Single Cell Force Measurement Using Suspended Fiber Nanonets

J. Wang, K. Sheets, B. Behkam and A. Nain
Virginia Tech

Introduction: Mechanical communication between a cell and its substrate occurs bi-directionally. Cells generate forces which act on their surroundings to interrogate substrate stiffness and topography (inside-out) but also respond to forces transmitted through the ECM which elicit reactionary cellular response (outside-in)[1–4]. Force measurement platforms that are able to capture both modes can allow one to distinguish the forces that cells apply from those that they can withstand, with implications to disease states where progression from normal function to failure either occurs rapidly or with minimal warning or detection (bone fracture, muscle or ligament tears, blood vessel aneurysms). In recent years, numerous force measurement (*inside-out* and *outside-in*) techniques have been developed to investigate single cell behavior. While these methods have yielded fundamental insights, they lack ECM mimicking fibrous environment for capturing cell-fiber interactions. Fiber based biophysical interactions, involving parameters of curvature, structural stiffness (N/m), alignment and hierarchy, have been shown in studies by others and us to play key roles in disease and developmental biology. Here, using our non-electrospinning fiber manufacturing platform (STEP: Spinneret based Tunable Engineered Parameters[5–7]), we demonstrate development and application of fused-fiber nanonets as force probe sensors for measuring single and population level cell forces. Using these nanonets, we demonstrate the ability to measure contractile forces exerted by single cells attached to and migrating along the fiber axis (*inside-out*). This is followed by an investigation of force response of single cells attached to two parallel fibers using a probe capable of deflecting the leading fiber at different strain rates (*outside-in*). In both techniques, forces are calculated using beam deflection within the elastic limit.

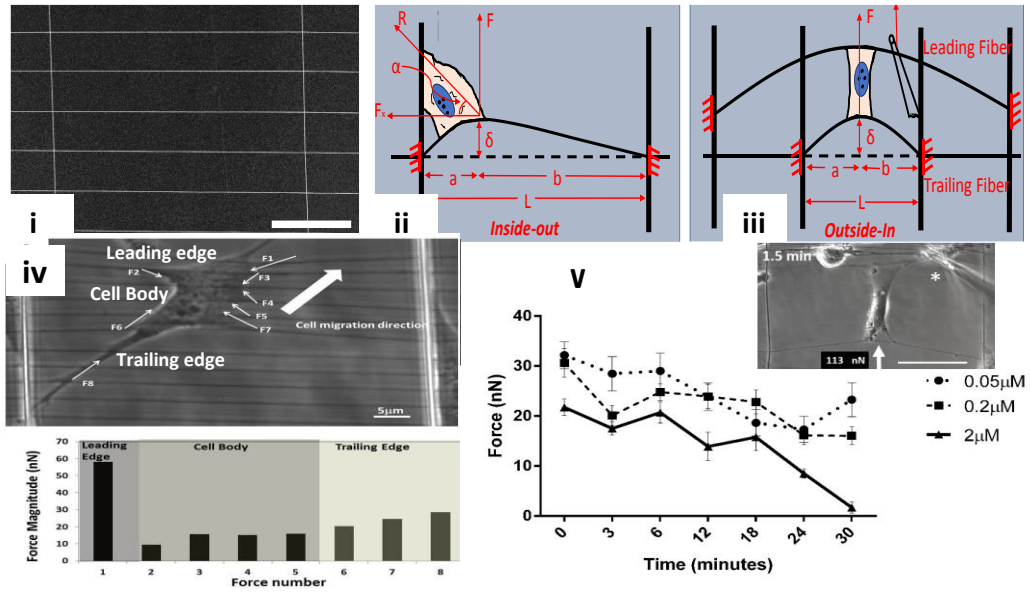


Figure 1. Scanning electron microscopy (SEM) image of suspended STEP nanonets showing with scale bars of 100 µm. STEP based force measurement probes: (iv) inside-out method showing single cell force measurement during migration, and (v) outside-in. Effect of cytochalasin D drug dosage on cell force (iv) inside-out, and (v) outside-in with inset respective optical images of single cell force response.

Materials and Methods: STEP nanonets of varying diameters were deposited in parallel and cross hatch patterns and fused at the intersections using an in-house fusing technique (Fig. 1). Cells of different lineages were investigated for force response with and without the presence of cytoskeletal altering drugs using a microscope with incubating capability (Zeiss AxioObserver Z1, Thornwood, NY).

Results and Discussion: One of the important goals in this cell force study is to achieve a force map for a single cell by increasing the spatial force resolution. Microdimensional force sensor arrays (µFSA) with large pillar densities and polymer gels with densely embedded beads have been used to achieve detailed single cell force maps. Currently, the spatial force resolution ranges from 2-9 µm depending on center to center distance between pillars and beads. In our study, densely spaced nanofiber arrays (fiber spacing ~5 µm) have been prepared for single MSC force mapping as shown in **Figure 1 (iv)** (A)-(D). It was found that the largest contractile forces

always localized at the edge of the cell, indicating that much of the mechanical work underlying cell locomotion is generated near the cell boundary. This observation of traction force decline with distance away from the edge is in agreement with previous cell force studies based on micropillar arrays and polymer gels.[8,9] In addition, we find that the advancing edge of the cell is always exerting larger forces than the mid body and posterior. Similar results have been reported by Dembo *et al*, who argues that the mid-body and posterior are more like passive structures being pulled forward by the contractions generated at the leading edge.[9] Note that our current spatial resolution (5 μm) is comparable to most micro-pillar arrays (2-9 μm) and polymer gels (5μm). For the highly aggressive glioma model (DBTRG-05MG), we investigated single cell force modulation with and without the presence of a cytoskeleton altering drug (cytochalasin D) using outside-in perturbation. Cytochalasin D compromises the spreading ability (area) of single glioma cells, eventually decreasing their 'inside-out' contractile forces, and 'outside-in' force response to external strain (Figure 1). Additionally, we have recently demonstrated measurement of cell-cell junctional forces and forces involved in cellular blebbing using *outside-in* perturbation.

Conclusions: Through time lapse microscopy and immunofluorescence imaging, we present our findings on the role of suspended fiber geometry (curvature and structural stiffness) on single and population level cell force modulation. The suspended and aligned fiber networks act as ultra-sensitive force probes (~ nanoNewtons) for investigating temporal drug response and efficacy in disease models at the single cell resolution. Furthermore, measurement of stem cell forces involved in life-cycle of a single and population of cells provides new insights in the role of forces in cell differentiation and development biology. Combined, these two platforms provide new pathways to investigate cell behavior on synthetic ECM fiber mimics which present cells with simultaneous 1, 2 and 3D environments. Through engineering and study of these simple systems of exceedingly complex interactions, we hope to contribute to development of mechanistic in vitro assays combined with relevant biochemical growth factors, thus approaching in vivo physiological relevance for studying development biology and disease.

Acknowledgements: The authors would like to acknowledge support received from the Institute for Critical Technology and Applied Sciences (ICTAS) at Virginia Tech. This research was funded in part by the Bill and Andrea Wade Research Fund.

[1] Goldfinger, L. E., 2013, Integrin Signaling, Elsevier Inc.

[2] Holle, A. W., and Engler, A. J., 2011, "More than a feeling: discovering, understanding, and influencing mechanosensing pathways.," Curr. Opin. Biotechnol., **22**(5), pp. 648–654.

[3] Eyckmans, J., Boudou, T., Yu, X., and Chen, C. S., 2011, "A hitchhiker's guide to mechanobiology.," Dev. Cell, **21**(1), pp. 35–47.

[4] Chen, C. S., 2008, "Mechanotransduction - a field pulling together?," J. Cell Sci., **121**(20), pp. 3285–3292.

[5] Nain, A. S., Sitti, M., Jacobson, A., Kowalewski, T., and Amon, C., 2009, "Dry Spinning Based Spinneret Based Tunable Engineered Parameters (STEP) Technique for Controlled and Aligned Deposition of Polymeric Nanofibers.," Macromol. Rapid Commun., **30**(16), pp. 1406–12.

[6] Nain, A. S., and Wang, J., 2013, "Polymeric nanofibers: isodiametric design space and methodology for depositing aligned nanofiber arrays in single and multiple layers," Polym. J., **45**(7), pp. 695–700.

[7] Sheets, K., Wunsch, S., Ng, C., and Nain, A. S., 2013, "Shape-dependent cell migration and focal adhesion organization on suspended and aligned nanofiber scaffolds.," Acta Biomater., **9**(7), pp. 7169–77.

[8] Du Roure, O., Saez, A., Buguin, A., Austin, R. H., Chavrier, P., Silberzan, P., Siberzan, P., and Ladoux, B., 2005, "Force mapping in epithelial cell migration.," Proc. Natl. Acad. Sci. U. S. A., **102**(7), pp. 2390–5.

[9] Dembo, M., and Wang, Y. L., 1999, "Stresses at the cell-to-substrate interface during locomotion of fibroblasts.," Biophys. J., **76**(4), pp. 2307–16.