Icahn School of Medicine at Mount Sinai

Atomic Force Microscopy Core Facility
Atran Berg Laboratory Building, Rm 3-23
1428 Madison Avenue
New York, NY 10029
T 212-241-2362 (x4AFMC)
F 212-241-4080
kevin.costa@mssm.edu

Atomic Force Microscopy Core Facility
Current Research Projects

From cellular signaling to molecular imaging, our laboratories are home to a broad spectrum of clinical and translational cardiology research using Atomic Force Microscopy (AFM). By taking advantage of AFM technology in a variety of research projects, our investigators are advancing the prevention, diagnosis, and treatment of cardiovascular diseases.

1. Micromechanics of Cardiomyocytes and Intact Tissue

Principal Investigator: Kevin D. Costa, PhD, Department of Cardiology

In the Costa Lab, we investigate how global pump function of the heart relates to the micro-scale mechanical and structural properties of underlying cardiovascular cells and tissues. With our atomic force microscope, we conduct high-resolution imaging and elastographic mapping of sample viscoelastic properties, obtaining molecular specificity by combining confocal microscopy. We use finite element analysis to help interpret measurements, develop new data analysis methods, and improve experimental protocols to maximize the amount of information we can extract from AFM tests.

Relevant Papers:

- Azeloglu, E.U. and Costa, K.D., Cross-bridge cycling gives rise to spatiotemporal heterogeneity of dynamic subcellular mechanics in cardiac myocytes probed with atomic force microscopy, Am J Physiol Heart Circ Physiol, 298 (H853), 2010.
- Azeloglu, E.U., Bhattacharya, J. and Costa, K.D., Atomic force microscope elastography reveals phenotypic differences in alveolar cell stiffness, J Appl Physiol, 105 (652), 2008.
- Costa, K.D., Sim, A. J. and Yin, F. C-P., Non-Hertzian approach to analyzing mechanical properties of endothelial cells probed by atomic force microscopy, J Biomech Eng, 128 (176), 2006.

2. Structural Bases of High Fidelity of DNA Polymerase Delta

Principal Investigator: Aneel K. Aggarwal, PhD, Departments of Structural and Chemical Biology and Oncological Sciences

Our Aggarwal Lab is focused on the crucial role of DNA polymerase δ (Polδ) in replication, and in mutations that lower the fidelity of Polδ and cause cancers in mice and humans. We utilize the AFM to image Polδ and its interaction with DNA at the molecular level. Our aim is to identify high-resolution structures of Polδ and other components involved in replication. By taking advantage of the AFM system's single molecule imaging capabilities, we strive to answer questions about DNA arrangement around these purified components.

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3. **Nanoparticles for Molecular MRI of Atherosclerosis**
Principal Investigator: Zahi A. Fayad, PhD, Departments of Radiology and Cardiology

In the Fayad Lab, we are developing multifunctional lipid-based nanoparticles for molecular imaging. Our platforms are either fluorescent or magnetic, allowing for high contrast detection with optical techniques and MRI. One of our aims is to develop HDL mimicking nanoparticles to detect atherosclerosis in vivo. We employ AFM to visualize molecular surface structure of HDL nanoparticles; to track the real time process of their uptake by macrophage cells; and to characterize surface structure and micromechanical properties of atherosclerotic lesions in vascular tissue sections exposed to HDL nanoparticles.

4. **Functions of Regulatory Motifs in Signaling Networks**
Principal Investigator: Ravi Iyengar, PhD, Department of Pharmacology and Systems Therapeutics

The Iyengar Lab utilizes system level approaches to study how multiple cellular signals can be integrated to stimulate and sustain neurite outgrowth in Neuro2A cells. We are interested in how signals from G protein coupled receptors and those from membrane forces transduced by the integrin receptor, integrate in real time to control the outgrowth processes in neurons. We use the AFM in force mode to apply targeted mechanical stimuli along the length of the neurite while simultaneously measuring the intracellular signaling response using integrated FRET probes. Our goal is to understand the spatial basis for the signal integration process. In doing so, we are striving to develop novel spatially specified networks to define the integration of mechanical and chemical signals.

5. **Mechanisms Underlying Mitochondrial Dysfunction in the Diabetic Heart**
Principal Investigator: Fadi G. Akar, PhD, Department of Cardiology

The Akar Group recognizes that mitochondrial dysfunction is the hallmark of a wide range of human pathologies, and is the origin of many cardiovascular disorders. With that in mind, our goal is to identify the mechanistic inter-relationship between altered mitochondrial energetics and arrhythmias in cardiovascular disorders associated with oxidative stress, such as diabetes. We employ AFM techniques to apply targeted mechanical stressors to isolated cardiac cells, including physiologically relevant biochemical and mitochondrial oscillations. Our goal is to determine the critical threshold of mechanical stress required for pathological mitochondrial membrane potential oscillations in normal and diabetic animals. By probing at the subcellular level with the AFM, we hope to uncover mechanisms by which these targeted mechanical perturbations scale to the entire mitochondrial network. We are also exploring how these mechanisms are affected by the disease process itself.

6. **Control of Local Calcium Signaling in the Heart**
Principal Investigator: Eric A. Sobie, PhD, Department of Pharmacology & Systems Therapeutics

Our research in the Sobie group concerns mechanisms by which calcium release from intracellular stores is controlled in the heart, and how this process is disrupted in disease

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states. We focus on the elementary unit of calcium release, Ca^{2+} sparks, and particularly the coordinated opening of ryanodine receptors that facilitate these sparks. We have discovered that heart failure can disrupt the association between these receptors and the cell membrane because of severe alterations to the membrane structure. Fluorescence imaging provides us with an overview of the gross changes in myocyte structure. The limited optical resolution limits our access to quantitative information, such as the organization of transverse tubules, details which could lead to better modeling of their role in calcium signaling. Instead, we utilize AFM to provide a high resolution characterization of these subcellular structures. We also perform targeted physical disruption, such as indenting the transverse tubules to study the effect on calcium release in the myocyte.

7. PICOT and Cardiac Hypertrophy

Pricipal Investigator: Roger J. Hajjar, MD, Department of Cardiology

The Hajjar group is concerned with the fact that pressure-overload induced hypertrophy during valvular or hypertensive heart diseases is one of the most common causes of congestive heart failure. Past studies have suggested roles for protein kinase C (PKC) in the hypertrophic response. Our researchers have demonstrated that elevated PKC activity in cardiac hypertrophy is counteracted by a feedback inhibitor mechanism dependent on a cytosolic PKC inhibitor protein PKC-Interacting Cousin of Thioredoxin (PICOT). We have characterized the molecular mechanism of PICOT activity in PKC regulation and evaluated the physiological consequences of PICOT overexpression in rodent models of cardiac hypertrophy and heart failure. We hypothesize that PICOT overexpression can also lead to alterations in sarcomere structure, which promotes ventricular dilation. We use AFM to study the effects of PICOT on the elastic properties of beating isolated cardiomyocytes and intact mouse hearts. Our goal is to better understand PICOT signaling mechanisms, allowing us to design novel therapeutic strategies to block the development of cardiac hypetrophy and the progression to heart failure.

8. Tendon Response to In Vivo Fatigue Damage

Principal Investigator: Evan L. Flatow, MD, Department of Orthopaedics

In the Flatow Lab, we are keenly aware that understanding local tendon response to pre-rupture fatigue is critical to the prevention of tendinopathies. We have shown an increase in structural damage associated with higher levels of tendon fatigue by evaluating temporal response of the tendon to low-level fatigue applied by in vivo cyclic loading of the patellar tendon of rats. We detected an early transient response, followed by stabilizing of the bulk mechanics of the tendon. Thus, there is an unknown connection between the micro- and macromechanical responses of the tendon. We use AFM indentation techniques to measure the structural and mechanical effects of the in vivo cyclic fatigue protocol at the micro level along with second harmonic generation imaging to evaluate the level of damage at the test site.