Micron 43 (2012) 1380–1389

Contents lists available at SciVerse ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Fabricated cantilever for AFM measurements and manipulations: Pre-stress analysis of stress fibers

Shinichi Machida[a,b,*], Takahiro Watanabe-Nakayama[a], Masakazu Saito[a], Rehana Afrin[a], Atsushi Ikai[a]

[a] Innovation Laboratory, Tokyo Institute of Technology, 4259-S2-8, Nagatsuta-cho, Midori-ku, Yokohama 226-8503, Japan
[b] International Center for Materials Nanoarchitectonics (MANA), National Institute for Materials Science (NIMS), 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan

ARTICLE INFO

Article history:
Received 30 December 2011
Received in revised form 30 March 2012
Accepted 30 March 2012

Keywords:
Cytoskeleton
Pre-stress
Atomic force microscope (AFM)
Focused ion beam (FIB)
Fabricated cantilever
Force curve

ABSTRACT

The atomic force microscope (AFM) is a highly successful instrument for imaging of nanometer-sized samples and measurement of pico- to nano-Newton forces acting between atoms and molecules, especially in liquid. Generally, commercial AFM cantilevers, which have a sharp tip, are used for AFM experiments. In this review, we introduce micro-fabricated AFM cantilevers and show several applications for cell biology. In manipulation of samples on a cellular scale with a force of tens to hundreds of nano-Newtons, attempts have been made to secure the formation of covalent/non-covalent linkages between the AFM probe and the sample surface. However, present chemistry-based modification protocols of cantilevers do not produce strong enough bonds. To measure the tensile strength and other mechanical properties of actin-based thin filaments in both living and semi-intact fibroblast cells, we fabricated a probe with a hooking function by focused ion beam technology and used it to capture, pull and eventually break a chosen thin filament, which was made visible through fusion with fluorescent proteins. Furthermore, we fabricated a microscoop cantilever specifically designed for pulling a microbead attached to a cell. The microscoop cantilevers can realize high-throughput measurements of cell stiffness.

1. Introduction

As the importance of quantifying cellular responses toward mechanical stimuli from the environment has become increasingly recognized, the measurement of the mechanical properties of biological materials and structures made thereof, is gaining impetus in biological research (Ji and Gao, 2004; Tamura et al., 2007; Dufrêne, 2001). Biomechanics is an established field specialized in the measurement of the mechanical properties of macroscopic biological structures and the functional assimilation of such measurements (Fung, 1993). As our understanding of cellular and tissue level biological functions has been advanced, our explanations of many such functions are now based on observations made at the molecular level. Consequently, the measurement of mechanical stresses and the cellular response against such stresses are being performed at the nano-meter and nano-Newton (nN) level, or even at finer precisions (Tsuda et al., 1996; Deguchi et al., 2006; Afrin et al., 2009). A variety of concepts and tools developed for broader fields of nano-science and nano-technology are now actively used in nano-biomechanics research including molecular handling technology based on scanning probe microscopy and/or optical or magnetic tweezers (Dufrêne, 2001; Binnig et al., 1986; Haber and Wirtz, 2000; Hosu et al., 2003; Ashkin et al., 1986; Svoboda and Block, 1994). Among them, atomic force microscopy (AFM) is one of the most frequently used techniques, and many successful attempts of molecular manipulations such as stretching of single molecules of DNA (Lee et al., 1994), polypeptides (Idiris et al., 2000), proteins (Rief et al., 1997) and polysaccharides (Li et al., 1999), forced unbinding of ligand–receptor pairs (Florin et al., 1994), mapping of membrane proteins on a live cell surface (Kim et al., 2003), extraction of membrane proteins (Afrin et al., 2003), protein expression by means of transfection of cells with plasmid DNA (Afrin et al., 2009; Cuerrier et al., 2007), and hole creation on a live cell surface or nuclear surface, have been reported (Afrin et al., 2009; Obataya et al., 2005). While performing such manipulations at the molecular and cellular level, we realized that in cantilever based manipulations using AFM, the AFM probe could only push the sample. When the probe was modified with specific ligands or covalent cross-linkers, it could pull a specific part of the sample, but the force of pulling was limited to a few tenths of nN in the case where specific antibodies or lectins were used as ligands, and a few nN when covalent cross-linkers were used (Granbois et al., 1999). If we want to apply a larger tensile force than a few nN, it is necessary to resort to multiple bonding of an unspecified nature. In our study of cytoskeletal mechanics, we found it was necessary to manipulate the thin filaments found in the cell with a force as

* Corresponding author. Tel.: +81 45 924 5828; fax: +81 45 924 5828.
E-mail address: smachida@bio.titech.ac.jp (S. Machida).

0968-4328/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.micron.2012.03.025

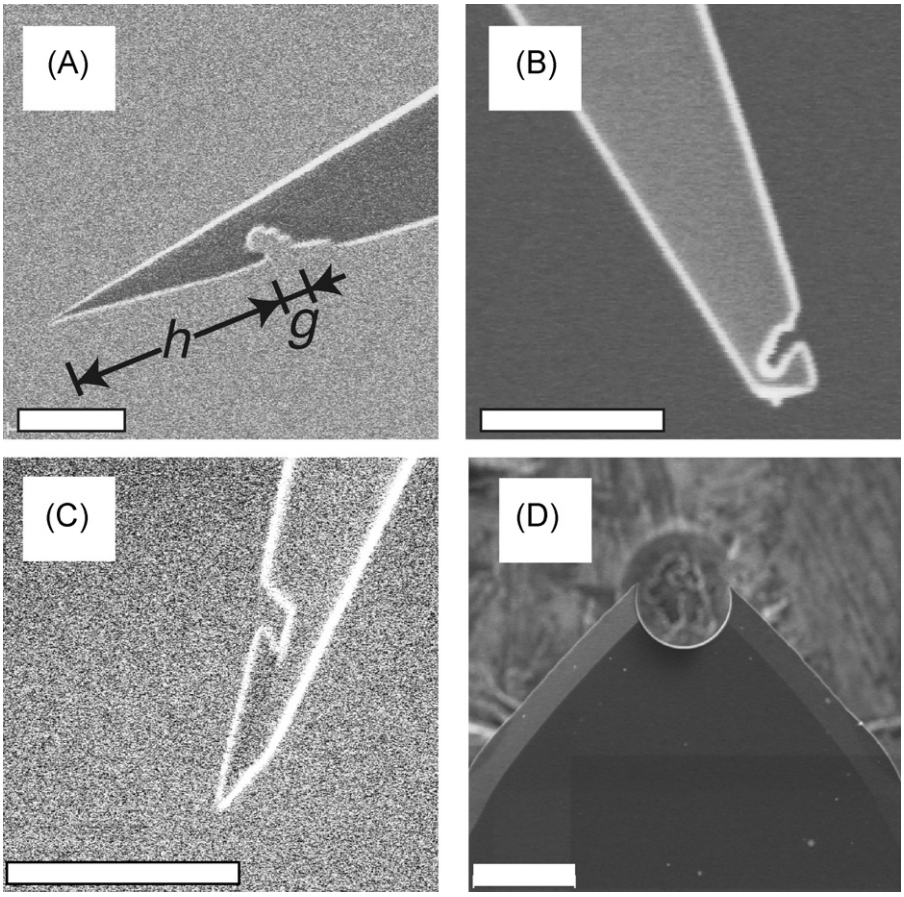

Fig. 1. Scanning ion microscopy images of an as-fabricated nano-hook on AFM cantilever tips (A–C) (Machida et al., 2010) and microscoop cantilever (D) (Watanabe-Nakayama et al., 2010). Each scale bar indicates 1 μm for A–C, and 10 μm for D. The gap size "*g*" and bait point height "*h*" are (A) 0.6 μm and 2.2 μm, (B) 0.09 μm and 0.3 μm, (C) 0.1 μm and 0.8 μm, respectively.

large as several tens to hundreds of nano-Newtons. In our previous report of such manipulations, we employed the lateral torsion mode of the AFM cantilever to measure the tensile strength of a stress fiber and record the deformation of the fiber with a force of tens to hundreds of nN (Hakari et al., 2011). The lateral manipulation of the stress fibers was, however, limited to those lying very close to the solid substrate, and it required a more indirect way of calibration of the cantilever force constant of the torsion mode than in the case of vertical deflections. To effect the pulling of stress fibers with more freedom and accuracy, we described the fabrication of a probe physically modified into a hook shape by focused ion beam (FIB) technology (Machida et al., 2010).

The cytoskeleton of a cell is a major cellular structure, which serves many functions such as maintaining the shape of the cell and covering intracellular transportations (Weeds et al., 1991; Gelfand and Bershadsky, 1991; Avila, 1992; Schroer and Sheetz, 1991). Actin is a major cytoskeletal protein that acts as the driving force to enable the associated cells to move and divide. Knowledge of the mechanical property of the actin filaments is thus important to understand cell dynamics. The mechanical properties of isolated single actin filaments and stress fibers (SFs) have previously been reported by *in vitro* studies (Tsuda et al., 1996; Deguchi et al., 2006).

In this review, we show the results of an *in situ* measurement of the properties of fibroblast SFs, namely those fibers present in semi-intact cells, and the stiffness of the corresponding cells using an AFM system with fabricated AFM cantilevers.

Several hook-shaped AFM cantilevers were specifically designed and fabricated by FIB to assist us in picking up and pulling fibrous structures in the cell and to record the corresponding force curves. A distinct advantage of using such a hook-shaped probe is the possibility of being able to apply much larger forces than those currently used in conventional chemical/biochemical bonding methods (antigen–antibody, lectin–sugar chain, *etc.*). To analyze the measured force curves, one of the force curves was compared with a calculated force curve based on a simple model.

To measure the mechanical response of a cell, a round-shaped tip or an AFM cantilever with an attached bead, are often used because AFM cantilevers with sharp tips tend to scratch the cell surface. AFM cantilevers modified with ligand molecules are often used to detect the interactions between the ligand molecules with the receptors on the cell surface (Florin et al., 1994). However, such modified AFM cantilevers are not suitable for repeated force measurements because the surface of the once-used cantilever is easily contaminated with fragments from the cell sample. Thus, we devised a combination of a microscoop cantilever and a modified microbead for high throughput measurement (Watanabe-Nakayama et al., 2010).

2. Experimental

2.1. Probe processing with focused ion beam technology

As a starting material for focused ion beam (FIB) fabrication, we chose the NANOSENSORS^{TM} Advanced TEC^{TM} series cantilevers (ATEC-CONT and ATEC-FM) for hooks and tip-less cantilevers for scoops (TL-CONT and TL-FM) (NANOSENSORS^{TM}, Neuchatel, Switzerland). The nominal spring constants of the series of cantilevers were 0.02–0.75 N/m (ATEC-CONT), 0.7–9.0 N/m (ATEC-FM), 0.02–0.77 N/m (TL-CONT) and 0.5–9.5 N/m (TL-FM), respectively. The actual spring constants of the cantilevers used in the