P-2.4

Exploring Cortex Polarity: Mechanics And Fluidity Via Inverted Cell Culture & Image Analysis of Cell Sheets

Niklas Klatt [1]

[1] University of Goettingen, Goettingen, Germany

Epithelial cells are essential for tissue formation and remodeling, with their mechanical properties determined by the viscoelastic actin cortex. Madin-Darby Canine Kidney II (MDCK II) cells are an ideal model for studying epithelial polarity and actin dynamics. While studies focus on apical mechanics, basal mechanics remain less explored. To address this, we use an inverted cell culture approach on TEM grids, enabling access to both apical and basolateral sides. This setup allows us to study viscoelastic changes related to cell-cell junction integrity, using genetic variants and a γ-actin knockout (KO) cell line. Our findings show that γ-actin forms stiffer networks compared to β-actin, which is more flexible. The sandwich cleavage method isolated cell cortices for image analysis, including bubble analysis, watershed segmentation, and Detrended Spatial Variance Analysis (DSVA), revealing insights into actin network properties such as mesh size and entanglement length. These results link mechanical changes to structural alterations in the actin cortex, highlighting the mechanical polarity of epithelial cells and its dependence on functional cell-cell contacts. This approach integrates whole-cell and isolated fragment studies, advancing our understanding of epithelial cell mechanics.