# Biomimetic Platform to Study Effects of Surface Topography and Flow-induced Shear Stress on Renal Epithelial Cells

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**Statement of Purpose:** Similar to the way the extracellular matrix (ECM) influences and controls cell behavior *in vivo*, cell-substrate interactions strongly govern the behavior of cells *in vitro*. The interaction of cells with surrounding physical parameters, such as sub-micron substrate topography and flow-induced shear stress (FSS), play an important role in the alignment, migration, differentiation and phenotypic expression of cells [1, 2]. Although traditional cell culture techniques offer a convenient method for growing cells, the flat surfaces and static conditions do not provide a microenvironment with biomimetic cues. In the current study, we focus on directing cell response using multiple physical parameters in a well-controlled, *in vitro* microenvironment. In particular we concentrate on guiding junctional complex and tissue structure formation in renal tubule epithelial cells using surface topography and FSS as physical environmental cues.

**Methods:** We designed a layered microfluidic device to emulate a kidney tubule environment in which topographically-patterned polystyrene served as the cell substrate and a polydimethylsiloxane (PDMS) top layer formed an array of channels. The sub-micron topography was formed by photolithography, reactive ion etching, and hot embossing imprint lithography. A contact printing method was used to pattern chemical domains on blank and topographical substrates to restrict cell growth to areas spanned by the channels and with uniform shear stress. Cells from the human renal proximal tubule cell line (HK-2) were seeded on the surfaces at 400 cells/mm$^2$ and cultured to confluency for 5 days under standard growth conditions. Prior to the flow tests, microfluidic channels were aligned and assembled to the substrates. During flow tests, cells were exposed to shear stress values of either 0.01 dyne/cm$^2$ or 1 dyne/cm$^2$. Control samples consisted of blank and topographical substrates exposed to static conditions (zero FSS). Cells were subsequently fixed and immunofluorescently stained for analysis via confocal microscopy.

**Results:** Hot embossing resulted in topographical grooves 0.75 µm wide and 1 µm deep with a 1.5 µm pitch. Using controlled flow rates, the flow chamber produced shear stress values equal to those typically experienced in the adult renal proximal tubule and uniform over cell adhesion area. Controlled amounts of shear stress are possible in the range from 0.01 to 3.0 dynes/cm$^2$ by adjusting inlet flow rates. Computer simulation predicted the FSS across the cell adhesion areas will vary less than 5%. The device channels were sterilized after use and reused successfully without leaking. In the absence of FSS, cells on flat substrates tended to be randomly oriented and spread out whereas cells on topographical substrates were elongated and aligned parallel to groove direction. Preliminary results showed ZO-1 expression, the protein marker for tight junctions, changed under FSS values of 1 dyne/cm$^2$ as opposed to low shear stress conditions, indicating stronger, tighter junctions between cells under the presence of fluid flow.

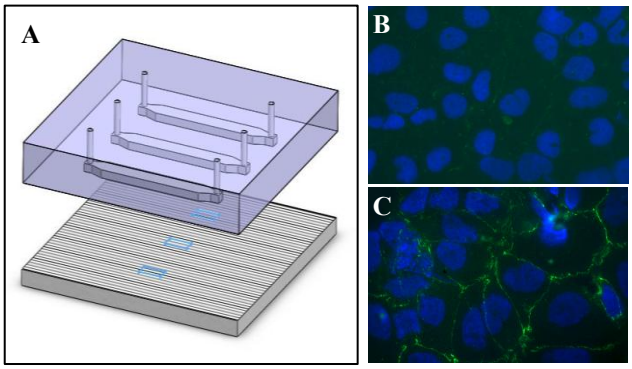


**Figure 1 Assembly and operation of biomimetic platform. (A) The PDMS channels (top) are aligned and sealed over the topographically-patterned substrate (bottom) prior to flow tests. Blue rectangles represent cell adhesion areas. Cells stained for ZO-1 (green) and Hoechst (blue) after 5 hours of 0.01 (B) and 1.0 (C) dynes/cm$^2$ FSS show altered cell-cell junctions.**

**Conclusion:** Unlike traditional cell culture environments, our platform offers the well-controlled, biomimetic cues of topography and FSS in which we can study and influence cell response to multiple physical parameters. This will significantly contribute to research surrounding cellular superstructure formations, tissue engineering and regenerative medicine.

**References:** [1] Teixeira AI. Biomaterials. 2006:3945-3954. [2] Dalby MJ. Nature Mat. 2007: 997-1002.