Measuring Cell Forces by a Photoelastic Method

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ABSTRACT A new method for measuring the mechanical forces exerted by cells on the substratum and through the substratum to act on other cells is described. This method depends upon the growth of cells on a photoelastic substratum, polydimethylsiloxane coated with a near monolayer of fibronectin. Changes in the forces applied by the cells to the substratum lead to changes in birefringence, which can be measured and recorded by the Polscope computer-controlled polarizing microscope. The changes in azimuth and retardance can be measured. A method for calibrating the stress is described. The method is sensitive down to forces of 1 pN per square microns. Fairly rapid changes with time can be recorded with a time resolution of ~1 s. The observations show that both isolated adhering, spread cells and also cells close to contact exert stresses on the substratum and that the stresses are those that would be produced by forces of 10–1000 pN per cell. The forces are almost certainly exerted on nearby cells since movement of one cell causes strains to appear around other nearby cells. The method has the defect that strains under the cells, though detectable in principle, are unclear due to birefringence of the components of the cytoplasm and nucleus. It is of special interest that the strains on the substratum can change in the time course of a few seconds and appear to be concentrated near the base of the lamellopodium of the cell as though they originated there. As well as exerting forces on the substratum in the direction of the long axis of the cell, appreciable forces are exerted from the lateral sides of the cell. The observations and measurements tend to argue that microtopography and embedded beads can concentrate the forces.

INTRODUCTION

Mechanical forces acting on cells affect many processes such as proliferation, cytoskeletal expression (1), and gene activity (2) Often these forces may be applied from external sources, e.g., muscular activity in remote parts of the body. But in addition the cells can act on their near neighbors (3).

Recently a number of novel methods for measuring the forces generated by individual cells have been reported (4–7). These methods, though highly ingenious and useful, measure the forces required either to deform microstructures on the substratum beneath the cell (5,6) or displace marker beads embedded in the substratum close to the cell (7). The possibility exists, as has been allowed by Bershadsky et al. (4), that the heterogeneous mechanical nature of the substratum (pillars or embedded particles) modifies the forces the cell can exert, almost as exercise machines in the gymnasium influence the athlete to develop greater forces. The aim of those measurements has been directed mostly at the questions relevant to cell movement.

But forces between cells are well known to be important in events such as wound healing, especially in contracture and the pulling open of wounds, in development, and perhaps in remodeling of tissues (3).

We describe a new method, based on photoelastic measurements, particularly directed at observing the effects of pairs of cells on each other or on small groups and also providing a method unimpaired by the presence of a heterogeneous substrate. Thus we test whether such substrates with micrometric topographic details modify cell force generation or application. The method has already been used (8) to detect active transverse contractions in fibroblasts in addition to the well-known longitudinal contractions in such cells. It can also detect forces acting between cells when there is microscopically visible separation between them. A further possible advantage of this technique is that observations can be made at fairly short -term and repetitive intervals over long periods, thus revealing whether rapid changes or fluctuations occur.

Measurement of the forces exerted by cells on their surroundings has been achieved by a variety of methods. Basically nearly all these methods set up situations where the cells distort their surroundings in a detectable way and where the situation is sufficiently simple and reproducible to allow calibration of the distortion that a given force applies. Harris (9) introduced the idea of growing cells in a thin membrane and observing the distortion. Those early results were not calibrated but indicated that fibroblasts develop forces sufficient to distort a thin elastic membrane.

Thus it would be appropriate to introduce a method where the substratum is effectively isotropic at least at the start of the experiment. We have grown cells on an isotropic substratum and looked for distortion of that substratum by forces exerted on it by the cells. The method we describe relies on the detection of such distortion by photoelasticity; see Huard (10) for a description of the principles of photoelasticity or Zhao et al. (11) for details of the method applied to strain distribution in a composite material.

MATERIALS AND METHODS

Cells' h-tert fibroblasts (human) (Clontech, UK), B10 D2 (otherwise known as LeII) mouse capillary endothelia (from laboratory stocks), and HGTFN (human granuloma endothelia also derived from laboratory stocks) were

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grown on the polydimethylsiloxane surfaces which had been coated with appropriate adhesion proteins such as fibronectin for various periods (1–4 days) and the cultures then examined at 37° C with the Polscope microscope.

The Polscope Microscope (CRI International, Woburn, MA) was mainly used with 40× and 100× strain-free objectives. The microscope with its associated software can measure retardances down to 0.1 nm (manufacturer's handbook) and azimuths as well. Pseudocolor images can be obtained which are useful for rapid survey observations and for revealing any mis-set up of the optical system; but for serious observation, images stamped with retardances at various points on the image are most useful. Images of birefringence were obtained after a number of images of the bare poly (dimethyl siloxane) (PDMS) surfaces and a number of phase contrast images of the cells at areas where the birefringence was to be measured. The images of the bare PDMS surface were obtained to check that there was no strain in the substratum in the absence of cells. This check validates the points on the origin in the graph in Fig. 1. The phase contrast images of the sampling areas were acquired so that the exact boundaries of the cell or cells could be established. Three images of each area to be measured were taken in quick succession, one for acquiring the raw birefringence data and two for further processing (for pseudocoloring, obtaining retardance transects across parts of the field, and stamping of calculated retardance values). This method can also be used for measuring forces over a time series. Time- lapse video image sets were acquired over short periods (30– 90 min) to record possible rapid changes in forces.

On many of the images in this work code numbers appear in the bottom right-hand corner: these are the original image filing names from the Polscope. Parts of the date and time may also appear just to the left of the file name.

The images obtained with the Polscope, running in the retardance mode, of isotropic materials were effectively black with a noise level of $<0.2\%$. Each set of measurements made started with a test of isotropic PDMS to show that the noise level met this criterion. When examining cultures of cells on PDMS with the display in the retardance mode, any areas whiter than the noise level indicate birefringence with the whitest parts of the display corresponding to appreciable retardances.

Calibration

The calibration of the Polscope measurements was based on the measured retardance which developed in PDMS films as a response to a known applied force and its comparison with modeling of the same structure and its loading. Finite element modeling allows determination of the strain and stress distribution due to a given set of loading and boundary conditions applied to a structure whose material properties are known.

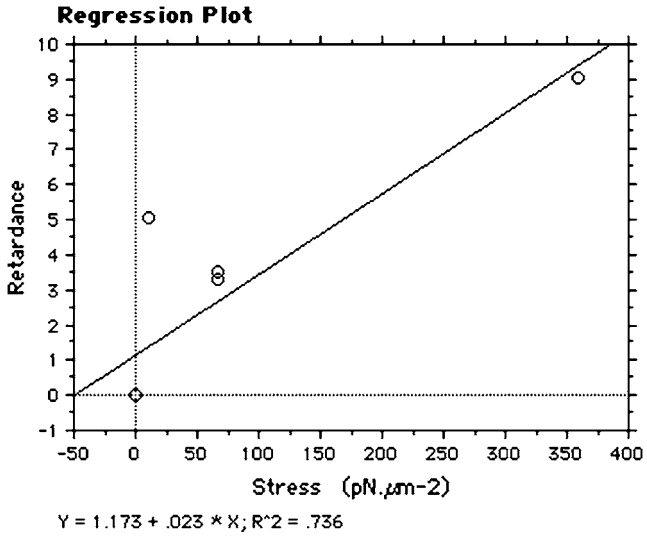

FIGURE 1 Calibration of stress against retardance. Note that retardances measured (see later) mostly are in the piconewton range. Retardance in nanometers. Stress in units of 10^{-12} N/ μm.

The PDMS structure for Polscope measurements was prepared as follows: PDMS uncured was made by mixing 10 parts of SYLGARD 184 and 1 part of curing agent (both supplied as type 184 by Dow Corning, Wiesbaden, Germany) and sandwiching a thin film between two polystyrene coverslips or alternatively (and better) polystyrene flexible sheets. These ''sandwiches'' were then placed in a heated oven at 90° C for 16 h and the coverslips or covering sheets removed to acquire sheets of cured PDMS. The sheets were allowed to overhang a glass coverslip by a known distance. The force was thus applied by the weight of a free edge of Sylgard. Retardance values were measured along transects crossing the edge of the film and the edge of its support at or close to the central axis of the film.

The structure described above was modeled with finite element analysis, using an elasticity modulus of 2.02 MPa and a Poisson ratio of 0.49 (5). Elasticity moduli were measured according to Pelham and Wang (12) by suspending known masses at the end of PDMS strips. Elasticity moduli were measured according to Pelham and Wang (12) by suspending known masses at the end of PDMS strips. Finite element analysis (Abaqus program, Hibbit, Karlsson & Sorensen, Providence, RI, available under academic license) was used in the process of calibration. First, the PDMS sheet was loaded with a known force and resulting retardance values were measured experimentally. Then, the whole situation was modeled by finte element analysis with corresponding loading and boundary conditions, and the PDMS was modeled as a mesh of nodes having appropriate elastic properties. The stress values caused by the applied force were calculated in each node, and then these are linked to the measured retardance values as there is a relation between stresses and retardance for birefringence imaging.

The loading caused by gravity acting on overhanging PDMS sheets results in an axial stress field. Stress values in nodes of the top plane of the model mesh were used for comparison with the measured retardance based on the following relationship: $R = C \times d \times \Delta s$, where C is the optical stress coefficient being the material constant, d is the thickness of the PDMS film, and Δs is the difference between values of principal stresses. In this case, principal stresses are in-plane stresses in x and y directions and, the z stress component is at least by one order of magnitude smaller and is neglected. As changes in thickness are small ($<0.01\%$ according to the model), d is considered constant, and a relationship between retardance values measured at a certain position of the film and values of calculated principal stresses difference at this position should exist. The area at the border of a free-hanging part of the film and the fixed part was used for this purpose as the strain should reach maximum values at that position. Only the data from the smallest overhangs were used since it is under these conditions that strains will be nearest to uniaxial.

Based on our modeling, measured retardance values of units of nanometers of retardance correspond to the stress range of 10^{-12} to 10^{-11} N/μm^2. It is necessary to bear in mind that in case of cells exerting biaxial stress at a certain point, the resulting measured retardance will correspond to the difference of these two stress components and hence the stress caused by the cell can appear smaller. An alternative and less rigorous method of relating retardances to stresses is to use the Coulomb approach (13), which results in similar values. The azimuths of the strongest retardances were also recorded, which allows the experimenter to observe the directions of strongest force development.

RESULTS

Cell death

The first requirement is to show that the strain seen in the substrate disappears when the cell is dead. To test this, images are collected of a cell before killing and afterwards.

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