Nano Life Science Institute (WPI-NanoLSI) Open Seminar

Studying cell-matrix adhesion using atomic force microscopy

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Abstract

In tissues cells are typically surrounded by the extracellular matrix (ECM), a well-structured and flexible scaffold composed of different fibrillar macromolecules, including collagen and fibronectin. Characterizing the complex adhesive, mechanical and signalling interactions between cells and ECM is essential for better understanding how cells perform their tissue-specific function.

Here I review how we have used atomic force microscopy (AFM) in combination with optical microscopy to study cell-matrix interactions at the nanoscale. Importantly, by performing time-lapse AFM imaging, the dynamics of cell-induced reorganization of matrix fibrils can be directly visualized in real-time at the molecular scale. Fast AFM scanning can also visualize individual steps during the self-assembly process of collagen fibrils from its molecular building blocks, providing unique insight into mechanisms driving collagen matrix assembly. Furthermore, we have used AFM imaging to generate the first high-resolution images of both the intracellular and extracellular side of cell-matrix focal adhesion contacts in either *de-roofed* or *inverted* cells under physiological conditions, revealing a co-alignment between the intracellular actin-based cytoskeleton and extracellular matrix fibrils.

In addition, AFM-based single-cell force spectroscopy (SCFS) is a sensitive method for measuring cell-matrix adhesion forces down to the single-molecule level. However, these experiments commonly use rigid surfaces homogeneously coated with ECM components as artificial cell adhesion substrates, while ECM molecules in their natural cellular environment, are arranged into complex elastic scaffolds displaying a high

degree of nano- and microscale structuring. To systematically investigate the effect of matrix structure and mechanics on cell adhesion, we employed different micro-patterning techniques, including *microcontact printing* and *direct laser writing*, to produce complex 2D and 3D cell adhesion substrates with tuneable adhesive and mechanical properties. These patterned substrates mimic the cellular environment more closely than homogeneous substrates and ensure that results from SCFS adhesion experiments can be applied for understanding physiological interactions between cells and ECM. Furthermore, the mechanical properties of such flexible cell culture substrates can be determined by AFM indentation measurements, providing important insight into the mechanical interplay between cells and matrix.

Together, these experiments demonstrate that the complementary use of AFM and light microscopy serves as a useful and versatile toolbox to study the interaction of living cells with their matrix surrounding quantitatively and across dimensions.

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