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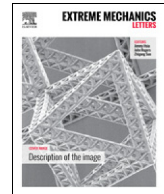

Light induced, localized, and abrupt force relaxations in fibroblast cells on soft substrates

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ABSTRACT

Exposure to fluorescent excitation light is known to adversely affect living cells. Here, we provide experimental evidence showing fibroblast cells relax abruptly during illumination with fluorescent excitation light. Cells were plated on polyacrylamide (PA) gels embedded with fluorescent microbeads. The beads served as fiducial markers to track substrate displacements induced by cell force. When the cells adhere, they apply traction forces to their underlying surface, causing the substrate to contract inward with respect to the cell boundary. We find that contractile cell forces are sporadically interrupted by abrupt force relaxation events, characterized by outward "jumps" in displacement ($\geqslant$ 30 nm in 0.5 s) of the underlying substrate during exposure to fluorescent light. Jumps occur more frequently on softer substrates. Additionally, the speed and direction of displacements of particles before and after jumps are conserved, suggesting that the jumps do not involve any change in cytoskeletal or adhesion structure of the cell, and are possibly mediated by disengagement and re-engagement of myosin motors with actin filaments. Interestingly, we find that the frequency of jumps is also modulated by the energy introduced by the excitation light source. We propose a mechanistic hypothesis to explain the observations.

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1. Introduction

Living cells respond to their surrounding mechanical microenvironment. Factors such as substrate stiffness [1–4], applied tension [5–7], and extracellular matrix composition [7–11] affect cell behavior. Cells are also known to respond mechanically to their surroundings, by altering the forces they apply in response to environmental conditions. Recently it has been shown that cells modulate their contractile forces in response to various illumination conditions [12]. This mechanical response manifests within seconds of exposure to fluorescent excitation light and consists of contractile pulling of the substrate interrupted by sporadic instances of large-scale force relaxation.

Previous studies have reported similar intermittent force relaxation events in various biological contexts. In reconstituted systems containing actin and myosin molecules, actomyosin interactions exhibit periodic relaxation composed of intermittent myosin-motor detachment verified through computational and experimental studies [13,14]. Additionally, focal adhesion slippage has been identified during periodic extension and retraction of lamellipodia in migrating epithelial cells [15]. Filopodia forces in epithelial cells have been shown to stall abruptly during retraction [16], exhibiting an interruption to contractile forces. Other studies have identified stiffness-dependent load-and-fail dynamics exhibited by a molecular clutch that transmits force from a cell to its underlying substrate [17]. Small-scale force fluctuations have also been identified within individual focal adhesions. One study reported the location and magnitude of peak traction force within a focal adhesion stalls and

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occasionally abruptly snaps outward, representing intermittent relaxation [18]. These fluctuations were also shown to mediate rigidity sensing within the cell, representing a mechanosensitive process.

These studies provide contextual precedent for abrupt relaxation events in adherent cells. However, no prior study has investigated the effect of excitation light on abrupt relaxation events, to our knowledge. Living cells are known to respond adversely to illumination by fluorescent excitation light. Photo-induced cellular responses include blebbing [19], mitochondrial swelling [20], and stimulation or interruption of cell division [21,22]. Furthermore, photo-induced force relaxation yields nearly complete detachment of cells from their substrate, compromising cell viability [12]. Because excitation light is commonly used in experimental studies, it is critical to understand the process by which cells alter their forces in response to observation with light. This highlights the need to further characterize the mechanical response of adherent cells to fluorescent excitation light.

In this study, we have utilized a common experimental platform for measuring cell traction forces in order to study the effect of excitation light on abrupt relaxation events in fibroblast cells. Cells were plated on polyacrylamide (PA) hydrogels embedded with a monolayer of fluorescent microbeads [23]. Adherent cells apply forces to their underlying surface [24,25], resulting in deformation of the substrate and motion of the embedded microbeads [26,27]. Displacement of the microbeads serves as a measure of cell force dynamics. We illuminated cells with fluorescent excitation light in order to induce cell force relaxation, and quantified the displacement of the beads as previously reported [12]. Intermittent outward (relative to the cell area centroid) jumps in displacement indicate abrupt relaxation events during illumination. Our findings demonstrate that increasing substrate rigidity reduces jump frequency, indicating that the mechanical microenvironment influences relaxation events. Jumps occur in localized spatial regions (<5 μm from non-jumping neighboring particles), and particles maintain the same contractile-pulling speed and direction before and after a jump event. This finding suggests the substrate adhesion linkage is maintained and a disruption to the internal cell force-generating machinery is responsible. Finally, we show that modulation of excitation light intensity alters jump frequency patterns, indicating that the observed force-disruption process is energy-dependent. Understanding the broad context within which abrupt changes to force-generating mechanisms in cells has been studied previously, we propose a hypothesis by which displacement jumps occur as a result of excitation light intensity.

2. Materials and methods

2.1. Substrate fabrication and cell culture

PA gel substrates were fabricated with red (100 nm-dia., 580/605 nm ex/em) or dark red (200 nm-dia., 580/605 nm ex/em) fluorescent microbeads (Fluospheres®, Life Technologies, Carlsbad, CA) embedded just below ($\leq$1.6 μm) the cell culture surface [23]. Substrates were fabricated with elastic moduli 2, 5, and 10 kPa by mixing appropriate proportions of acrylamide and bisacrylamide according to a well-established protocol [28]. The extracellular matrix protein, fibronectin, was linked to the surface using *N*-hydroxysuccinimide (NHS) and 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) (Thermo Scientific, Waltham, MA) [29]. Cell culture media was formulated with Dulbecco's Modified Eagle's Medium (Corning, Corning, NY) (4.5 g/L glucose, 4 mM L-glutamine), 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO), and 1% Penicillin Streptomycin (Corning, Corning, NY).

2.2. Experimental procedure

Monkey Kidney Fibroblast cells (ATCC, Manassas, VA) were plated at an approximate density of 2500 cells/cm^2 on PA gel substrates and allowed to adhere (attaining a fully spread state) for 4 to 6 h. Individual cells were identified and illuminated for 60 s with fluorescent excitation light. During the illumination period, a video of the embedded beads underlying the cell was recorded at a sampling rate of 10 Hz. All experiments utilized an Olympus IX81 and 40x UApo N340 water immersion objective (NA 1.15) (Olympus America Inc., Center Valley, PA) mounted on a vibration isolation table (Newport Corporation, Irvine, CA). The microscope imaging platform was enclosed by an environmental chamber, and culture conditions were maintained throughout experimentation (5% CO_2, 60% humidity and 37 °C). All image acquisition was conducted with a Neo sCMOS camera (active pixels 1392 × 1040, 165 nm/pixel) (Andor Technology, Belfast, Northern Ireland). Three fluorescent excitation light sources were employed to evaluate the effect of varying levels of light intensity on displacement dynamics: (1) A wide field fluorescent metal halide lamp (X-Cite® 120, Excelitas Technologies, Waltham, MA) coupled with an mCherry filter (Semrock Brightline mCherry-M-OMF, $\lambda = 540 - 585/600 - 682$ nm ex/em, $I = 12.5$ W/m^2, Rochester, NY), (2) a neutral density filter (32ND25, Olympus America Inc., $I = 3.0$ W/m^2, Center Valley, PA) coupled with the light source listed in (1), and (3) a deep red collimated LED ($\lambda = 625 - 650/650$ (high pass) nm ex/em, $I = 1.9$ W/m^2) (Thorlabs, Inc., Newton, NJ). Throughout the remainder of this paper, the light sources will be referred to as follows (1) mCherry, (2) ND25, and (3) LED.

2.3. Displacement and jump analyses

Cell-induced substrate deformation was quantified using a single-particle tracking algorithm. [30] with ±10 nm spatial resolution [12]. Displacements of embedded particles represent changes in cell force dynamics. Particles were classified as either inside or outside of the cell spread area boundary. Motion of particles outside the cell boundary represents measurement noise. Drift was approximated as an average of all particle motion outside the cell area at a given time during illumination, and was removed from all displacements. Particle motion was characterized

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