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# Cellular level robotic surgery: Nanodissection of intermediate filaments in live keratinocytes

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**Abstract**

We present the nanosurgery on the cytoskeleton of live cells using AFM based nanorobotics to achieve adhesiolysis and mimic the effect of pathophysiological modulation of intercellular adhesion. Nanosurgery successfully severs the intermediate filament bundles and disrupts cell–cell adhesion similar to the desmosomal protein disassembly in autoimmune disease, or the cationic modulation of desmosome formation. Our nanomechanical analysis revealed that adhesion loss results in a decrease in cellular stiffness in both cases of biochemical modulation of the desmosome junctions and mechanical disruption of intercellular adhesion, supporting the notion that intercellular adhesion through intermediate filaments anchors the cell structure as focal adhesion does and that intermediate filaments are integral components in cell mechanical integrity. The surgical process could potentially help reveal the mechanism of autoimmune pathology-induced cell–cell adhesion loss as well as its related pathways that lead to cell apoptosis.

***From the Clinical Editor:*** This team of authors performed nanosurgery on the cytoskeleton of live cells using AFM based nanorobotics to achieve adhesiolysis, and mimic the effect of pathophysiological modulation of intercellular adhesions. This method could potentially help reveal the mechanism of autoimmune pathology-induced cell–cell adhesion loss as well as its related pathways that lead to cell apoptosis.


*Key words:* Atomic Force Microscopy; Nanosurgery; Intermediate filament; Mechanical property; Cell–cell adhesion; Desmosome

Surgery is a centuries-old medical technique that uses tissue/organ specific physical intervention to treat illness. Modern surgery has been taken to new levels with engineering technology that places robots at the center of operations to achieve higher precision, thus the name robotic surgery.[1] Microsurgery has been successfully used on small blood vessels or nerves with dimensions of 1 $mm^2$. New technology has been pushing surgical precision to new limits. A new medical frontier looms in the horizon—surgery on cells, the smallest living unit, with precision down to the nanoscale and operation at the molecular level. Femto-second laser nanosurgery can achieve the desired non-invasiveness, only at the sub-micron resolution.[2]

We have implemented atomic force microscopy (AFM) as a surgical instrument to operate on living cells with resolution down to nanometers. AFM has been applied in investigations of biological processes with high resolution imaging and mechanical property characterization.[3] With a sharp probe as its main imaging unit, it is naturally a surgery instrument at the nanoscale. AFM was first developed as an imaging instrument, and a number of biological samples have been visualized ranging from tissues, to cells, to cellular components, and even molecules with vertical resolution as high as an angstrom.[4,5] Imaging by AFM is the direct result of the scanning motion driven by the piezo

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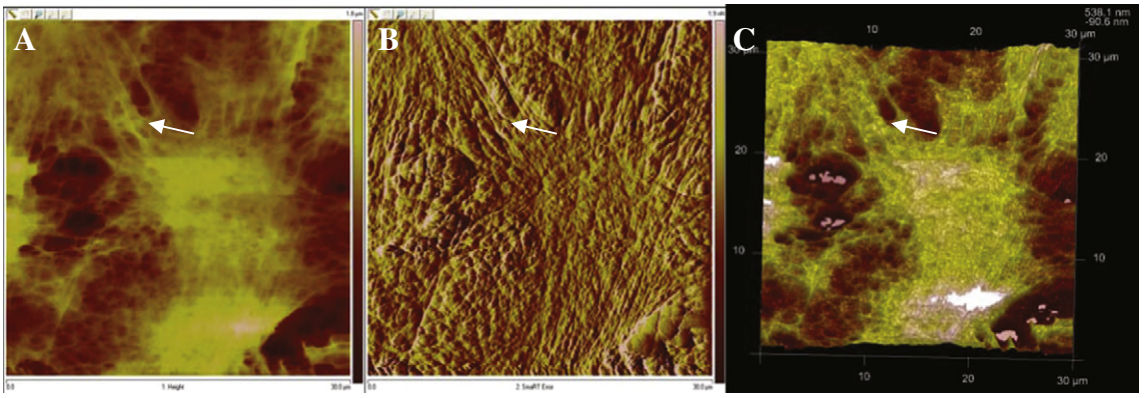


Figure 1. Intermediate filament bundles imaged with AFM show stranded filamentous structures at the peripheral of the cells (arrows). **(A)** Topography image. **(B)** Deflection error image. **(C)** Three dimensional rendering. Scan size: 30 µm.

actuation unit. To perform the nanosurgery, the position of the tip is not linearly related to applied signal and the applied voltage should be an arbitrary shape. Besides, most operations on biological samples are performed in liquid. Thus, the viscoelasticity of the sample requires a higher response frequency, and a nanorobotic system was developed to address these issues.[6] Studies in cell biology have been taken to a whole new level with the introduction of AFM based nanorobotics. Equipped with the precision control capabilities, the nanorobot can have a workspace of nanoscale, but the controllability of a traditional robot. Thus, the handling of matters in the nanoscale would be achieved with stability, accuracy and efficiency. AFM nanorobotic systems allow human-directed position, velocity and force control with high frequency feedback. Most importantly, it can provide the operator with the real-time imaging of manipulation results from the fast-imaging based local scanning.[7]

Researchers have reported on the dissection of DNA molecules, proteins, cell membranes, intermediate filaments, among other structures.[8–12] Studies have shown the capability of AFM nanosurgery on fixed cell membranes by making incisions with a resolution of 100 nm or less.[13,14] Isolated gap junctions were dissected to resolve their detailed structures.[12] In addition, isolated intermediate filaments were stretched with an AFM probe to test their tensile strength.[15] At the current stage, most of these nanomanipulation experiments were performed to demonstrate the basic capability of AFM based nanomanipulation systems in handling and changing biological matters, since it remains challenging in controlling the tip-live cell interaction in living conditions. We report the use of nanorobotics for the nanodissection of cellular structures on living keratinocytes *in situ* to achieve adhesiolysis and to mimic the effect of pathophysiological modulations of intercellular adhesion.

Keratinocytes are the major component of the epidermis, or top layer of skin, and contains desmosome-based cell–cell adhesion structures. Desmosomes are cadherin based intercellular junctions in epithelial cells to maintain their mechanical integrity and provide strength,[16] acting like a "spot-welding" point connecting intermediate filaments from neighboring keratinocytes. The AFM images in Figure 1, *A* and *B* captured the intermediate filaments underneath the cell membrane between neighboring cells. The structures agree well with the fluorescence images from the work published by Godsel and coworkers,[17] in which the yellow dots display the desmosomal complexes surrounding cells at the periphery and the red shows the intermediate filaments. There is a narrow area a few hundred nanometers in length and approximately 50 nm in width when characterized by an electron microscope that comprises a cluster of proteins. Among them, trans-membrane desmosomal cadherins, desmogleins (Dsg) and desmocollins (Dsc) bind to the armadillo family protein plakoglobin (PG), which anchors the plakin family member desmoplakin (DP) and plakophilins (PKP).[18] The lateral interactions among these proteins allow tethering to intermediate filaments. Dsc molecules from neighboring cells form covalent bonds in a $Ca^{2+}$ dependent manner.

Desmosomes are the target of autoimmune antibodies in several skin disorders, including pemphigus vulgaris (PV), in which intercellular adhesion is disrupted through disassembly of desmosomal proteins, especially Dsg3.[19] We have previously investigated the structural characterization of desmosomes in cultured keratinocytes lines and shown that the loss of intercellular adhesion via desmosomal disruption[20] can lead to mechanical property change.[21] According to the cellular tensegrity model, the cell cytoskeleton is a tensional integrity structure bounded by the cell membrane, in which microtubules are the compressive element while actin filaments and intermediate filaments are tensional elements.[22,23] The structure bears most external forces on the cell and is able to maintain force balance through interaction with extracellular matrix (ECM), via mainly focal adhesions, serving as the anchoring point for the cell body.[24,25] Desmosomes link the intermediate filaments of neighboring cells through cell–cell adhesions, and the intermediate filaments are the tensional elements. Therefore, it is logical to consider the roles desmosomes play in the tensegrity structure.

In this study, we sought to investigate the integral role of desmosomes in maintaining the cellular structure by modulating the desmosome mechanically using nanosurgery. We applied the AFM based nanorobotic system to mechanically disrupt the intercellular adhesion to mimic the pathophysiological treatment. Nanosurgery successfully dissected the cellular connection by severing the intermediate filaments underneath the cell membrane. The biochemical and mechanical modulation were verified by AFM nanomechanical analysis which shows the decrease of cellular stiffness after the three mechanisms of modulation, indicating that the tension in the cytoskeleton structure was released by loss of intercellular connection. A mechanical model