A validated solution for shipping primary human proximal tubule cell monolayers between laboratories

Kidney

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Introduction

- aProximate™ is a highly sophisticated, near-physiological proximal tubule cell (PTC) model that is a proven market-leader in the study of transporters and drug-drug interactions.
- One issue with primary cell cultures is the short time window they remain differentiated and functional, which has hindered the sharing of primary cell models between laboratories.
- To overcome this and ship cells globally in an assay-ready format, a safe and reliable short-term preservation method was developed and internally validated, pausing cells at hypothermic temperatures for up to 5 days.

Methods

- Human PTCs were isolated from the cortex tissue of 17 nephrectomies by Percoll gradient separation and grown on Transwell® filter supports to functional maturity (Figure 1.)
- Membrane barrier integrity was quantified by transepithelial electrical resistance (TEER), with values of more than 60 Ω.cm^2 used as quality control to indicate a fully established, polarized monolayer. Cultures typically took 5-6 days to reach confluence.
- Cells were placed into metabolic stasis, using a formulated biogel and maintained at 4°C for 3 or 5 days to simulate shipping conditions.
- Subsequently, cells were revived to 37°C and left to recover until the minimum TEER QC value was reached.

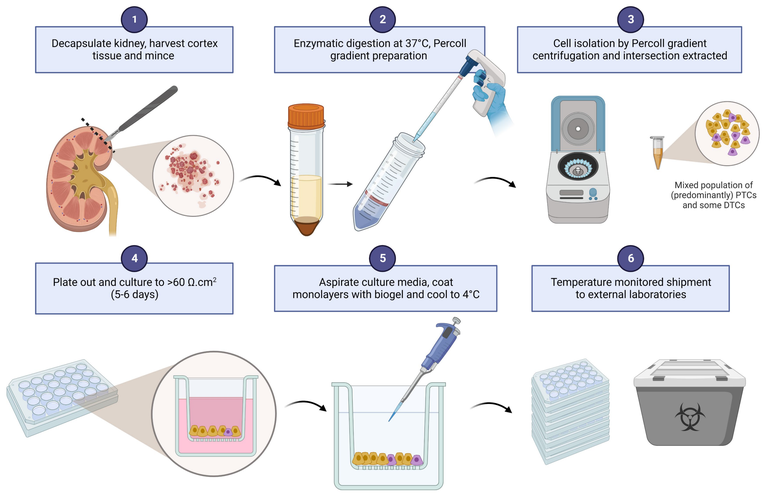

Figure 1. *Illustration of methodology* Cells were isolated by enzymatic digestion and Percoll gradient preparation, cultured to confluence, then coated in a formulated biogel and placed into hypothermic preservation for up to 5 days. Upon revival and TEER recovery, monolayer viability and functionality were assessed.

Results

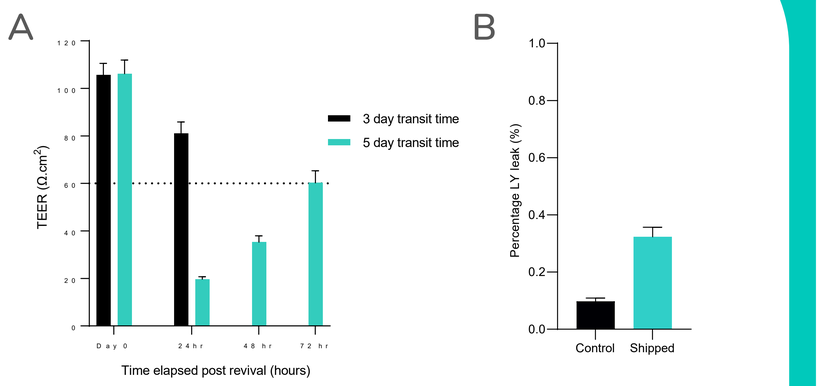

Figure 2. *Membrane barrier integrity assessment.* **(A)** TEER recovery was cold-time dependent, with plates shipped for 3 (n = 7 kidneys) and 5 days (*n* = 12 kidneys) taking 24hr and up to 72hr respectively to reach QC threshold. **(B)** Lucifer Yellow (LY) paracellular leak in monolayers shipped for 5 days was 0.32 % (*n* = 4 kidneys) and remained well below the upper accepted limit of 2%.

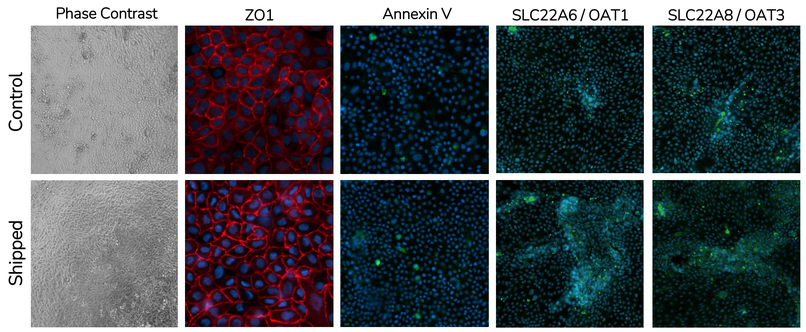

Figure 3. *Morphology & Protein Expression.* **(Phase Contrast)** Following 5 days of hypothermic preservation and a 48-72hr recovery phase, monolayers remained differentiated and exhibited a single-layer cobblestoned epithelium, morphologically akin to pre-shipment control. **(ZO1)** Extensive positive immunofluorescent staining of the tight junction protein, ZO1 (red), was demonstrative of an uncompromised cell membrane barrier. **(Annexin V)** Expression of the apoptotic marker, Annexin V was not affected by shipment. **(SLC22A6 / OAT1) & (SLC22A8 / OAT3)** Expression of Organic Anion Transporters 1 and 3 were not affected by shipment. All nuclei counterstained with Hoescht (blue) and images taken on the ImageXpess Pico (Molecular Devices).

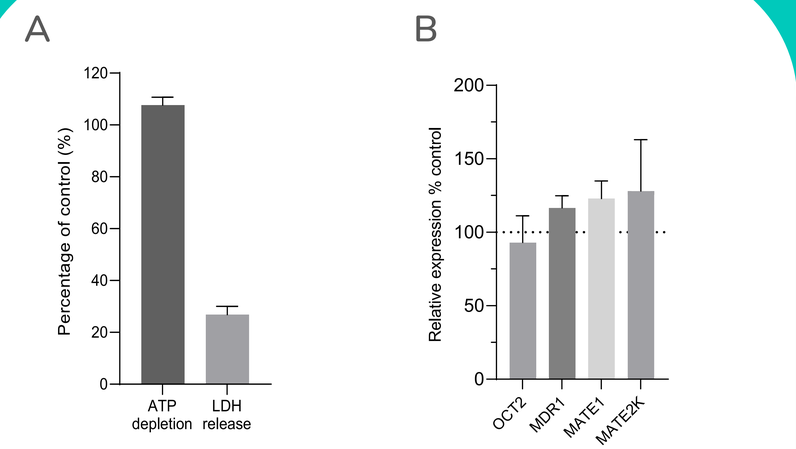

Figure 4. **(A)** *Cell health assessment.* Monolayers were viable and metabolically active, indicated by way of ATP assay (>100 % of control , *n* = 9 kidneys). LDH cytoxicity revealed cell loss of approximately 26% upon initial monolayer revival but PTCs grew back to full confluency during the 1-3 day recovery phase. **(B)** *Transporter Expression.* At the mRNA level, expression of the key drug transporters OCT2, MDR1, MATE1 and MATE2K were stable or upregulated relative to pre-shipment control (93%, 117% 123% and 128% respectively, *n* = 5 kidneys).

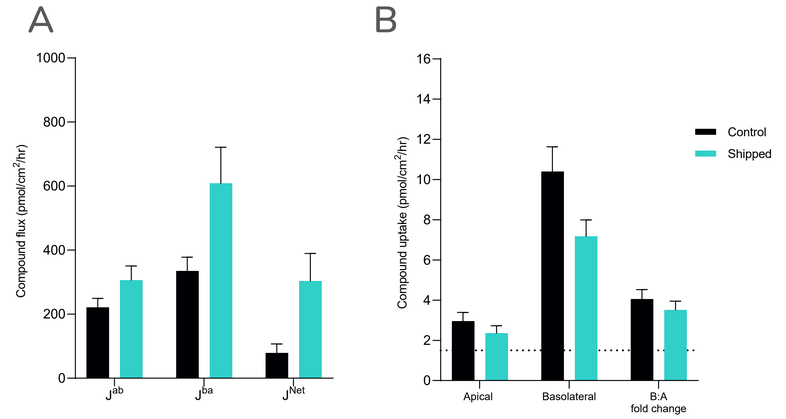

Figure 5. *Bidirectional transcellular flux and uptake of a radiolabelled compound* **(A)** Functionally, monolayers conserved the mechanics to facilitate the OCT2-mediated basolateral uptake and MATE-mediated efflux of ^{14}C creatinine. The efflux ratio for creatinine in shipped cells was 1.8 ± 0.16 fold and was not significantly different from time-matched control monolayers (*n* = 10 kidneys, paired t test, *P* > 0.01). **(B)** Likewise, the basolateral : apical creatinine uptake ratio was 3.5 ± 0.43 and was not significant from time-matched control monolayers (*n* = 17 kidneys, paired t test, *P* > 0.05).

Conclusion

Taken together, these data suggest that coating PTCs at hypothermic temperatures offers the possibility of shipping a viable and functional primary cell model across the world with improved confidence, overcoming a major limitation in the widespread use of primary cell models.

Conflict of interest statement: All authors are employees of Newcells Biotech