

Long-Term Water Transport and Barrier Function of Proximal Tubule Cells Cultured Under Apical Shear Flow Condit

Peter Soler¹, Nick Ferrell², William Fissell³, Shuvo Roy⁴, Paul Brakeman⁵

¹*Department of Chemical & Biomolecular Engineering, UCB*

²*Department of Medicine, Vanderbilt University*

³*Division of Nephrology, Vanderbilt University*

⁴*Department of Bioengineering & Therapeutic Sciences, UCSF*

⁵*Department of Pediatrics, UCSF*

Background:

Development of a bioartificial kidney (BAK) comprising a hemofilter and a proximal tubule cell bioreactor for treatment of chronic kidney disease requires long-term survival of proximal tubule cells under shear flow conditions. Previous work has only characterized transport properties of proximal tubule cells for 2-3 weeks in perfusion culture. We investigated fluid transport characteristics of proximal tubule cells in a shear flow bioreactor in long-term culture for >60 days.

Methods:

LLC-PK1 cells were cultured on polycarbonate filter supports for 14 days and then exposed to apical shear stress. Cells were perfused with media containing urea (40 mg/dL) and creatinine (10 mg/dL) on the apical side and media alone was used on the basal side. The change in volume of the compartments and concentrations of sodium, potassium, phosphorus, urea and creatinine were measured.

Results:

Water transport for proximal tubule cells under shear flow conditions increased significantly from 34+/- 10 ul/cm²/day on day 7 of low shear flow (0.2 dyn/cm²) to 119+/-12 ul/cm²/day on day 63 (p=.002) with high shear flow (2 dyn/cm²) and was stable for 14 days at high shear flow from days 49 to 63. We characterized the barrier function of the cells by looking at the leakage of creatinine. On day 63, the leakage of creatinine was 0.012+/- 0.009 mg/cm²/day and did not differ significantly from the leakage of creatinine on day 7.

Conclusion:

Proximal tubule cells can maintain barrier function and water transport under shear flow conditions for >60 days. Water transport increases with increased shear flow and time in culture.