Methods xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

For whom the cells pull: Hydrogel and micropost devices for measuring traction forces

Alexandre J.S. Ribeiro [a,b], Aleksandra K. Denisin [a,c], Robin E. Wilson [a], Beth L. Pruitt [a,b,d,*]

[a] Department of Mechanical Engineering, Stanford University, Stanford, CA 94305, United States
[b] Stanford Cardiovascular Institute, Stanford University, Stanford, CA 94305, United States
[c] Stanford Bioengineering, Stanford University, Stanford, CA 94305, United States
[d] Department of Molecular and Cellular Physiology, Stanford University, Stanford, CA 94305, United States

ARTICLE INFO

Article history:
Received 17 May 2015
Received in revised form 10 July 2015
Accepted 6 August 2015
Available online xxxx

Keywords:
Traction force microscopy
Polyacrylamide hydrogels
PDMS microposts
Displacements
Mechanical calibration
Surface functionalization

ABSTRACT

While performing several functions, adherent cells deform their surrounding substrate via stable adhesions that connect the intracellular cytoskeleton to the extracellular matrix. The traction forces that deform the substrate are studied in mechanotrasduction because they are affected by the mechanics of the extracellular milieu. We review the development and application of two methods widely used to measure traction forces generated by cells on 2D substrates: (i) traction force microscopy with polyacrylamide hydrogels and (ii) calculation of traction forces with arrays of deformable microposts. Measuring forces with these methods relies on measuring substrate displacements and converting them into forces. We describe approaches to determine force from displacements and elaborate on the necessary experimental conditions for this type of analysis. We emphasize device fabrication, mechanical calibration of substrates and covalent attachment of extracellular matrix proteins to substrates as key features in the design of experiments to measure cell traction forces with polyacrylamide hydrogels or microposts. We also report the challenges and achievements in integrating these methods with platforms for the mechanical stimulation of adherent cells. The approaches described here will enable new studies to understand cell mechanical outputs as a function of mechanical inputs and advance the understanding of mechanotransduction mechanisms.

1. Introduction

Mechanical forces between cells and the extracellular matrix (ECM) evolve during growth and development [1], tissue homeostasis [2,3], and wound healing [4]. Changes in tissue mechanical properties have been linked to cancer metastasis [5,6] and disease progression [7–9]. Understanding how mechanical cues affect cellular responses in these processes requires methods to measure cell-generated forces while modulating their mechanical environment. Forces between cells and their environment are transmitted across cell–ECM and cell–cell adhesions (Fig. 1, inset). These forces are either generated externally and applied to cells or generated by cells and applied to the ECM. Cells convert externally applied forces to biological signals by mechanotransduction mechanisms, leading to changes in cell phenotypes (reviewed concisely in Vogel & Sheetz [10]). Some of the same structures involved in mechanotransduction also transmit forces generated by the contractile machinery of cells to the ECM. By generating forces, cells deform their local environment [9,11], which can lead to remodeling of the extracellular environment [12].

In this review, we describe methods to measure traction forces exerted by cells through substrate interactions in 2D using hydrogel substrates and elastomeric micropost arrays. We discuss the importance of protein functionalization methods for both platforms and highlight their applications in mechanobiology studies. To guide those interested in measuring cell traction forces with these methods, we note key considerations and assumptions in: (1) calculation of force from acquired data, (2) fabrication of devices, (3) design of experiments, (4) engineering cell attachment to the surfaces of devices and (5) integration of these methods with platforms for mechanical stimulation. For detailed step-by-step fabrication and data analysis discussions, we refer readers to more targeted methods reviews. Style and Plotnikov provide methods and sample analysis code for traction force microscopy (TFM) [13,14]. Yang, Fu and colleagues offer in-depth discussions of micropost arrays [15,16].

* Corresponding author at: Department of Mechanical Engineering, Stanford University, Stanford, CA 94305, United States.
E-mail address: pruitt@stanford.edu (B.L. Pruitt).

http://dx.doi.org/10.1016/j.ymeth.2015.08.005
1046-2023/

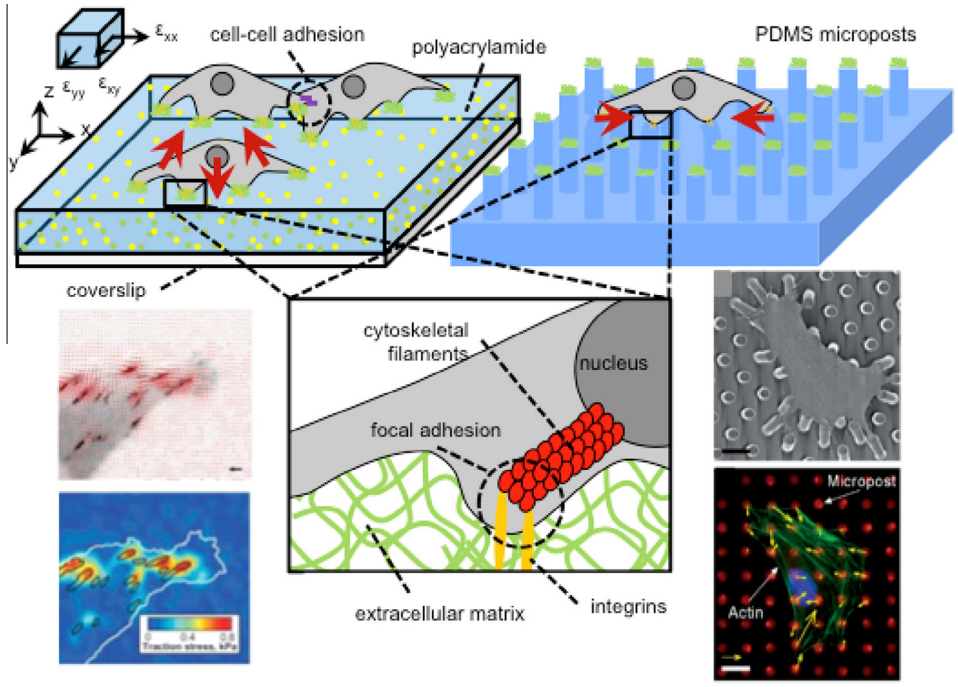

Fig. 1. Measurement of cell traction forces with hydrogel traction force microscopy (TFM) and arrays of elastomeric microposts. Cell contractile machinery produces traction forces (shown as red vectors), which are transmitted to the extracellular environment via focal adhesions, which transmit forces to the nucleus via cytoskeletal (figure inset) to enable mechanotransduction. Hydrogel platforms offer continuous substrates for cell adhesion whereas microposts provide discrete binding "islands." In both systems, cell tractions are calculated from substrate displacements. 3D tractions can be computed on hydrogels if *x*-, *y*-, and *z*-axis deflections of fiducial markers are visualized by confocal microscopy. Figure adapted with permissions from [14,19,199].

2. Traction force measurements using hydrogel TFM and elastomeric micropost arrays

The first qualitative reports of cell–ECM forces began in the 1980s when Harris and colleagues observed wrinkling of a silicone membrane due to fibroblast traction forces [17]. These observations inspired the design and fabrication of systems that could quantitatively measure traction forces. In 1999, Dembo and Wang introduced "traction force microscopy" as a method to quantify forces exerted by adherent cells on a hydrogel substrate with fiducial markers [18] (Fig. 1). Fiducial markers are observed with a microscope, and their movement is computationally determined from images acquired before and after changes in cell contractile activity. Adherent cells deform the substrate, and the resulting surface deformation field is determined from the displacement of fiducials and used to calculate cell traction forces using continuum mechanics models. In 2003, Tan and colleagues developed another commonly used system for measuring cell–ECM forces [19]. This system relies on an array of compliant microposts of constant controllable dimensions (diameter and height). Cells attach and contract to displace the microposts. Researchers observe the microposts using microscopy, and the traction forces exerted by the cell are estimated from micropost displacements using beam bending theory.

TFM on hydrogel substrates and micropost deflection measurements allow for cell traction force analysis with spatial and temporal resolution set by microscopy acquisition parameters. These methods have similar aims, but they are fundamentally different systems, which may lead to differences in force calculations between both methods. Table 1 shows the range of forces that have been calculated in a set of studies with the two systems and highlights that the force values vary considerably between studies, even when using similar cell types. Comparisons across these methods and studies are difficult because experiments for both

Table 1
Cell tractions reported using hydrogel and micropost platforms vary widely with cell type, ECM, and substrate conditions. Comparison between these systems is complicated due to assumptions required about the substrate and cell–ECM adhesions.

Substrate stiffness (if reported, kPa)	Range of traction stress (kPa)	Cell type	Refs.
Polyacrylamide hydrogels analyzed by traction force microscopy			
2.8–30	0.25–0.5	T3T fibroblasts	[188]
15.6	0.5–10	Mouse embryo fibroblast	[43]
10	0.15–0.80	Invasive epithelial bladder cancer cells (T24)	[189]
1.95–9.9	0.2	Invasive epithelial bladder cancer cells (T24)	[50]
6.2	1.32–2.48	3T3 fibroblasts	[47]
Micropost stiffness (nN/μm)	Force per post (nN)	Cell type	Refs.
PDMS micropost arrays			
32	2–78	Bovine pulmonary artery smooth muscle cells, 3T3 fibroblasts	[19]
31	1–32	Human pulmonary artery endothelial cells	[190]
1.9	0.14–1.2	Murine dendritic cells	[191]

Please cite this article in press as: A.J.S. Ribeiro et al., Methods (2015), http://dx.doi.org/10.1016/j.ymeth.2015.08.005