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Review

Atomic force microscopy for the investigation of molecular and cellular behavior

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ABSTRACT

The present review details the methods used for the measurement of cells and their exudates using atomic force microscopy (AFM) and outlines the general conclusions drawn by the mechanical characterization of biological materials through this method. AFM is a material characterization technique that can be operated in liquid conditions, allowing its use for the investigation of the mechanical properties of biological materials in their native environments. AFM has been used for the mechanical investigation of proteins, nucleic acids, biofilms, secretions, membrane bilayers, tissues and bacterial or eukaryotic cells; however, comparison between studies is difficult due to variances between tip sizes and morphologies, sample fixation and immobilization strategies, conditions of measurement and the mechanical parameters used for the quantification of biomaterial response. Although standard protocols for the AFM investigation of biological materials are limited and minor differences in measurement conditions may create large discrepancies, the method is nonetheless highly effective for comparatively evaluating the mechanical integrity of biomaterials and can be used for the real-time acquisition of elasticity data following the introduction of a chemical or mechanical stimulus. While it is currently of limited diagnostic value, the technique is also useful for basic research in cancer biology and the characterization of disease progression and wound healing processes.

Contents

1. Introduction

Both uni- and multicellular organisms coordinate their behavior using a network of chemical, electrical and mechanical signals, and employ a variety of sensory mechanisms to perceive and respond to internal or external regulatory cues (Ricca et al., 2013; Johnson, 2013). In unicellular organisms, such signals may assist in feeding, attracting conspecifics, synchronizing reproductive cycles or initiating defense mechanisms in a hostile environment (Dufour and Levesque, 2013); while multicellular life utilizes cell signaling networks to regulate cell recruitment, adhesion, differentiation, proliferation and death (Watt and Huck, 2013; Ravichandran, 2003; Zoranovic et al., 2013; Jaenisch and Bird, 2003; Owens and Wise, 1997). As the latter category of processes are integral to sustain complex life, the characterization of regulatory signals is of great

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importance to the medical and biological sciences, and much work has been performed to elucidate the links between environmental cues and cellular processes (Ando et al., 2013; Carvalho et al., 2013; Dorobantu et al., 2012). However, while the chemical and biological environment of cells are relatively well-defined, the mechanical properties of cells and their immediate environment are investigated only to a lesser degree; partly because of the high complexity and variability of the mechanical interactions exhibited by cells and partly due to limitations associated with the high-resolution mechanical probing of cell surfaces and interiors (Cohen and Kalfon-Cohen, 2013). Nonetheless, considerable effort has been spent to establish how cells perceive and act upon the physical characteristics of nearby substrates (Shao et al., 1996), and to determine how the mechanical properties of cells and tissues are altered in response to disease state or environmental factors, using material characterization tools such as magnetic twisting cytometry, optical tweezers, microneedle probes, scanning acoustic microscopy and atomic force microscopy (Neuman and Nagy, 2008).

Atomic force microscopy (AFM) is a characterization tool that measures the topology and material properties of surfaces by recording the deflection of a metallic probe (or "tip") as it moves over the target surface. AFM can be operated under three principal modes: In contact mode, the tip is dragged directly over the surface and deflects away due to a repulsive Coulombic interaction, while in non-contact mode it is held at a short (typically <100 nm) distance over the sample and oscillates at a frequency that depends on the attractive van der Waals forces acting upon it. In tapping or intermittent contact mode, the tip is kept oscillating above the sample, and the oscillation frequency changes as the tip approaches the surface at regular intervals (Giessibl, 2003). Contact and intermittent modes are particularly suitable for the probing of biological samples, due to their applicability in liquid media (Danino, 2008). Despite considerable losses in resolution, a liquid sample environment allows cellular imaging in a native (or pseudo-native) environment and, more importantly, permits the direct investigation of mechanical changes on a live cell surface in response to an introduced stimulus (Liu et al., 2005). Time-lapse elastographs taken in this fashion have been utilized for a diverse array of applications, including to visualize the formation of amyloid (Harper et al., 1997) or collagen (Revenko et al., 1994) fibers under different environmental conditions, determine how membrane integrity is altered in the presence of antibiotics (Fantner et al., 2010a), or record the production and dissolution of cytoskeletal elements during cell movement (Rotsch and Radmacher, 2000). In addition, it is possible to utilize the AFM tip as a stimulus to elicit a response from the target cell, and the probe itself can be functionalized with ligand molecules to determine the affinity of the cell membrane to a particular biological moiety.

Due to the versatility and potential application areas of AFM, the technique has attracted substantial interest in biomechanical research, and has been used in the characterization of a great variety of tissues, cells and sub-cellular structures in both live condition and following fixing and drying. The present review aims to cover those studies that focus on the differences in mechanical properties associated with pathological conditions or changes in environmental cues, and emphasizes the importance of the mechanical Umwelt in modulating the behavior of both single-celled and multicellular systems.

2. Effect of probe morphology, composition and surface chemistry

Before discussing the AFM imaging of biological materials, the importance of AFM tip choice should be underlined. The diameters, materials, morphologies and cantilever lengths of commercial AFM probes show considerable variance, and optimal performance requires the use of a probe conductive to the task at hand. The composition of the sample material should be taken into consideration to choose the spring constant of the AFM probe, as softer materials, such as cells, may be damaged over repeated contact with the AFM tip (Costa, 2003). In addition, depending on the area to be scanned, it may be desirable to increase or decrease the tip diameter. Larger tips are associated with lower resolution, but can be utilized to scan larger sample areas without compromising tip integrity, as sharper tips may experience significant wear over long scanning distances, such as when scanning cells. On the other hand, sharper tips are capable of resolving smaller features to a greater extent, which is invaluable when measuring proteins and other nanoscale biological materials. Consequently, differences in material stiffness that are evident under nanoscale investigation may be unmeasurable using microscale tips (Stolz et al., 2009a). If adhesion data is to be collected, the material and morphology of the AFM tip (alongside substrate properties) also determines the suitable model for use in elasticity calculations (Fig. 1).

AFM probes can also be functionalized in order to characterize the interaction between two specific types of biological moieties, such as between a receptor and its ligand. This type of interaction is best exemplified by biotin and avidin, used by Colton et al. in their hallmark paper to illustrate the possibility of using AFM to directly evaluate the strength of molecular interactions (Lee et al., 1994). Mechanical properties of a wide variety of proteins have now been elucidated, including the interactions between antibodies and their corresponding antigens (Allen et al., 1997), actin and myosin (Kodera et al., 2010), osteopontin and integrin (Lee et al., 2007), and various cell adhesion proteoglycans (Dammer et al., 1995). Such proteins can either be covalently tethered to the target material (Ebner et al., 2007; Kamruzzahan et al., 2006) or attached by drying the protein sample on the surface (Florin et al., 1995). In addition, the mechanical strength of the constituent domains of a single protein can be evaluated by attaching that protein to a surface and using the AFM tip to stretch it (Li et al., 2003). This results in the gradual unfolding of the protein, and the unwinding of each domain is associated with a momentary drop in force. Tensile characteristics of the immunoglobulin and fibronectin III domains of titin were investigated using this method (Rief et al., 1998), and the ability of the bacterial ribonuclease barnase to withstand force was likewise evaluated by incorporating this protein into a chimeric construct consisting of four TI I27 and three barnase subunits (Best et al., 2001).

DNA and RNA can also be immobilized and characterized in a similar manner, and the mechanical investigation of DNA molecules of varying lengths and configurations has been performed using AFM (Mao et al., 1999; Hansma et al., 1995). In addition to the determination of covalent bond strength in ssDNA or dsDNA, it is also possible to evaluate the strength of the bonds between complementary strands in a short dsDNA piece, or to determine the forces necessary to stretch an intact DNA molecule (Hansma et al., 1996). High-resolution AFM imaging can also be used to characterize the physical structure of a DNA helix (Fig. 2), and more complex DNA architectures and DNA – protein interactions can be visualized and characterized using atomic force microscopy. Yaneva et al., for example, confirmed that DNA-dependent protein kinase (DNA-PK) can bind to DNA without the assistance of Ku proteins, and that the latter shows a time-dependent preference for strand ends, by visualizing DNA-Ku and DNA-DNA-PK interactions using AFM (Yaneva et al., 1997). The affinity between cells and specific proteins can also be assessed by indenting the cell of interest with an AFM tip functionalized with the protein of interest (Han et al., 1995). Gaub et al. reported a method to distinguish between individual red blood cell origins in a mixture of A- and O-group erythrocytes, using an AFM tip functionalized with *Helix pomatia* lectin (Grandbois et al., 2000).