Single-shot In Vitro Phenotypic Characterization of Kidney Cells for Cell Therapy

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Background:
Cell therapy in tissue engineering is predicated on the idea that cells will retain or adopt a particular phenotype with quantitative fidelity to the cells in the original organ. In vitro cell culture protocols are concessions to practicality that differ from in vivo conditions in almost every way. As part of our efforts to implement a bioreactor of human renal epithelial cells, we developed a rapid technique to measure key aspects of renal tubule cell function. We assessed active transport of sodium, secretion of organic anions, and barrier function in a single experiment.

Methods:
Primary renal tubule cells and cell lines (e.g. LLCPK1) were grown to confluence on permeable supports. Apical culture medium was supplemented with 10mmol/L lithium chloride and 0.1 mmol/L sodium diatrozoate, and basolateral media was supplemented with 0.1mmol/L para-amino hippuric acid and culture continued for 24 hours. Cells were allowed to recover and the experiment was repeated with addition of ouabain to the media. Aliquots were sampled at 6 and 24 hours. Lithium concentration in the basolateral media was measured by atomic absorption spectroscopy, and diatrozoate and PAH in basolateral and apical media were measured by C-18 HPLC with UV absorption.

Results:
Diatrozoate concentrations in basolateral media were low unless cells were injured or subconfluent. PAH concentrations in apical media remained low, reflecting low OAT-1 expression in LLCPK1 cells. Lithium concentrations rose over time in basolateral media, and rose sharply when cells were injured. We have streamlined our functional assessment of cultured renal tubule cells and now have a straightforward go/no-go assay with which to define release criteria for tubule cell bioreactors.