Traction Forces Generated by Locomoting Keratocytes

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Abstract. Traction forces produced by moving fibroblasts have been observed as distortions in flexible substrata including wrinkling of thin, silicone rubber films. Traction forces generated by fibroblast lamellae were thought to represent the forces required to move the cell forwards. However, traction forces could not be detected with faster moving cell types such as leukocytes and growth cones (Harris, A. K., D. Stopak, and P. Wild. 1981. Nature (Lond.). 290:249-251). We have developed a new assay in which traction forces produced by rapidly locomoting fish keratocytes can be detected by the two-dimensional displacements of small beads embedded in the plane of an elastic substratum. Traction forces were not detected at the rapidly extending front edge of the cell. Instead the largest traction forces were exerted perpendicular to the left and right cell margins. The maximum traction forces exerted by keratocytes were estimated to be \( \sim 2 \times 10^{-8} \) N. The pattern of traction forces can be related to the locomotion of a single keratocyte in terms of lamellar contractility and area of close cell–substratum contact.

To move cells must exert traction forces upon the substratum. This involves the temporal and spatial regulation of numerous force generating molecular motors. Yet an understanding of how and where moving cells generate traction forces, represents a major gap in our knowledge of cell locomotion. This paper presents the first measurements of the traction forces generated by rapidly moving cells.

Traction forces produced by moving fibroblasts were first observed as distortions in flexible substrata that caused wrinkling of thin, silicone rubber films (Harris et al., 1980). These traction forces act inwards, relative to the extending lamella and retracting edge, leading to compression of the substratum such that wrinkles are formed perpendicular to the direction of lamellar extension. Wrinkles were thought to be formed by an actomyosin-based contraction of the cytoskeleton which is transmitted to the substratum via focal adhesions located just behind the extending edge and trailing cell edge. Traction forces generated by fibroblast lamellae were thought to represent the forces required to move the cell forwards along the substratum. It was therefore surprising to find that traction forces generated by faster moving cell types such as leukocytes and growth cones could not be detected (Harris, 1981), since it was assumed that larger traction forces would be required for faster locomotion. However, slow moving cells such as fibroblasts form strong focal adhesions to the substratum whereas faster moving cells tend to form weaker close contacts (Couchman and Reese, 1979). In addition large numbers of actin stress fibers are found in slower moving cells, implying greater cytoskeletal contractility. Therefore rapid cell locomotion appears to rely on both weaker cell substratum adhesions and cytoskeletal contractility.

To learn more about the traction forces required for rapid locomotion, we have modified the traction force assay of Harris to detect the size and orientation of traction forces generated by fish epithelial keratocytes, without wrinkling the substratum. Instead these forces can be detected as two-dimensional displacements of marker beads embedded in the substratum. Such displacements are easier to quantify than wrinkles and so provide more accurate information about the location and orientation of traction forces. In addition the use of a simple-shaped cell type such as the fish keratocyte allows the distribution of the traction forces to be related to the movement of the entire cell. The fact that keratocyte cell shape is maintained during locomotion means that the steady-state distribution of traction forces may be observed.

Materials and Methods

Silicone Substrata

A Rappaport chamber was used consisting of a thick walled Pyrex cylinder (22 × 8 mm) to which a circular glass coverslip has been sealed to its base with vacuum grease (see Fig. 1 a). Silicone fluid (dimethylpolysiloxane, viscosity = 12,500 centipoise; Sigma Chemical Co., St. Louis, MO) was poured into the base of the chamber and allowed to spread evenly. Latex beads (1-μm diam) were sprinkled over the surface with a fine brush. Cross-linking of the top layer of silicone oil was achieved by placing the chamber into a modified glow discharge apparatus (Polaron sputter coater ES100).
at an air pressure of 0.15 Torr and a target height of 60 mm. Voltage was applied to maintain a constant current of \( \sim 2 \text{ mA} \) for 2 s. Weaker "hyper-compliant" substrates were produced by reducing the time in the glow discharge.

**Calibration of Substratum**

Force calibration needles that were highly flexible with sharply pointed tips were made using a programmable pipette puller (P87 Brown Flaming). Stiffer reference needles were pulled by hand and calibrated by plotting the amount of tip deflection produced by single weights in the range of 0.35–6.1 mg made from varying lengths of copper wire. The force required to displace a bead in a silicone substrate was measured by reproducing the deflection of the tip of the highly flexible microneedle with the stiffer calibrated reference microneedle. Tip deflection in both needles was proportional to the force applied. Video images of the calibration experiments were recorded and used to obtain tip deflection for a given bead displacement. This was repeated about 20 times with beads randomly selected throughout the substratum. The compliance of a substratum was given by the slope of the displacement versus force plot (see Fig. 1 B). The percent recovery of a bead from its original undisplaced position was measured 15 s after the bead was released from the microneedle. Percent recovery = \( (p - q)/p \times 100 \), where \( p = \) original bead displacement and \( q = \) bead displacement after recovery.

**Cell Culture**

Keratocytes from hybrid populations of the Molly fish Poecilia sphenops were cultured as described previously for goldfish Carassius auratus (Lee et al., 1993a). They were then disaggregated by adding Ca\(^{2+}\)-free PBS and trypsin in a 1:1 mixture and resuspended in a small (\( \sim 50 \mu\text{l} \)) volume of culture medium which was then added to the Rappaport chambers that had been prefilled with medium.

**Video Microscopy and Analysis of Bead Displacements**

Observations of keratocytes were made at room temperature within glass Rappaport chambers (see Fig. 1 A) using differential interference microscopy. Interference reflection microscopy was performed according to Bereiter-Hahn et al. (1981). The video microscope system was composed of a Zeiss inverted microscope (Axiovert 10), a 20\( \times \)0.5 NA plan neofluor

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**Figure 1.** (A) A schematic diagram of modified traction force assay. The Rappaport chamber consists of a thick-walled Pyrex cylinder to which a circular glass coverslip has been sealed to its base with vacuum grease. Inside, resting on silicone fluid, is a thin film of crosslinked silicone into which small latex beads have been embedded. (B) Applied forces required for various bead displacements on an elastic substrate. (C) Percent recovery of the bead towards its original undisplaced position after it is released from the microneedle.

**Figure 2.** (A–C) Computer images of selected bead displacements (numbered 1–5) induced by keratocyte locomotion on an elastic substrate. The path of the cell's centroid (c) is shown consisting of three sections (red, green and blue) with each color representing \( \sim 40 \text{ s} \) of cell locomotion that match corresponding time intervals for bead displacements with respect to the substratum. (A) Bead displacements (red) are negligible as the cell approaches. (B) Maximum bead (1, 2, 4, and 5) displacements (green) occur inwards, perpendicular to the lateral cell margins, at the rear, below the equator of the cell (dotted line). (C) The beads return toward their original positions (blue). (D–F) Bead displacements induced by the locomotion of a keratocyte on a hyper-compliant substrate. Colored sections correspond to consecutive, 26-s time intervals. (D) Beads 2 and 3 are displaced forwards (red). Beads 1 and 4 are displaced inwards, perpendicular to lateral cell margins. (E) Forward displacement (green) of beads 2 and 3 continues beneath the extending lamella, and to a lesser extent for bead 1. Bead 4 is displaced inwards, perpendicular to the rear, lateral cell margin. (F) Beads 1–3 make their largest inward displacements (blue) beneath the cell rear. Bead 4 continues to be displaced in the direction of cell locomotion. Bar, 10 \( \mu\text{m} \).
Figure 3. (a) A vector map of traction forces exerted on an elastic substratum (compliance = $8 \times 10^{-9}$ N/$\mu$m) with respect to the centroid (C) of the cell in Figure 2a. The change in magnitude and direction of traction forces (indicated by length and direction of arrows, respectively) exerted at a given bead location, with increasing time, can be seen by reading downward, opposite to the direction of locomotion (arrow). The largest traction forces are directed inwards, perpendicular to the lateral cell margins. They are not detected at the front of the extending lamella. Small traction forces are oriented inwards, perpendicular to the lateral edges of the extending lamella (asterisks). (b and c) Vector maps of traction forces exerted on elastic substrata. The compliances of the substrata were $1.06 \times 10^{-9}$ and $2.14 \times 10^{-8}$ N/$\mu$m, respectively. Because of smaller displacements the vectors shown in the figures b and c were amplified fivefold. (d) Bead displacement vectors occurring on a hyper-compliant substratum, with respect to the centroid (C) of the cell in Figure 2d. They increase in size towards the rear of the cell and are oriented inwards, perpendicular to the cell edge. Displacement vectors (e.g., bead 3) that exist beneath the extending lamella tend to be oriented in the direction of cell motion. This is thought to result indirectly from lateral compression of the substratum by the moving keratocyte. Bar, 10 $\mu$m.

Objective, a 0.55 NA long-distance condenser, and a Hamamatsu C2400 video camera with a 4x adapter. Images of moving keratocytes (16 frames averaged) were acquired every 5 s and recorded on an optical memory disc recorder (Panasonic TQ-2028F) during about 10 min of locomotion. Image-i software (Universal Imaging, West Chester, PA) was used to acquire, record, and process images.

Vector diagrams were produced by calculating the displaced and undisturbed bead positions with respect to the moving cell’s centroid at every time interval (see Fig. 3, a and b, 20 s; c, 50 s; and d, 10 s). These relative bead positions were replotted in reference to a given cell centroid position (C). Lines drawn from the each bead’s undisturbed position to its displaced position gave the size and direction of bead displacement with respect to the cell’s stationary frame of reference at C. The cell centroid positions were obtained by manually fitting ellipsoids onto the keratocyte cell margin. Although keratocytes generally maintain an almost constant shape during locomotion, some small changes in shape do occur. To estimate errors from such shape changes, cell centroid positions were obtained in three independent measurements. The maximum uncertainty was ±1.84 $\mu$m either side of the original trajectory. Vector diagrams drawn from the three different centroid measurements did not vary significantly.

Results

Elastic substrata were made by using a glow discharge device to cross-link a thin film of silicone oil within a glass Rapaport chamber (Fig. 1A). This technique allows the reproducible manufacture of elastic substrata with similar compliances. In addition substrata with a range of compliances can be made by varying the degree of crosslinking. Compliances were measured with a calibrated glass microneedle (Harris et al., 1980; Yoneda, 1960) and were found to be similar within a given substratum. Substrata with compliances of ei-
ther $\sim 2 \times 10^{-4}$ or $\sim 5 \times 10^{-9}$ N/µm were preselected prior to plating keratocytes. These substrata are referred as "elastic" and "hyper-compliant" substrata, respectively. On the elastic substrata, bead displacements were proportional to the force applied to them (Fig. 1 B). In addition nearly all beads recovered to their original positions (Fig. 1 C). The half time for recovery was $\sim 0.5$ s indicating a small amount of viscous drag. On the hyper-compliant substrata, while bead displacements were proportional to the applied force, the beads recovered only 30–60% towards their original position (data not shown). In addition the half-time for recovery was $\sim 3$ s. These observations indicate that hypercompliant substrata exhibit both plastic and viscoelastic behavior.

Lcomot ing keratocytes produced a symmetrical, steady-state pattern of bead displacements on both elastic (Fig. 2, A–C) and hyper-compliant substrata (Fig. 2, D–F) without any apparent effect on cell morphology or speed. Use of hypercompliant substrata was essential in determining whether our modified traction force assay was working since bead displacements are large and easily visible during playback of video sequences. In addition they allowed the detection of traction forces. This is because the effects of their viscous and plastic properties cannot be separated from the elastic behaviour of these substrata. Thus smaller traction forces (detected as bead displacements of beads were measured for several cells (Table I). The calculated traction forces range from $1 \times 10^{-4}$ to $2 \times 10^{-4}$ N.

Bead displacement vectors within hyper-compliant substrata (Fig. 3 d) are not assumed to be proportional to applied traction forces. This is because the effects of their viscous and plastic properties cannot be separated from the elastic behaviour of these substrata. Thus smaller traction forces (detected as bead displacement vectors) could be observed that would otherwise not be visible within elastic substrata. Of all the cells observed ($\sim 20$) no rearward oriented vectors were found at the

### Table I. Estimation of Maximum Traction Forces

| Experiment number | Substratum type | Compliance ($\times 10^{-9}$ N/µm) | Bead # | Maximal displacement (µm) | Force ($\times 10^{-9}$ N) |
|-------------------|----------------|------------------------------------|--------|------------------------|--------------------------|
| 1                 | Elastic        | 1.06                               | 1      | 0.914                  | 0.97                     |
| 2                 | Elastic        | 1.06                               | 2      | 0.750                  | 0.79                     |
| 3                 | Elastic        | 1.06                               | 3      | 1.172                  | 1.24                     |
| 4                 | Elastic        | 1.26                               | 1      | 0.990                  | 1.05                     |
| 5                 | Elastic        | 0.80*                              | 2      | 1.260                  | 1.33                     |
| 6                 | Elastic        | 0.80                               | 3      | 1.104                  | 2.36                     |
| 7                 | Hyper-compliant| 0.762                              | 1      | 0.762                  | 1.63                     |
| 8                 | Hyper-compliant| 1.172                              | 2      | 1.459                  | 1.84                     |
| 9                 | Hyper-compliant| 1.172                              | 3      | 4.350                  | 2.03                     |
| 10                | Hyper-compliant| 0.762                              | 1      | 2.880                  | 1.32                     |
| 11                | Hyper-compliant| 2.34                               | 2      | 2.930                  | 2.34                     |
| 12                | Hyper-compliant| 2.34                               | 3      | 2.930                  | 2.34                     |

In each experiment two or three beads which displayed the largest displacements were selected and their maximal displacements obtained. The traction force exerted on each bead was calculated by multiplying the substratum compliance and the maximal displacement.

* The compliance of this substratum was not measured but estimated according to its relative elasticity.

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Our traction force assay allows the detection of traction forces generated by rapidly moving cells that are an order of magnitude less than those generated by fibroblasts. The traction forces exerted by keratocytes are estimated as \( \sim 2 \times 10^{-4} \) N, while those exerted by fibroblasts are estimated as \( \sim 2 \times 10^{-7} \) N (Harris, A., personal communication). The method not only detects weaker forces but also estimates the direction and the location of forces.

**Discussion**

Our traction force assay allows the detection of traction forces generated by rapidly moving cells that are an order of magnitude less than those generated by fibroblasts.
tractility and relatively high cell–substratum adhesion favor lamellar extension (Fig. 5 B, white region).

The generation of traction forces is dependent both on the contractile strength of the cytoskeleton and cell–substratum adhesion. The requirement for a contractile cytoskeleton is indicated by finding that photoactivated actin marks move inwards with respect to the substratum (and cell edge) at the lateral edges of the keratocyte, corresponding to where the largest traction forces are seen (Lee, J., and J. A. Theriot, unpublished data). Increasing contractility of the cytoskeleton, toward the rear of the keratocyte is shown by the progressive, inward curvature of photoactivated actin marks at the rear of the cell (Lee et al., 1993a). Not only must contractility be great enough to deform the substratum but sufficient adhesions must exist to transmit contractile forces to the substratum. Thus although a large region of close contact exists at the front of the cell no traction forces are detected because contractility of the cytoskeleton is too low. The relative magnitude and position of inward oriented traction forces depends on the varying ratio of cytoskeletal contractility and adhesion strength (assuming area of close contact is proportional to adhesion strength at the cell margin) at different locations along the cell margin.

The largest traction forces are found at the equatorial region of the cell (Fig. 5 B, light grey region). The position of these forces coincides with a region of high cytoskeletal con-
tractility but which is counterbalanced by close cell–substratum contacts on either side of the cell. This leads to the development of tension perpendicular to the direction of cell motion as shown by parallel arrays of actin stress fibers that span the width of the cell (Bereiter-Hahn et al., 1981; Euteneur and Schliwa, 1984; Heath and Holifield, 1991). Increased tension within the cytoskeleton inhibits lamellar extension (Kolega, 1986; Lee et al., 1993b) such that the lateral cell margins neither extend nor retract in accord with the Graded Radial Extension model of keratocyte locomotion (Lee et al., 1993a).

At the rear of the keratocyte the contractility of the cytoskeleton is greatest (Fig. 5 B), but cell–substratum adhesion becomes weaker as indicated by the decreasing area of close contact towards the rear edge (Fig. 4). Increasing contractility together with decreasing adhesion strength leads to progressively increased rates of lamellar retraction towards the rear of the cell (Lee et al., 1993a). Thus, the traction forces generated by keratocytes provide the necessary mechanical force to overcome adhesion forces between cell and substratum (Fig. 5 C).

It is interesting to note that the traction forces produced by keratocytes are similar in magnitude to the contractile forces measured in retracting neurites and phagocytosing leukocytes (Heidemann, et al., 1990; Evans, et al., 1993). This suggests that actomyosin based contractility can generate forces of similar magnitudes in diverse motile phenomena (Oliver et al., 1994).

The Pattern of Traction Forces Is Related to the Mode of Cell Locomotion

In keratocytes broad close cell–substratum contacts and negligible lamellar contractility at the front of the cell favor rapid lamellar extension. Conversely, high contractility and an absence of close cell–substratum adhesions at the rear favor rapid retraction of the cell margin (Fig. 5 B). This marked asymmetry between front and rear of the keratocyte appears to be essential for the rapid, forward locomotion of these cells. Such asymmetry is much less apparent in fibroblasts (Fig. 5 D) where strong cell–substratum adhesions and high lamellar contractility (1 × 10⁻⁷ N as inferred from wrinkling silicone substrata; Harris, A., personal communication) exist at front and rear cell edges. Increased tension in the actin meshwork between front and rear of the cell may thus impede forward locomotion of fibroblasts. This is consistent with the observation that forward movement is slow and occurs in two discrete phases. Thus lamella extension proceeds until inhibited by increasing tension between front and rear of the cell. Following a short lag, retraction of the cell rear occurs and tension is released, leading to another phase of lamellar extension (Chen, 1979).

Although traction forces are generated by the same processes in keratocytes and fibroblasts, the spatial arrangement of cell–substratum adhesions and degree of lamellar contractility are the key determinants of the differences in pattern of traction forces generated by these two cell types and in their modes of locomotion.

The development of an assay to detect the traction forces generated by moving keratocytes provides an important new tool with which to study cell locomotion. In addition, traction forces detected by this assay are amenable to quantitative analyses, that can produce a more detailed map of the traction forces generated beneath a moving cell (Oliver, T., M. Dembo, A. Ishihara, and K. Jacobson, manuscript in preparation). The ability of manufacture substrata of various compliances means that this assay may be used to detect traction forces over a range of magnitudes and in various cell types.

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