Piezoresponse Force Microscopy of Biological Materials and Cells

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Statement of Purpose: Imaging nanoscale piezoresponses in biological materials may reveal their functional hierarchy from the molecular to the cellular levels, progressing towards modeling the coupling between electrical and mechanical phenomena in living biological systems across all levels. Recently, piezoresponse force microscopy (PFM) demonstrated potential for electromechanical imaging the structure of connective and calcified tissues with sub-10 nm resolution [1]. Furthermore, high-resolution electromechanical imaging of a number of model inorganic and biological systems has been demonstrated in aqueous solutions [2,3]. The ability to map electromechanical properties in aqueous media points the way to the electromechanical characterization of live cells and tissues in native-like environments. Here we demonstrate PFM imaging of living mammalian (myocytes, myoblasts and fibroblasts) and algal (diatoms) cells in native-like conditions.

In the future, we plan to use solution PFM for modeling the electromechanical responses of living cells. It can be expected that electromechanical mapping of individual cells will help to understand complex electromechanical responses related to their physiological activity and will possibly serve as the basis for novel diagnostic methods and therapeutic interventions.

Methods: PFM utilizes the inverse piezoeffect to image local polarization orientation. In PFM, a local oscillatory electric field is generated by applying an *ac* voltage to a conducting tip in contact with a sample, and the deformation due to the piezoelectric effect is detected. The imaging paradigm in PFM is complementary to conventional atomic force microscopy (AFM) and scanning tunneling microscopy (STM): while AFM is sensitive to tip-surface forces through the mechanical motion of the cantilever (mechanical detection) and STM is sensitive to tip-bias induced current (current detection), PFM detects bias-induced surface displacement (electromechanical detection).

An Asylum Research (Santa Barbara, CA) MFP3D Atomic Force Microscope (AFM) set up for PFM operation was used with IgorPro version 6.01 software (Wavemetrics). The MFP3D tip holder allowed the tip to be directly biased in liquid. Measurements were performed using Au-coated SiN tips (Olympus TR400PB) in a static fluid cell. Rat skeletal muscle myoblasts (line L8, from ATCC) were grown on glass coverslips. Chicken embryo primary cardiac myocytes and diatoms were attached to collagen type I- and poly-L-lysine-coated ITO (indium tin oxide)-glass coverslips, respectively. Control samples were fixed using 4% paraformaldehyde.

Results: Figure 1 shows PFM images of a fixed rat myoblast (L8) in phosphate buffered saline (PBS). The PFM amplitude image (b) of the myoblast only shows noticeable responses at the edges of the cell, while the PFM phase image (c) shows a prominent response from much of the cell, suggestive of an elastic contribution to the PFM signal. As this cell has been fixed, an electromechanical response from the entire cell has been precluded, and the mechanical properties may have been altered from that of a live cell.

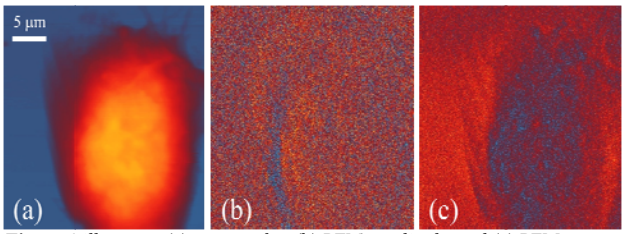

Figure 1 illustrates (a) topography, (b) PFM amplitude, and (c) PFM phase images of a fixed rat myoblast on a glass coverslip in PBS. The Z-ranges are (a) 8 um, (b) 120 pm, and (c) 90 degrees. The images are 30 um x 30 um. For PFM, the tip was biased with 10 V at 422.81 kHz.

Figure 2 demonstrates preliminary data of a living, electromechanically active chicken embryo primary cardiomyocyte on ITO-coated glass in growth media. A pulsed bias of +50 mV was applied to the stationary tip for 10 ms at the spot indicated by the green square on the cell. The deflection rises about 5 seconds after the pulse and returns to the initial value. Over the next minute, smaller oscillations continue regularly.

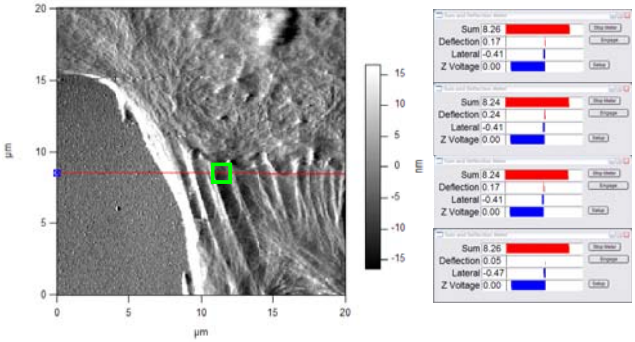

Figure 2 illustrates the electromechanical response of a cardiomyocyte in media. The deflection oscillates regularly after a pulsed bias is applied to the cell, suggesting that the cell beats for about a minute after being electrically stimulated.

Summary: Piezoelectric phenomena at the molecular level may be transduced into electromechanical coupling at the cellular and tissue scales. Piezoresponse Force Microscopy (PFM) imaging of individual living cells allows for demonstration of electromechanical transduction within cells.

References:

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