Dynamic Culture on an Orbital Shaker Alters the Phenotype of Primary Human Renal Tubular Epithelial Cells

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Background:
Primary cells cultured in vitro gradually lose features characteristic to the in vivo cell type, variously termed “senescence” or “culture stress." Culture conditions that help maintain cell-specific phenotype are advantageous for cell biology and tissue engineering. Here we evaluated the phenotype of primary renal tubular epithelial cells after applying apical fluid shear stress using an orbital shaker.

Methods:
Human renal tubular epithelial cells were isolated from donor kidneys not suitable for transplant. Cells were cultured on Transwell inserts under static conditions or on an orbital shaker at a frequency producing fluid shear stresses of 2 dyn/cm2. Transepithelial resistance was measured daily. After 2 weeks in culture, cell density was analyzed by counting DAPI stained nuclei, and expression of tubule-specific markers was measured by PCR and western blotting.

Results:
Dynamic culture significantly increased steady state transepithelial resistance from 344±31 to 544±32 Ω-cm2 ($p<0.001$) and resulted in a 21.5±2.1% ($p<0.001$) increase in cell density. Gene expression of tubule epithelial cell markers (GGT1, COL4A1, COL4A2, NHE3, and NAPP2) increased with fold changes in expression of 4.3±0.3, 4.1±0.3, 3.1±0.2, 2.1±0.4, and 1.9±0.2, respectively. Cells grown under shear also showed increased protein expression of gamma-glutamyl transpeptidase.

Conclusions:
Primary renal tubular epithelial cells grown on an orbital shaker with physiological levels of fluid shear stress appear to express proximal tubule markers more than cells grown in static conditions. This may be due to increased nutrient delivery and waste removal with improved mixing at the apical brush border, or due to specific gene regulation related to mechanotransduction. Further mechanistic insight may allow investigators to develop improved in vitro culture systems for cell biology and tissue engineering and more accurate in vitro models of disease.