found only in the apical media.
This corresponds to the in vivo release of biomarkers into the urine

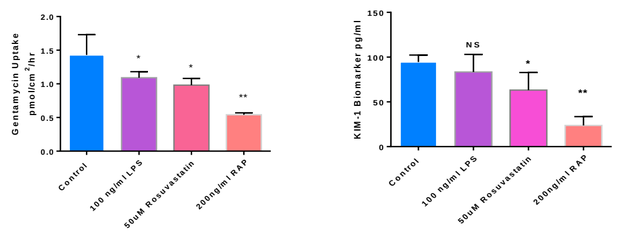
Results

Renal Biomarkers Response in aProximate™ PTC cells is Polarised and Correlates with Exposure to Nephrotoxin



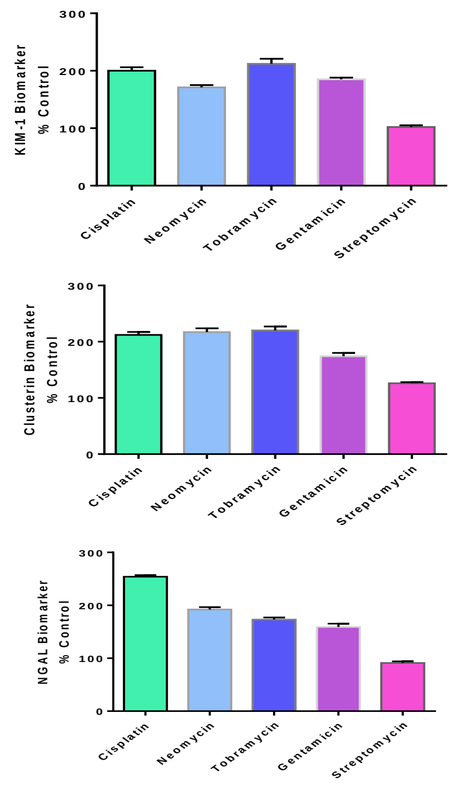
Gentamicin is predominately taken up across apical membrane. Only apical exposure to gentamicin elicits release of biomarkers of damage. Cisplatin is predominately taken up across basolateral membrane. Only basolateral exposure to cisplatin elicits release of biomarkers of damage.

Inhibition of Gentamicin Uptake reduces Biomarker response to Gentamicin



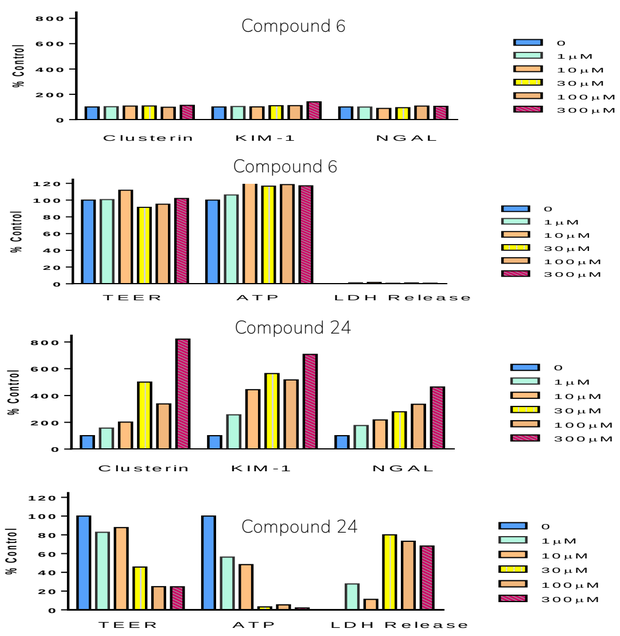
Inhibition of gentamicin uptake by inhibitors of megalin, cubulin result in a significant reduction in Biomarker release

Renal Biomarkers Response to Aminoglycoside Challenge



Cells were challenged with either cisplatin (10**µ**M) or a range of aminoglycosides (3000**µ**M) for 72 hours.
Significant increases in KIM-1, NGAL and Clusterin were found with exposure to Neomycin, Tobramycin and Gentacin. In contrast, challenge with streptomycin had no significant effect on biomarkers production

Validation of aProximate™ PTC monolayer as a Predictive model of Toxicity



Effect of a non toxic (compound 6) and toxic (compound 24 upon endpoints measured

Only AC_{50} value

	TEER	LDH	ATP	KIM-1	NGAL	Clusterin
Cut-off (µM)	< 15	< 10	< 50	< 30	< 22	< 95
AuROC	0.52	0.56	0.61	0.61	0.64	0.66
Sensitivity (%)	5.3	26.3	47.4	47.4	47.4	63.2
Specificity (%)	88.2	88.2	88.2	88.2	88.2	88.2

In vitro safety margin (AC_{50}/C_{max})

	TEER	LDH	ATP	Clusterin	KIM-1	NGAL
Cut-off (X)	< 5	< 10	< 25	< 31	< 30	< 30
AuROC	0.53	0.62	0.64	0.75	0.74	0.78
Sensitivity (%)	15.8	31.5	42.1	57.9	63.2	63.2
Specificity (%)	88.2	88.2	88.2	88.2	88.2	88.2

Injury specific biomarkers were more predictive than non specific endpoints (TEER, ATP depletion, LDH release)
In vitro safety margin (AC_{50}/C_{max}) was more predictive than AC_{50} alone
Best Predictivity came from combination of two biomarkers

In vitro safety margin (AC_{50}/C_{max})

	TP	FN	TN	FP	Sensitivity (%)	Specificity (%)	Accuracy (%)
All 6 end-points	13	6	12	5	68.4	70.6	69.4
Biomarkers + ATP	13	6	13	4	68.4	76.5	72.2
Any biomarkers	13	6	14	3	68.4	82.4	75.0
At least 2 biomarkers	**13**	**6**	**15**	**2**	**68.4**	**88.2**	**77.8**

Conclusion

Human proximal tubule cell monolayers retain a remarkable degree of differentiation and express a range of functional transporters and clinically relevant biomarkers of nephrotoxicity that are sensitive to nephrotoxin challenge over time. Human PTC monolayers show excellent potential as an in vitro predictive screening platform.

Further details available online or contact us at
enquiries@newcellsbiotech.co.uk

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