Applications of Atomic Force Microscopy and Single-Cell Force Spectroscopy in Cell Adhesion Research

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INTRODUCTION

Atomic force microscopy (AFM) generates topographic images by raster-scanning a sharp tip over a sample surface. Initially invented as a force imaging device, today AFM is also used as an imaging and force spectroscopy device, which allows measurements on a molecular resolution. AFM has been used in many different fields, including cell adhesion, collagen, TIRF microscopy, and single-cell force spectroscopy, expanding the use of SPM in cell biology.

Key Words

scanning probe microscopy, atomic force microscopy, single-cell force-spectroscopy, cell adhesion, collagen, TIRF microscopy
Figure 2: Molecular-scale interactions between cells and collagen microfibrils revealed by atomic force microscope (AFM) scanning. (A) Highly-ordered array of collagen microfibrils. Bar = 500 nm. (B) Fibroblasts polarizing along the collagen microfibrils. Bar = 10 µm. (C) AFM and (D) scanning electron microscope images of a fixed cell contacting and rearranging collagen fibrils. Bars = 1 µm. (E-H) Time-lapse AFM imaging reveals dynamic matrix remodelling by a living fibroblast cell. Bar = 2 µm.

EXAMPLE APPLICATIONS

High-Resolution Imaging of Living Cells by Atomic Force Microscopy

It is becoming increasingly evident that cells recognize and respond to nanotopographic cues in their environment. However, the mechanisms by which cells sense these small structural features are still under investigation.

We have been particularly interested in interactions of different mammalian cell types with arrays of nanoscopic, parallel collagen microfibrils [7] (Figure 2A). On these artificial matrices, different cell types efficiently align and migrate in the direction of the fibrils (Figure 2B) [8]. Clarifying the mechanisms by which cells adapt to the preset fibril orientation required a closer look at the cell-matrix interactions. However, individual collagen microfibrils are typically 5-15 nm wide, so that they cannot be resolved by diffraction-limited light microscopy.

In contrast, AFM scanning of chemically-fixed cells on the collagen surface reveals extensive matrix remodelling (Figure 2C). During polarization, cells apparently contact individual fibrils and deform them noticeably. Although similar processes involving cellular extensions contacting collagen fibrils can be visualized by scanning electron microscopy (Figure 2D), only time-lapse imaging by AFM in liquid is able to visualize the dynamics of fibril rearrangement by living cells (Figure 2A-D).

These time-lapse images generated by continuous AFM scanning reveals asymmetric matrix deformation during cell alignment due to an anisotropy in matrix rigidity [9]. As a result, cells develop traction forces and polarize only along the fibril direction. Better understanding such molecular-scale interactions between cells and their environment may provide important groundwork for using artificial matrices for future applications in tissue engineering and biotechnology.

Integrating Single-Cell Scanning Force and Total Internal Reflection Microscopy

Initial contact points between cells and macromolecules in their environment are frequently established by individual adhesion receptors. Cells then reinforce adhesion by clustering these adhesion receptors into larger adhesive units. The formation of such large adhesion clusters is most commonly studied by light microscopy using cells expressing fluorescently labelled adhesion molecules. However, with the high force resolution of AFM, the dynamics of receptor clustering can also be monitored on the force level [10, 11].

An ongoing experimental challenge has been to combine sensitive adhesion force measurements by AFM-SCFS with established fluorescence microscopy techniques, such as confocal laser scanning (CLS) or total internal reflection (TIRF) microscopy. The initial formation of adhesion clusters and the subsequent rupture during cell retraction can thus be monitored visually and correlated to the adhesion force information provided by AFM-SCFS [12]. In this way, unique quantitative information about the strength of individual adhesion clusters is becoming available.

A schematic depiction of a combined AFM/TIRF microscopy platform suitable to perform these experiments is shown in Figure 3B. A single cell expressing a fluorescently labelled adhesion molecule is attached to the AFM cantilever (Figure 3C). The formation of adhesion clusters during cell-surface can then be visualized by TIRF during cell-surface contact.

Microstructured Cell Adhesion Substrates

Many established adhesion assays measure cell attachment to surfaces homogeneously-coated with a single component of the extracellular matrix (ECM). In tissues, however, cells are usually exposed to a complex mixture of ECM molecules with which they interact in a spatially and temporally controlled manner. As model systems to emulate some of the structural complexity of natural cell environments, structured adhesion surfaces can be produced by microcontact printing (µC) (Figure 4A). Microstructured adhesion surfaces can also be used to directly compare the adhesion strength of an individual cell to two different adhesive ECM coatings.

We have prepared bifunctional adhesion substrates featuring alternating stripes of two different adhesion molecules (Figure 4B). Single living cells immobilized on an AFM cantilever are then alternately pressed on both coatings and detachment forces are determined by SCFS. Performing SCFS on heterofunctional adhesion surfaces therefore provides quantitative and comparative information on the adhesion profile of individual cells not obtainable from bulk measurements on homogeneous adhesion substrates.

Using AFM to Calibrate Novel Cell Scaffolds for Cell Biology and Tissue Engineering

In the laboratory, cells are most commonly cultured on flat and rigid supports, such as glass or tissue culture plastic, although the natural environment of many cells is formed by pliable, three-dimensional scaffolds. Recent studies suggest that the shape and flexibility of the extracellular environment governs important cellular processes, such as differentiation and growth. Understanding the complex mechan-
ical interplay between cells and their environment therefore requires an accurate quantitation of cellular traction forces in 3D.

To examine the scale of such traction forces, flexible three-dimensional cell culture substrates were produced by direct laser writing in the research groups of Martin Wegner and Martin Bastmeyer at the Karlsruhe Institute for Technology (Figure 5A). When functionalized with appropriate adhesion molecules, these scaffolds are readily populated by different cell types, such as fibroblast cells or even rhythmically beating heart muscle cells. Light microscopy observations demonstrated that the traction forces exerted by even single cells are sufficient to deform these structures visibly, highlighting the outstanding pliability of the scaffold polymer. However, quantitatively correlating scaffold pliability to cellular traction forces requires that the mechanical properties of the scaffold is known precisely.

Using the AFM cantilever as a microindenter, we could determine the mechanical properties of the scaffolds. Using the piezo-driven approach mechanism of the AFM, the cantilever was lowered onto a beam element until a preset contact force was reached. Mounting the AFM on top of an inverted light microscope allowed precise positioning of the AFM cantilever over flexible elements of the cell-culture scaffold (Figure 5B). From a simultaneously recorded force-distance curve, the piezo extension distance was determined between the moment of cantilever/beam contact and achievement of the preset contact force. Using this information to generate a calibration force/indentation curve, we could approximate cellular traction forces in the range of 10 to 100 nN (Figure 5C).

Thus, advanced microstructuring techniques in combination with light microscopy and AFM-based force spectroscopy provide an integrated platform to fabricate and characterize novel cell culture scaffolds. In this way, these experiments set the stage for future studies systematically investigating the influence of three-dimensionality and elasticity on the differentiation of individual cells and on tissue formation.

OUTLOOK

Due to its superior resolution, AFM imaging has provided important structural information on native biological samples and cells. Recently, ingenious new light microscopy techniques, such as photo-activated localization microscopy (PALM) [13, 14] stimulated emission depletion (STED) microscopy [15] or structured illumination have increased the resolution of fluorescence microscopy far beyond the diffraction limit. In the X-Y direction, several of these advanced light microscopy techniques are now offering a similar resolution typically achieved by AFM on biological samples in liquid.

Nevertheless, in many cases AFM still provides superior sample height information with a resolution below 1 nanometer. A promising approach is to overlay high-resolution AFM

Figure 3: Combining AFM-based single-cell force spectroscopy (SCFS) and total internal reflection fluorescence (TIRF) microscopy
(A) Sensitive adhesion force measurements on single cells. A single living cell attached to an AFM cantilever (I) is moved towards an adhesive surface (II). After a pre-set contact time, the cantilever is retracted (III) until the cell is fully separated from the surface (IV). From a simultaneously recorded force curve, cellular adhesion forces down to the single-molecule level can be determined.
(B) Schematic depiction of an AFM head scanner mounted onto an inverted optical microscope. A cell expressing a fluorescently tagged adhesion protein is attached to the AFM cantilever (C) and positioned so that the cell-substrate interface can be imaged by TIRF.
(C) TIRF imaging visualizes the formation of early adhesion sites appearing within the cell-substrate adhesion site during SCFS.
Cells can exert forces on their surrounding matrix through myocytes populating a biocompatible cell culture scaffold. Cellular contraction forces deform beam elements within the scaffold (see insert).

Mechanical properties of the scaffold are determined using AFM indentation measurements, recording scaffold bending in response to an applied external force.

From the resulting bending curves, cellular traction forces in the range of ~10 to 100 nanonewtons (nN) can be approximated.

The topographies with images showing the localization of specifically-labeled biomolecules as determined by light microscopy. Likewise, it will be interesting to combine AFM cell adhesion force measurements with high-resolution optical imaging of adhesion receptors in the adhesion contact zone.

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**REFERENCES**