Biochemical and Biophysical Research Communications xxx (2016) 1–5

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

New wrinkling substrate assay reveals traction force fields of leader and follower cells undergoing collective migration

Sho Yokoyama [a, 1], Tsubasa S. Matsui [a, b], Shinji Deguchi [a, b, *]

[a] Department of Nanopharmaceutical Sciences, Nagoya Institute of Technology, Japan
[b] Division of Bioengineering, Osaka University, Japan

ARTICLE INFO

Article history:
Received 14 November 2016
Accepted 25 November 2016
Available online xxx

Keywords:
Collective cell migration
Epithelial cells
MDCK-II cells
Traction force microscopy
Wrinkling substrate assay

ABSTRACT

Physical forces play crucial roles in coordinating collective migration of epithelial cells, but details of such force-related phenomena remain unclear partly due to the lack of robust methodologies to probe the underlying force fields. Here we develop a method for fabricating silicone substrates that detect cellular traction forces with a high sensitivity. Specifically, a silicone elastomer is exposed to oxygen plasma under heating. Removal of the heat shrinks the substrate so as to reduce its critical buckling strain in a spatially uniform manner. Thus, even small cellular traction forces can be visualized as micro-wrinkles that are reversibly emerged on the substrate in a direction orthogonal to the applied forces. Using this technique, we show that so-called leader cells in MDCK-II cell clusters exert significant magnitudes of traction forces distinct from those of follower cells. We reveal that the direction of traction forces is highly correlated with the long axis of the local, individual cells within clusters. These results suggest that the force fields in collective migration of MDCK-II cells are predominantly determined locally at individual cell scale rather than globally at the whole cell cluster scale.

1. Introduction

Cells often move in cohesive groups during embryonic morphogenesis, wound repair, and cancer invasion. For better understanding of such collective cell behaviors, robust methodologies to probe underlying cellular traction force fields are helpful. To this end, fluorescent microbead-based traction force microscopy [1] and micropillar assay [2,3] have been developed to visualize cell-driven elastic deformations of the substrates. However, details regarding the whole view of multicellular traction force fields still remain elusive partly because these experiments and analyses are not always easy to practice.

Cellular traction forces were first visualized using wrinkling of deformable silicone substrates [4]. In this method, prepolymer silicone fluid was coated on a cell culture dish and polymerized by contacting with a burner flame for a few seconds so that micro-wrinkles were generated on the planar surface upon exertion of traction forces. This technique is not suited to strictly quantifying the traction forces with a unit of Newton or Pascal because of complicated mechanical analyses of the wrinkle generation. Besides, the local burner treatment would result in yielding spatially nonuniform material properties. Consequently, the sensitivity to forces to display wrinkles would be heterogeneous in space, thus inappropriate for observing wide-spanned collective cell migration. Yet, the wrinkling technique has a huge merit of allowing for direct visualization of cellular forces as wrinkles in a manner promptly observed with conventional transmitted light microscopy.

Here we describe a method to modify the wrinkling substrate technique to improve the reproducibility and spatial homogeneity. We use oxygen plasma to create a thin oxide layer on a silicone elastomer in a reproducible and spatially uniform manner. The hydrophilic surface layer is mechanically stiffer compared to the underlying silicone solid and thus becomes buckled to display wrinkles when compressive stress is loaded. We also improve the sensitivity of the substrate to detect even small traction forces to expand the range of application to various cell types. Using this spatially uniform and highly force-sensitive substrate, we investigate traction force fields of epithelial cells undergoing collective migration.

* Corresponding author. Division of Bioengineering, Graduate School of Engineering Science, Osaka University, 1-3 Machikaneyama, Toyonaka, Osaka 560-8531, Japan.
E-mail address: deguchi@me.es.osaka-u.ac.jp (S. Deguchi).
[1] Present address: Micro/Nano Technology Center, Tokai University, Japan.

http://dx.doi.org/10.1016/j.bbrc.2016.11.142
0006-291X/

2. Results and discussion

2.1. Highly sensitive substrate enables detection of contractile forces of various cell types

We previously reported a method to detect cellular contractile forces as wrinkles with a pitch of subcellular length-scale, which was initially applied to MTD-1A epithelial cells elsewhere [5] and here characterized in details to demonstrate its usefulness in studying various cell types.

Cell culture dishes supporting a silicone solid were treated with oxygen plasma under heating (Fig. 1A, i–iii). We directly measured with light microscopy that the substrate thermally expands with this heating (Fig. 1B). The thermal expansion strain increased up to ~6×10^{-4} at 50 °C, corresponding to an expansion of 6 μm if the reference length is 10 mm. The removal of the heating back to the room temperature (24 °C; Fig. 1A, iv) results in generation of wrinkles on the substrate surface because of the following reasons: with the oxygen plasma treatment, the surface stiffened compared to the original state, implying that the range of elastic deformation is accordingly narrowed to some extent. Because the removal of the initial heating is followed by the shrink to the original length, a compression is loaded onto the substrate to induce buckling. Cell experiments are usually performed at 37 °C, and this moderate thermal expansion from the room temperature to the experimental condition again flattens the surface (Fig. 1A, v). Plating cells at this condition promptly elicits wrinkles on the surface because of the cellular intrinsic contraction (Fig. 1A, vi).

Our methodology is summarized as follows: assuming the reference length to be L (i), the substrate is modified with plasma under heating where the length is now $L + \delta_1$ due to thermal expansion (ii, iii). Returning the length to L at the room temperature is followed by generation of wrinkles (iv), but those wrinkles again disappear at the warmer temperature where cells are cultured because a slight thermal expansion of δ_2 is added (v). In this last condition, wrinkles are generated sensitively and locally where traction forces are applied by cells (vi). Here we determined the heating temperature 60 °C (and resulting expansion δ_1) to planarize the substrate surface just at the culture temperature 37 °C. This process minimizes the buckling strain, which is a threshold compressive strain required for causing wrinkling. Consequently, the sensitivity to cellular forces is significantly improved as shown below.

To evaluate the sensitivity, three distinct types of proliferative cells are used, i.e. mesenchymal (MEF, mouse embryonic fibroblasts), epithelial (MDCK-II, Madin-Darby canine kidney), and sarcoma (U2OS, human osteosarcoma) cells. Cells were seeded on the substrate at room temperature and placed in a stage incubator at 37 °C (Fig. 1C). Initially just after starting the imaging, isotropic wrinkles were observed all over the substrate because the dish is still close to room temperature, i.e., at condition iv in Fig. 1A. Here, the isotropic wrinkles are generated because the plasma is illuminated uniformly onto the substrate, and then a planar compression after the removal of heating is loaded on the round dish in a center-symmetric manner. As the temperature increased up to 37 °C, the random wrinkles gradually disappeared where cells were absent. Meanwhile, at the other places in the proximity of cells, wrinkles are rearranged to become oriented toward the cell axis as they were originated from pulling of the substrate by the individual cells. This traction force-induced wrinkling occurs on the substrate in an elastic, i.e. repeatable manner.

We quantified the sensitivity of the substrate to contractile forces, or specifically the percentage of how many cells generate wrinkles (Fig. 2). Most MEF (~90%) generated wrinkles even without the heating process. Meanwhile, only limited amounts (~10–20%) of MDCK-II and U2OS cells generated wrinkles on the substrates manufactured with oxygen plasma but without the heating process. Yet, marked wrinkles were observed even for these cells (~70–80%) if the dishes were manufactured with the thermal expansion process. These results indicate that, to detect cellular forces from the wrinkling, the heating process is dispensable for the mesenchymal cell types that exhibit marked traction forces on the substrate, but practically effective for the epithelial and cancer cells that exhibit relatively weaker forces.

2.2. Distinct force magnitudes between leader and follower cells

Traction force fields in collective migration of MDCK-II cells were previously investigated with the fluorescent microbead-based traction force microscopy [1], but this report did not focus on the mechanical role of so-called leader cells that take on mesenchymal cell-like morphology and large size [6–9]. Applying the present technique to observations of MDCK-II cell colonies, we found that leader cells that appear at the edge of their populations exert significant levels of traction forces distinct from those of follower cells (Fig. 3A). Because the substrate is spatially uniform in mechanical and chemical properties (Fig. 1), the present microscopy allows for visualization of the relative magnitude of traction forces among the cells imaged. Here, leader cells take on mesenchymal cell-like features not only for the apparent morphology but also for the presence of significant magnitudes of physical forces.

Intense wrinkles with clear stripe patterns are created under and at the front of leader cells (Fig. 3A). Morphology of leader cells always elongates in parallel along the border of cell clusters. The wrinkles just below the leader cells are orthogonal to the long axis of the cells, whereas those at the front run outward radially. Thus, the wrinkles just under cells are produced by centripetal "pinching" due to cellular contraction, whereas those at the outside of cells are caused by the pulling of the substrate toward the contracting cells and consequent Poisson's effect. Under follower cells, weaker but still obviously distinguishable wrinkles are observed over the range of the cells located within ~1–20 cell rows behind the leader cells. The angle between the long axis of follower cells and the direction of principal wrinkles under the cells was also almost normal, which will be discussed below regarding their specific contributions to the traction force fields (Fig. 4).

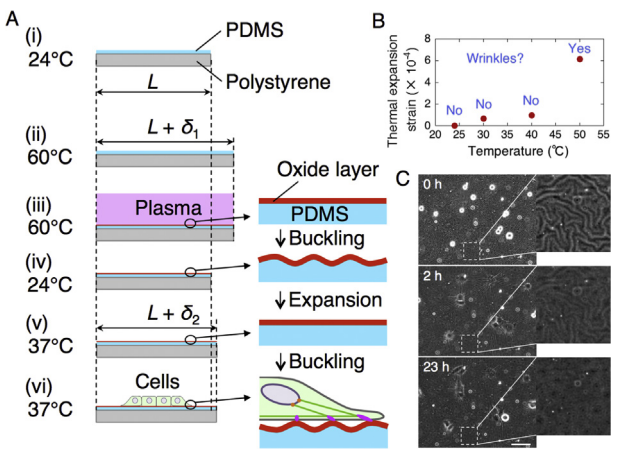

Fig. 1. Force-sensitive substrate. (A) Process for manufacturing the substrate. See text for details. (B) Wrinkles are generated on the substrate even in the absence of cells with a heating pretreatment up to more than 50 °C because a high thermal expansion strain is induced at Step iii in A. (C) Live imaging of U2OS cells on the substrate. Scale, 50 μm.

Please cite this article in press as: S. Yokoyama, et al., New wrinkling substrate assay reveals traction force fields of leader and follower cells undergoing collective migration, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.11.142