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Discussion "Cytoskeletal Mechanics Regulating Amoeboid Cell Locomotion"

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ABSTRACT

A virtually universal feature of adherent cells is their ability to exert traction forces. To measure these forces, several methods have been developed over the past fifteen years. In this issue of Applied Mechanics Reviews, Álvarez-González and co-workers review their own traction force microscopy approach and its application to the study of amoeboid cell locomotion. They show that the cycle of cell motility is exquisitely synchronized by a cycle of traction forces. In addition, they show how traction forces and cell cycle synchronization are affected by myosin and SCAR/WAVE mutants. Here I discuss some open questions that derive from the work of the authors and other laboratories as regards the relationship between cell motility and traction forces.

INTRODUCTION

For more than 250 years, physicists have known that motion of any object, living or inert, cannot be fully understood except in the context of physical forces. Strikingly,

the field of cell migration, arguably one of the most important fields in all biology and medicine, has ignored this basic principle until very recently. This is well illustrated by Álvarez-González and co-workers in the current issue of Applied Mechanics Reviews; the notion that cells migrate following gradients of soluble chemical cues was discovered in the second half of the XIXth century [1], yet the physical forces that ameoboid cells generate on their environment has evaded experimental observation until the past few years [2-4].

The delayed interest in the physical forces that drive cell migration is particularly surprising given that the most of the experimental and theoretical tools required for the implementation of traction microscopy have been available for decades if not a century. On the theoretical side, traction microscopy relies on a solution to a problem in continuum mechanics that was solved formally by Boussinesq in the XIXth century [5]. On the experimental side, the main tools required for the implementation of traction microscopy, such as polymer chemistry for the synthesis of soft substrates and light microscopy for the visualization of micron-sized fiducial bead markers, have also been available for decades. Even if relatively powerful computers are required to perform inverse calculations efficiently, approximate or even qualitative solutions could have been implemented many decades ago. However, it was not until the relatively recent works of Harris, Dembo, Butler and their co-workers, that traction forces became accessible to experimental observation [6-8].

THE IMPORTANCE OF TRACTIONS IN 3D

The earliest versions of traction microscopy assumed that forces applied by cells on their underlying flat substrates were essentially two-dimensional [6, 7]; forces in the the dimension normal to the plane of cell adhesion, were thought to be either negligible or biologically irrelevant. This notion was somehow supported by the observation that contractile stress fibers in adherent cells were essentially basal and parallel to the substrate. The group led by Del Alamo and Lasheras was one of the first to recognize that forces in the third dimension were neither negligible nor biologically irrelevant [9, 10]. They provided an elegant method to compute forces in 3D and recognized the importance of taking into account the finite thickness of the underlying cell substrate [10]. Thanks to these methods, we now know that the magnitude of traction forces in the normal dimension is similar to the magnitude of in-plane forces. The structural origin of forces in the normal dimension remains unclear, however. Recent experiments showed that surface tension of a simple liquid droplet on a soft polyacrylamide gel is sufficient to generate significant traction forces [11]. This finding raises the possibility that tractions generated by cells in 3D might also have their origin in the cortical tension of the cell. Alternatively, normal tractions might also be caused by transverse stress fibers that connect the substrate with the nucleus or with cell-cell junctions.

WHY DO CELLS GENERATE TRACTION FORCES?

Traction force microscopy was initially conceived to address the problem of cell migration [6], and several groups have now characterized traction forces generated by virtually every migrating cell type [12]. In their review, Álvarez-González *et al* focus on

Dictyostelium, one of the best studied models for gradient sensing and directed cell migration. The authors show that *Dictyostelium* cells move in a cycle of 4 steps: protrusion, contraction, retraction, and relaxation. While the cyclic nature of amoeboid migration has been known for a long time [13], the authors performed a systematic analysis of the traction forces generated during each step of the cycle. They showed, convincingly, that tractions were balanced internally throughout the cycle, and that variations in tractions were paralleled by changes in cell shape. In addition, they demonstrated that the migratory cycle and the underlying traction forces were perturbed by selective mutations in myosin and in the SCAR/WAVE complex [2].

Taken together, these experiments establish a physical picture in which traction forces and cell shape evolve synchronously during cyclic cell migration. A number of questions in this picture remain open, however. For example, what sets the magnitude of traction forces generated by a cell? For a single cell to migrate on a flat surface, it simply needs to overcome friction from the overlying fluid. In the case of a typical *Dictyostelium* cell, this frictional force is orders of magnitude smaller than the traction forces reported in the bibliography. Therefore, cells generate tractions that are much higher than those needed to migrate. This is perhaps best illustrated by experiments in which myosin was inhibited with blebbistatin, traction forces dropped below mesaurable levels, but cells migrated just fine [14].

If cells do not need large tractions to migrate, why do they exert them? One possible answer is adhesion: by applying high forces on their underlying gel substrate cells develop strong adhesions through reinforcement feedback loops [15]. Such strong

adhesions would ensure cell stability in mechanically active environments. A second advantage of generating large tractions is the possibility to perform mechanosensing, i.e., the ability of an adherent cell to probe the mechanical properties of the surrounding extracellular tissue. To perform mechanosensing, cells have access to a diverse repertoire of strategies [16], most of which rely on the ability of cells to apply high contractile forces on their surroundings. For mechanosensing to be efficient, such contractile forces need to be high enough to either deform the surrounding matrix or to deform the internal force-bearing structures of the cell [16]. Both reinforcement feedback loops and mechanosensing have been well established in mensenchymal cells, but the generalization of these concepts to amoeboid cells is unclear.

LINKING TRACTION FORCES AND CELLULAR VELOCITIES

Several methods to measure traction forces in 2D and 3D have now been made available. Despite the ever improving precision of these methods, the interplay between traction forces and biological function remains poorly understood. Basic questions such as whether there exists a quantitative relationship between the magnitude of traction forces and the speed of cell migration, remain wide open. In this regard, the so-called “clutch models” provide some of the most appealing predictions in the context of our current knowledge of cell-substrate interactions [17]. Clutch models are based on the realistic assumption that the contractile cytoskeleton is linked to the substrate though deformable proteins (clutches) with well-defined binding and unbinding rates. A dynamic force balance between cell contractility, clutch deformability, substrate

deformability, and clutch binding/unbinding rates determines retrograde flow of the actin cytoskeleton. When coupled with the rate of actin polymerization, clutch models predict a quantitative relationship between traction forces and the velocity at which the leading edge of a cell moves forward [17].

FROM LOCAL TRACTION TO GLOBAL TENSION

Traction forces are a local outcome of the global state of intracellular stress. Mapping intracellular stress is a major challenge in current biomechanics. By measuring cell geometry and assuming constitutive conditions for cell compressibility and homogeneity, the 2D stress tensor of a cell can be computed from traction forces [18]. The application of this approach to the single cell has led to insightful relationships between cell stiffness, cell geometry, cytoskeletal tension, and cytoskeletal structure[18]. More importantly, the generalization of this method to 2D epithelial tissues has unveiled how adjacent cells cooperate to build-up long-ranged gradients of intercellular tension [19, 20]. Despite these advances, a full understanding of cell and tissue biomechanics will require more precise methods to probe intracellular tension at the subcellular scale.

CONCLUSION

Fifteen years after its development, traction microscopy has now become a tool available to several laboratories in the field of cell and tissue mechanics. Forces exerted by cells during migration and chemotaxis can now be measured in exquisite detail.

Constitutive relationships between traction forces and cell velocities continue to evade theorists and experimentalists alike, however. The author’s approach to overlap maps of cell shape, structure, and 3D traction forces offers a great opportunity to explore systematic relationships between the physical properties that govern cell migration.

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