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# Biomechanics of cell reorientation in a three-dimensional matrix under compression

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ABSTRACT

Although a number of studies have reported that cells cultured on a stretchable substrate align away from or perpendicular to the stretch direction, how cells sense and respond to compression in a three-dimensional (3D) matrix remains an open question. We analyzed the reorientation of human prostatic normal tissue fibroblasts (NAFs) and cancer-associated fibroblasts (CAFs) in response to 3D compression using a Fast Fourier Transform (FFT) method. Results show that NAFs align to specific angles upon compression while CAFs exhibit a random distribution. In addition, NAFs with enhanced contractile force induced by transforming growth factor β (TGF-β) behave in a similar way as CAFs. Furthermore, a theoretical model based on the minimum energy principle has been developed to provide insights into these observations. The model prediction is in agreement with the observed cell orientation patterns in several different experimental conditions, disclosing the important role of stress fibers and inherent cell contractility in cell reorientation.

## 1. Introduction

Most cells and organs, from the simplest to the most complex, are mechanosensitive [1]. This property enables a cell to sense and respond to mechanical stimuli in its immediate surroundings, leading to biological signaling cascades that allow the cell to adapt to its local microenvironment through changes in cell morphology, proliferation, contractility, motility, orientation, and viability [1–10].

Numerous reports have demonstrated that cells cultured on an elastic substrate subjected to a uniaxial cyclic stretch tend to reorient themselves away from or perpendicular to the stretch direction [2,6,8,10–14], which is often referred to as stretch-avoidance or strain-avoidance [15]. Concurrent with cell reorientation, the remodeling of stress fibers (SFs) in response to a stretch stimulus has also been observed. Ventral SFs, as the most commonly observed SF type, are long and extend most of the length of cells [16]. The two ends of ventral SFs are anchored to the extracellular matrix (ECM) via focal adhesions (FAs), forming both outside-in and inside-out mechanotransduction pathways [17]. Numerous studies have suggested that SFs play a critical role in cell reorientation and morphogenesis under mechanical stimuli [3,6,7,10,11,18,19]. Furthermore, it has been shown that inhibition of SF formation leads to cell alignment with the stretch direction, indicating SFs are critical for cell stretch-avoidance or strain-avoidance [20].

The mechanism of the observed cell reorientation in response to a uniaxial stretch has been discussed. For example, one theoretical model proposed that actin filaments orient to the direction in which the deformation of the stretched substrate is a minimum so that the energy of SFs is least disturbed [4]. This hypothesis was confirmed with experiments conducted on both fibroblasts and endothelial cells. Another study suggested that the reorientation was driven by a dissipative process in which the passively stored cell elastic energy relaxed to a minimum through aligning cell bodies away from the stretch direction [10]. A statistical thermodynamics analysis considering passive mechanical responses and active subcellular dynamic remodeling predicted that cells would orient in the direction in which SF formation is energetically most favorable [21]. Other studies suggested that SFs would align in the direction in which the total energy reaches a minimum [7,14] and explained realignment of SF-FA assembly in response to substrate stretching [22] on the basis of Maxwell's global criterion for stability. In addition to these analyses based on an energetic argument, it has also been predicted that stretch-induced SF disassembly is an important factor in determining the rate of cell alignment [18]. In short, it is believed that cell reorientation

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under stress is driven by the tendency of cell energy minimization, and that SFs play a critical role in mediating this process.

Despite these significant findings, some important issues of cell behavior under stress still need to be addressed. First, most studies of cell reorientation in response to a mechanical stimulus, whether experimental or theoretical, are conducted with cells on a flexible two-dimensional (2D) substrate, which is different from the 3D microenvironment *in vivo*. To date, only a few investigations have been carried out to probe cellular responses to mechanical forces in a 3D environment, and the resulting cell behaviors differ from those observed in 2D studies. For example, two studies reported that fibroblasts aligned themselves along the stretch direction in 3D, which is contradictory to the stretch-avoidance displayed in 2D [23–25]. This apparently inconsistent behavior has been hypothesized as a result of cells in 3D interacting with the matrix, sensing the applied force, and reorienting in a different manner [26]. However, this speculation does not adequately address the mechanisms by which cells behave differently in 2D and 3D environments. Another report demonstrated that cells, as well as F-actin, exhibited stretch-avoidance at the surfaces of a collagen matrix but showed no preferable orientation in the core of the gel under stretching [27]. The authors hypothesized that the different cell behaviors observed at the surface and inside the gel are due to contact guidance from the collagen fibrils interfering with cell orientation in the bulk of the matrix; however, in another work [23], it was shown by scanning electron microscopy (SEM) for cell-seeded and cell-free collagen fibrils that contact guidance only plays a marginal role. As such, cell response to mechanical stimuli in 3D environments is different from and more complex than those observed on 2D substrates, and the underlying mechanism is still not clear.

Importantly, while the role of cell contractility in cell orientation has been recognized in previous stretching studies in 2D through inhibition of cell contractility [8,11,13,14,20], studies with 3D conditions are still lacking. Moreover, besides the tensile forces that have been examined in most previous studies, compression is another common type of mechanical stimulus *in vivo*. For example, articular cartilage and cardiac fibroblasts experience both tensile and compressive forces [28]. Uncontrolled tumor growth in a limited space can induce a continuous mechanical compressive stress in the tumor and the surrounding cells and tissue [29]. Reports have shown that compression alters gene expression [30–33], cell morphology [32,34], differentiation [35], alignment [26,32,36], and proliferation/apoptosis [33]. Interestingly, it has been shown that fibroblasts and smooth muscle cells realign themselves perpendicular to the direction of compression due to the contact guidance of collagen fibrils [35]. However, again, this possibility might be discounted by SEM images of collagen fibrils in other reports [23,26]. As such, the mechanism underlying cell alignment in response to compression is still largely unknown.

In view of the fact that it is currently not clear how and why cells of different contractility realign in response to 3D compression, we investigated the response of human prostatic normal tissue fibroblasts (NAFs) and cancer-associated fibroblasts (CAFs) to static compression in a 3D collagen I matrix. We experimentally demonstrate that inherent cell contractility affects cell realignment in response to compression and construct a theoretical model based on the minimum energy principle to provide insights into the different behaviors of NAFs and CAFs under compression.

## 2. Material and methods

### 2.1. Cell culture

The human prostatic NAFs and CAFs used in this study were isolated as previously described [37]. Cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium with 10% fetal bovine serum (FBS) and penicillin–streptomycin as previously described [38,39].

### 2.2. Preparation and loading of 3D collagen I gel and cell mixture for compression assays

NAFs and CAFs were labeled with CellTracker™ Green dye (Life technology, Carlsbad, CA) 24 h prior to being embedded in 3D collagen I gels (rat-tail type I collagen, BD Biosciences, Bedford, MA). Then, the collagen I was mixed in PBS to a final concentration of 2 mg/ml, and the pH was neutralized with 1 N NaOH on ice, per manufacturer's instructions. NAFs and CAFs were dissociated and re-suspended in RPMI culture media (250,000 cells/ml) on ice. Cells were then mixed with collagen I solution to a final suspension of ~75,000 cells/ml for each 2 mg/ml collagen I gel mixtures. Next, 700 μl of the cell-gel mixture was loaded into a 4-well Nunc™ Lab-Tek™ II Chamber Slide™ (Cole Parmer, Vernon Hills, IL) and incubated for 30 min at 37 °C to allow the collagen I gel with embedded cells to solidify. Subsequently, culture media was loaded into the cell chambers, and the cells were incubated at 37 °C overnight and then used for compression assays and imaging.

### 2.3. The cell-compression fixture

The cell-compression fixture is composed of three layers of glass slides (25 mm in width, 75 mm in length and 1.0 mm in height, VWR International, LLC, Suwanee, GA). The middle layer consists of two shorter glass slides (32.5 mm in length) that were stacked on top of the bottom glass slide with a ~10 mm gap between them (see Fig. 1a). For the laterally confined case, the gap was made the same as the width of the cell-gel mixture. In this case, when subjected to vertical compression by another glass slide from the top (Fig. 1b), the cell-gel mixture was restricted in the lateral direction denoted by the black dotted arrows in Fig. 1a, but could expand freely in the direction denoted by the red arrows. In the laterally unconfined scenario (see Fig. 1c), the gap between the two middle layer glass slides was wider than the cell-gel mixture, allowing the mixture to expand freely in both directions under vertical compression (see Fig. 1d). During the compression assay, the cell-gel mixture was carefully transferred from the Nunc™ Lab-Tek™ II Chamber Slide™ to the fixture, and another glass slide was placed on top as shown in Fig. 1b, d. The cell-gel mixture (1.4–1.5 mm thick) was compressed to 1 mm thick; thus, ~30% compressive strain was applied to the gel along the vertical direction. The assembly was secured using binder clips and submerged in a petri dish filled with media, and the cell-gel mixture was subjected to compression overnight (~18 h).

### 2.4. Determining cell orientation using fluorescence microscopy

Images of the cells in the 3D compression fixture were collected using a Quorum WaveFX-X1 spinning disk confocal system, which contained a Nikon Eclipse Ti microscope (Melville, NY), an EM-CCD camera (Hamamatsu, Hamamatsu City, Japan), and a 10X ADL objective (NA 0.25), with MetaMorph software. The Quorum confocal system has a Yokogawa CSU-X1 spinning disk (Yokogawa Electric Corporation, Newnan, GA) with Borealis upgrade/modifications (Guelph, Canada) and the CellTracker™ Green dye was excited using a 491 nm laser line. The emission filter used was 525/50 from Semrock (Rochester, NY).

### 2.5. Determining cell orientation using Fast Fourier Transform

Fast Fourier Transform (FFT) was used as previously reported [40] to statistically characterize the cell orientation for each compression result. The FFT was performed on a fluorescence confocal microscopy image of the cells using ImageJ software (NIH, http://rsb.info.nih.gov/ij) with the Oval Profile plug-in (authored by William O'Connnell). A