LS-2-P-3262 CHARACTERIZATION OF ELASTICITY OF WILD-TYPE AND MODIFIED CANCER CELLS USING ATOMIC FORCE MICROSCOPY-BASED METHODS

Crawford K.¹, Duan B.², Sevim S.³, Iyison N. B.², Torun H.¹

¹Department of Electrical and Electronics Engineering, Boğaziçi University, Bebek/Istanbul, Turkey, ²Department of Molecular Biology and Genetics, Boğaziçi University, Bebek/Istanbul, Turkey, ³Department of Mechanical Engineering, Boğaziçi University, Bebek/Istanbul, Turkey

Email of the presenting author: kcrawford@g.hmc.edu

Viscoelastic properties of diseased cells are often drastically altered from their healthy counterparts, most noticeably in cancer cells (1). However, as there is no unifying experimental method across studies, the reported values for Young’s modulus and viscosity obtained using atomic force microscopy (AFM)-based methods vary widely. This study attempts to provide a unifying methodology for AFM of cell material properties and reports on the effects of various experimental and data analysis methods on the reported cell stiffness. As cancer cells are often used as model systems with transfected genes for other biological experiments, the effect of transfection on the viscoelastic properties of the cells is presented. All experiments were performed with Huh7 hepatic cancer cells (donated by Dr. Mehmet Öztürk, Bilkent University, TR). With these methodological goals, the various groups probed were: living non-transfected cells, fixated non-transfected cells, living cells with the transfection reagent only (ThermoScientific, USA), living cells with an empty plasmid, living cells with a cytoplasmically-expressed protein (GFP, Clontech Laboratories, USA), and living cells with a membrane-expressed protein (AST, Genbiotek, TR). The basic method of probing mechanical properties of cells by AFM is shown in Figure 1. Force spectroscopy and AFM force-clamp methods were employed using a sharp-tip probe and colloidal probes with diameters of 10μm and 45μm (NovaScan, USA). However, because the 45μm bead was comparable to the cell diameter, the current Hertzian model must be revised. With the current model, the 45μm bead data yields Young’s moduli that are two orders of magnitude greater than the 10μm bead. Data analysis methods were: basic Hertzian contact model and a finite-thickness Hertzian corrected model (2). Figure 2 summarizes the differences in fit between the Hertzian contact model and the finite-thickness corrected model. Because of the better fit for the finite-thickness model, it was used in the remainder of the analysis. Figure 3 compares results across different types of AFM probes using a Hertzian model; this shows for both experimental groups tested, the sharp tip probe showed a 5x greater Young’s modulus than the 10μm probe. Figure 4 compares all the steps of transfection using the thin-film model; these results show that, from the empty plasmid transfection state to GFP, a 5x difference in cell stiffness was found.

References

Acknowledgement: The authors would like to acknowledge funding from the Whitaker Fellowship and TUBITAK grant no: 212TO11.
Fig. 1: Example set-up of an AFM cell stiffness experiment.

Fig. 2: A typical force curve from an AFM cell stiffness experiment with the Hertzian and thin-film corrected fits. The larger figure is the entire force curve, with the Hertzian fit in green and the thin-film correction fit in red. The inset figure is zoomed around the contact point (in black) to show the goodness of fit.

Fig. 3: The difference in Young’s moduli when using different types of cantilevers, as shown with two different transfection groups. The light grey bars are the 10μm colloidal probe, while the dark grey is the sharp-tipped probe.

Fig. 4: Comparison of Young’s moduli among different transfection groups. NT stands for ‘no transfection.’ TR stands for ‘transfection reagent only.’ EP stands for ‘empty plasmid.’ GFP stands for the cytoplasmically-expressed green fluorescent protein. AST stands for the membrane-expressed allatostatin protein.