Mechanical perturbation of cultured human endothelial cells causes rapid increases of intracellular calcium

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Sigurdson, Wade J., Fred Sachs, and Scott L. Diamond. Mechanical perturbation of cultured human endothelial cells causes rapid increases of intracellular calcium. *Am. J. Physiol.* 264 (*Heart Circ. Physiol.* 33): H1745–H1752, 1993.—In first-passage human umbilical vein endothelial cells (HUVEC) and bovine aortic endothelial cells (passages 13–16), exposure to gentle mechanical perturbation using a micropipette caused a transient rise in intracellular calcium concentration ($[Ca^{2+}]_i$). The increase in calcium concentration ($[Ca^{2+}]$) occurred each time the cell was nudged. Three responses were evoked in each of 27 cells using 5 independent HUVEC harvests. Increase in $[Ca^{2+}]$ returned to near baseline levels within ~30 s. The stimulus did not cause membrane puncture, as indicated by *1*) absence of rapid dye leakage, *2*) regulated nature of the $[Ca^{2+}]$ response, *3*) absence of membrane blebbing, and *4*) repeatable nature of the response in the same cell. As an alternative stimulus, we created very narrow fluid streams (1- to 2-μm diam) from a pressurized pipette that generated shear stresses of ~0.001–0.1 dyn/cm^2 on the cells. However, these low-shear streams had little effect on $[Ca^{2+}]_i$. The poke-induced change in $[Ca^{2+}]$ was not blocked by lowering extracellular $[Ca^{2+}]$ ($[Ca^{2+}]_o$; 10 μM). In the absence of $[Ca^{2+}]_o$, however, HUVEC did not respond to the first poke, indicating a requirement for some $[Ca^{2+}]_o$ as a mediator of signaling. After several poke-induced responses, $[Ca^{2+}]_i$ could still be released by caffeine (100 μM), indicating the integrity of the intracellular release mechanism(s). These studies indicate that the response of an endothelial cell to a membrane-deforming event involves a priming step utilizing $[Ca^{2+}]_o$, which facilitates the transient increase of $[Ca^{2+}]_i$.

endothelium; mechanical activation; shear stress; intracellular calcium; fluorescence video microscopy; biomechanics

ENDOTHELIAL CELLS EXIST in a mechanically active environment that includes both fluid shear stress generated by flowing blood and stretching forces generated by tension in the vessel wall due to blood pressure. During invasive procedures such as bypass surgery, grafting, or balloon angioplasty, the large mechanical forces imposed on the endothelium and underlying tissue structures are often correlated with vascular pathologies (11, 17). In normal arterial vessels, the endothelium is often aligned and elongated in the direction of flow (33). This alignment can be recreated in vitro when endothelial cells are exposed to unidirectional shearing forces greater than ~8 dyn/cm^2 for more than 24 h (9, 13).

It is likely that several intracellular signals occur to produce the wide variety of mechanically induced responses. Observations of flow-mediated dilation of whole vessels in vivo or ex vivo also suggest endothelial responsiveness to physical forces (18). Fluid shear forces can cause activation of nitric oxide (NO) synthase, which is largely membrane bound in endothelial cells. Flow-mediated dilation is blocked by L-arginine analogues (5) that inhibit NO production; however, the mechanisms are not fully resolved. Also, both stretching and shearing forces cause enhanced prostacyclin (PGI_2) production in cultured endothelial cells (3, 15). A shear stress-sensitive potassium current that saturates above 15 dyn/cm^2 has been identified (25) in bovine aortic endothelial cells (BAEC). Stretch-activated (SA) nonselective cation channels with conductances of 40 pS have been identified in cultured porcine aortic endothelial cells (21).

Inositol trisphosphate ($InsP_3$) levels are enhanced in shear-stressed endothelial cells, suggesting that phospholipase C activity is elevated and that intracellular calcium is possibly released in stressed endothelium (24). However, the initial report of shear-induced calcium mobilization (2) has recently been shown to be mediated predominately by free-flowing ATP in the medium 199 perfusion buffer (12, 20, 22, 23) and not by a direct shear-stress effect. Some researchers, however, have observed flow-induced calcium mobilization in the absense of ATP in the perfusion media (16, 30).

Important distinctions can be made regarding the nature of physical forces in substrate-stretching models (1–20% strain), fluid shear systems (1–30 dyn/cm^2 shear stress), and micropipette measurements of SA channels (0.1–10 dyn/cm of tension) (27). The endothelial responses to these different forces are heterogeneous. Whereas substrate-stretching assays with endothelial cells have been reported to produce calcium mobilization (K. Naruse and M. Sokabe, personal communication) and enhanced endothelin production (3), fluid shear stress has little effect on intracellular calcium (12, 20, 22, 23) and suppresses endothelin production (23, 29).

Our interest was to study the signal transduction events involving ion regulation and mechanically activated membranes. Using a micropipette in a micromanipulator, we could generate deformations on a single endothelial cell ranging from gentle membrane displacements of <1 μm to lethal damage of the cell involving bleb formation and cell detachment.

EXPERIMENTAL METHODS

Cell culture. Human umbilical vein endothelial cells (HUVEC) were cultured as previously described (10). In brief, endothelial cells were removed from the umbilical vein lumen by a 30-min collagenase incubation (Worthington Enzymes). The endothelial cells were pelleted and resuspended in medium 199 (GIBCO) with 20% heat-inactivated fetal calf serum (Hyclone), 0.1 mg/ml penicillin and streptomycin, and 0.3 mg/ml L-glutamine and seeded in T25 flasks. One day before the experiments, the confluent primary cells were passaged to NaOH-treated 25-mm cover slips. First-passage HUVEC were then used in the experiments. BAEC (passages 13–16) were used 1 day after passage. Cell thickness above the cover slip ranged from 2 to 5 μm in the cytoplasmic periphery to ~8 μm in nuclear regions of some cells. In the absence of extracellular

calcium, the endothelial cells retracted their cytoplasm, rounded, and finally detached from their substrate. Thus experiments conducted without extracellular calcium were limited to 20 min in duration.

Dye loading. Individual cultures were loaded for 30 min at room temperature in 4 μM fluo-3/AM (Molecular Probes) dispersed with 0.2% Pluronic F-127 (Molecular Probes) in normal buffered saline [NBS; composed of (in mM) 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, and 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid at pH 7.4] as previously described (31). The cells were incubated for an additional 20 min to allow hydrolysis of the ester form of the dye. The cells displayed a uniform and diffuse dye loading throughout the experimental period.

Fluorescence video microscopy. Cells were viewed with a Zeiss Axiovert using ×63 (NA 1.4) Plan-Apochromat or ×40 (NA 1.3) Achrostigmat objectives in either fluorescence or differential interference contrast (DIC) configurations using 100-W Zeiss Hg lamps and the fluorescein dichroic filter cube set. As previously described (31), images were recorded using a Videoscope Image Intensifier coupled with a Dage MTI-72 charge-coupled device camera. Images were stored on super-VHS video tape for analysis using a Data Translation frame grabber and a Sun TAAC board (Sun Microsystems) running Tacit software (SUNYAB Sponsored Programs). For analysis of average pixel intensities, digitized video frames were not processed in any way.

Cell stimulation. We stimulated the cells with a fire-polished patch pipette (Fig. 1). The pipette was mounted in a standard electrode holder stabilized with a quartz rod to reduce drift (32). The entire assembly was maneuvered with a Buleigh piezoelectric three-axis micromanipulator. Pipette position in the *z*-axis was monitored during fluorescence microscopy by observing the manipulator driving voltage, which had been previously calibrated in micrometers per volt. The entire microscopy assembly was mounted on a vibration-free table. The target cell was videotaped in DIC mode before fluorescence imaging to establish the cell morphology before stimulation. While in fluorescence mode, the pipette was slowly lowered to the cell surface until a response was observed, at which time the pipette was promptly withdrawn. The intracellular calcium concentration was allowed to return to near baseline levels before the next stimulation. After stimulating the cell, we carefully inspected the cell in DIC mode for evidence of cell damage, such as blebbing or vesicle formation. Intentional puncture of the cell membrane was easily verified by the rapid loss of all cellular fluorescence within ~5 s with concomitant membrane blebbing.

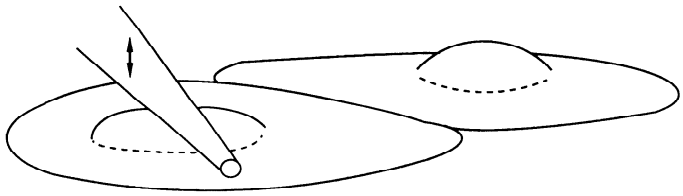

Fig. 1. Schematic diagram of mechanical perturbation of fluo-3/AM-loaded human endothelial cells using side (not tip) of a micropipette moving downward on top of nuclear region. Distal 1-mm end of electrodetip was bent to be perpendicular to optical axis.

RESULTS

When the micropipette was slowly lowered to the surface of the target cell, the cell membrane was displaced by <2 μm, causing a rapid increase of fluo-3 fluorescence (Fig. 2). Within 20–40 s, the cell restored its calcium to near resting levels. The process could be repeated three or four times on a single cell (Fig. 2) before the response desensitized. Typically, the peak intensity (relative to the prestimulus intensity) declined with each subsequent stimulus. After repeated stimuli of sufficient magnitude to cause calcium transients, we could see no morphological changes that could be associated with cell death (Fig. 3).

In 27 cells from 5 independent harvests, >90% of the cells responded to the first poke . Although the response rate for a second poke was also high, desensitization of the response was evident in the third and subsequent stimuli, as seen in Fig. 2. Very similar responses were also observed with BAEC. Oscillations in calcium were not seen under any conditions. In addition, we verified that intracellular calcium could be released after mechanical perturbation of cells by adding 100 μM caffeine to the bath.

Utilizing the temporal and spatial resolution of our video imaging, we analyzed the dynamics of the calcium waves that spread throughout the cell on stimulation. In Fig. 4*A*, the spread of calcium in a single cell originated near the point of contact with the electrode and then spread through the cell toward the periphery. In the sequence of cellular activation, regions of the cell near the

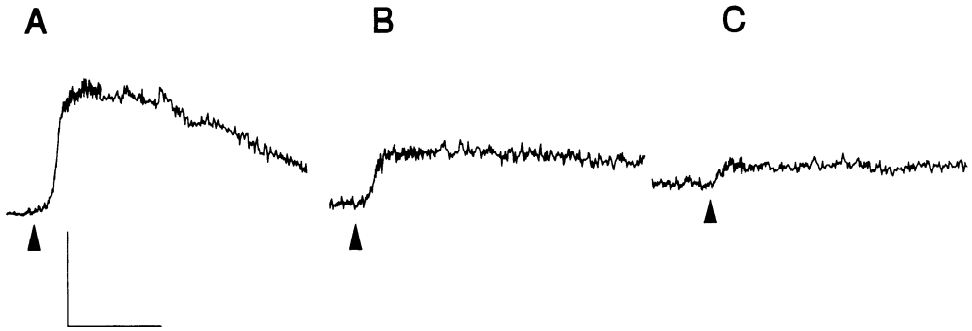

Fig. 2. Change in average cell fluorescence intensity with mechanical stimulation. Traces represent average pixel intensities of a 18-μm^2 perinuclear region sampled from individual video frames. For the 1st 10 s, all video frames were sampled, whereas for later times, frames were selected at 90-ms intervals. Tracings (34 s each) are shown for 3 separate mechanically activated responses in 1 endothelial cell. On activation of cell for the 1st time (*A*), intracellular calcium ($[Ca^{2+}]_i$) increased to maximal levels within 250 ms and then returned back to near baseline levels. After each stimulation, intensity was allowed to return to resting levels for 2–3 min before the next stimulation. Desensitization of mechanically induced increase in $[Ca^{2+}]_i$ is seen in 2nd (*B*) and 3rd (*C*) responses to stimulation. Arrowheads indicate times of contact with pipette. Scale bars indicate 10 s and 150% change in fluorescence intensity.

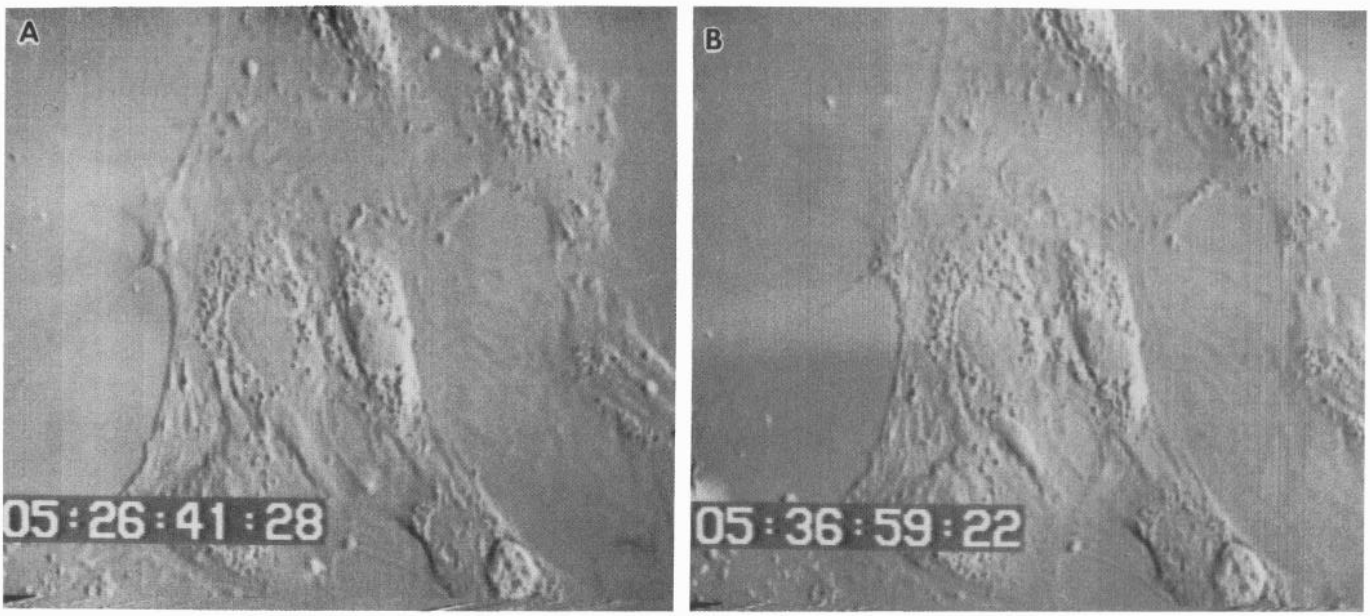

Fig. 3. Morphology of endothelial cells before (*A*) and after (*B*) stimulation; left cell of the pair was prodded 5 times. No changes in morphology were apparent in either cell as viewed using differential interference contrast (DIC) microscopy. Length of time-code bar represents 66 μm.

site of activation displayed a fluorescence that increased from resting to maximal levels within eight video frames corresponding to 250 ms (Fig. 4*A*). When a target cell with contiguous neighbors was activated, the neighbors also became excited, resulting in a wave of fluorescence increase spreading from cell to cell. Although a stimulated cell with contiguous neighbors displayed a similar time course to isolated cells (Fig. 2), there was a delay before neighboring cells began to exhibit a calcium increase. This is seen in Fig. 4*B*, in which the cell on the left experienced a delayed calcium increase after the cell on the right was mechanically activated. In experiments with cell pairs, care was taken to stimulate only one cell. Increases in intracellular calcium were observed when the periphery of a cell was prodded; however, these exceedingly thin regions of the cell were difficult to deform without puncture or bisection of the cell.

To quantify the fluo-3 fluorescence changes, we used pixel averages over 18-μm^2 regions of each video frame. Typical selected regions are indicated schematically in Fig. 5*A* (*inset*), in which a stimulus time course for one cell is shown. *Region 1* was near the initial stimulus site (2 μm), whereas *regions 2* and *3* were further away (4 and 16 μm, respectively). Within 150 ms of applying the stimulus, fluorescence increased 50% at *region 1*. This same increase in *region 2* was delayed by 50 ms. At the cell periphery (*region 3*), 16 μm away, a 50% increase in fluorescence occurred 600 ms after *region 1* had responded at the 50% level. The velocity of the calcium wave at the center of the cell, where the calcium gradients were largest, was ~50 μm/s. However, the average velocity for the calcium front to move across the entire cell was 23 μm/s. We analyzed two such cells and found similar velocities. The maximum fluorescence (F/F_o) decreased with distance from the stimulus site (Fig. 5*A*), probably indicating that the peak calcium concentration was less at the cell periphery than in the center.

In some experiments it was possible to stimulate single cells without touching their neighbors. Stimulating a single cell often caused its neighbors to display calcium mobilization. In the nearest neighbor, the calcium response occurred with a delay of 1–2 s after the target cell had responded (Fig. 5*B*). The dynamics of the response were similar in the mechanically activated cell and its neighbor. Within the microscope's field of view (outside the intensified camera's field of view) we could sometimes observe a wave of cellular activation spreading from the stimulus site for several cell diameters.

To explore the role of extracellular calcium in coupling the mechanical stimulus to the intracellular response, we varied the bath concentration of calcium. On a single cover slip, we verified that in the presence of extracellular calcium the monolayer contained cells responsive to the first poke (Fig. 6*A*; 2 cells display a total of 7 poke-induced responses). When the medium was exchanged with calcium-free NBS with 5 mM EGTA (Fig. 6*B*), none of the cells responded (5 of 5 cells tested). When calcium-containing medium was added back, the monolayer regained its ability to respond to mechanical stimulation (3 of 3 cells tested) as seen in Fig. 6*C*. Low extracellular calcium concentration (50 μM) did not prevent a response to the first poke, but it did attenuate the response to subsequent pokes.

To test the limits of mechanical stimulation and to confirm our standards for cellular damage, we applied purposefully large deformations of the cells. Intentional puncture of the cell with the pipette tip led to loss of all cellular fluorescence within a few seconds, indicative of dye leakage (Fig. 7*A*). The decrease in fluorescence was an order of magnitude faster under puncture conditions than the recovery time of a transient caused by a sublytic stimulus (Fig. 2). Under puncture conditions, the cells formed blebs (Fig. 7*B*) within 1–2 min.

Because endothelial cells have been reported to be sen-

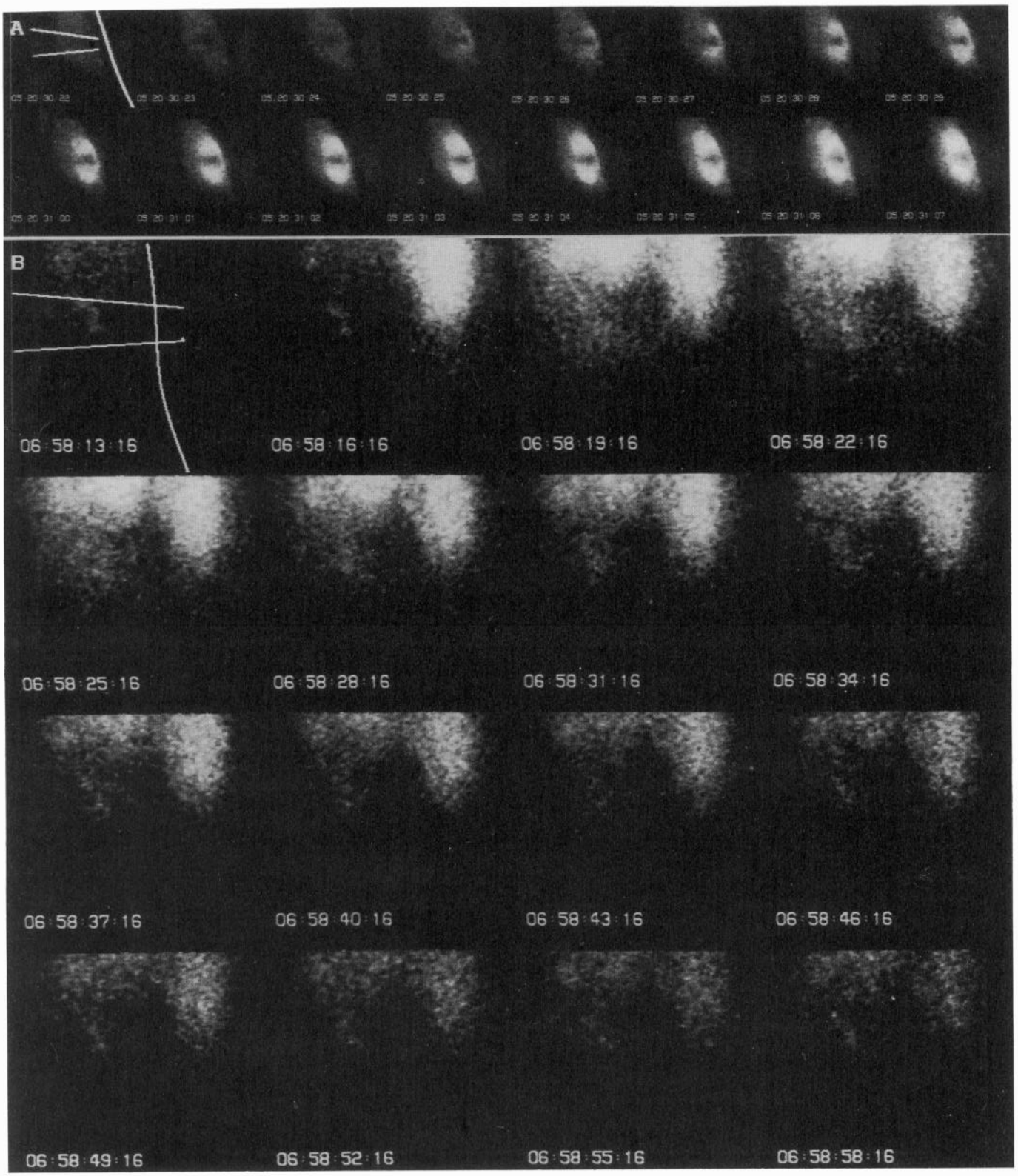

Fig. 4. Fluo-3 fluorescence in 2 human endothelial cells as recorded using intensified charge-coupled device video microscopy (see EXPERIMENTAL METHODS). Frame-by-frame images (33-ms intervals) demonstrate a rapid and large cellular activation on mechanical stimulation (*A*). Maximal Ca^{2+} response occurred in <250 ms. At lower time resolution with frames captured every 3 s (*B*), full time course is seen for right cell of the pair, which had been mechanically activated. Left cell activated after a 1.5-s delay, probably through gap-junctional communication. *A* and *B* (first frame): cell boundries and position of prodding pipette are indicated by vertical and horizontal lines, respectively. Length of time-code bar represents 23 μm.

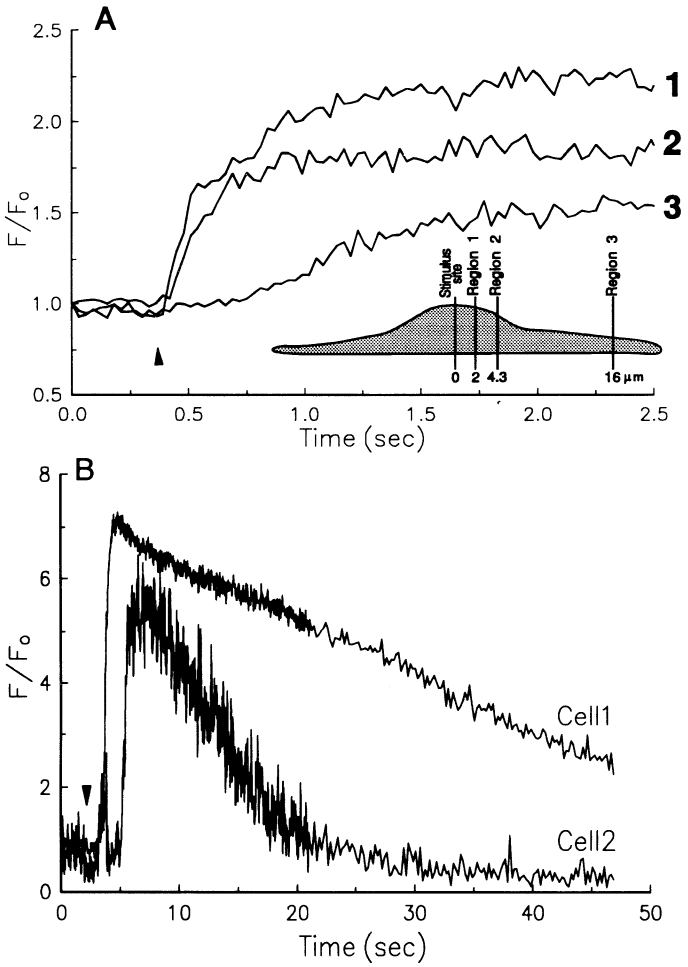

Fig. 5. Tracings obtained from mechanically activated endothelial cells demonstrating temporal and spatial gradients of $[Ca^{2+}]_i$. *A*: average fluorescence intensities normalized to initial fluorescence intensity (F/F_o) within a nuclear sample region (*1*), a perinuclear sample region (*2*), and a peripheral cytoplasmic region (*3*) (as indicated schematically in *inset*) are shown for an endothelial cell responding to mechanical activation. *Region 1* is 1st to achieve maximal Ca^{2+} levels, whereas *regions 2* and *3* achieve their maximal response with a time delay relative to *region 1* of 150 and 600 ms, respectively. *A, inset*: schematic of endothelial cell showing radial distances of *regions 1, 2,* and *3* from stimulus site. *B*: mechanical stimulation of *cell 1* resulted in increased fluo-3 fluorescence, which was followed by an increase in an unstimulated neighbor (*cell 2*). Time lag of ~1.5 s was required for cellular communication between cells with the mechanically activated cell having already achieved maximal response.

sitive to laminar shear stress as well as to changes in tension, we investigated the effect of shear stress on the calcium response. Flow was driven by a positive pressure between 10 and 100 mmHg on the pipette, the tip of which was positioned <5 μm from the cell surface. Wall shear stresses were estimated to be 0.001–0.1 dyn/cm^2 using the boundary layer solution for flow past a blunt object (27). These stresses did not cause calcium mobilization in any of several cells tested.

DISCUSSION

Vascular endothelial cells are known to contain a variety of ion channels including cation-selective mechanosensitive (21) as well as numerous exchangers (1) that may alter the internal calcium levels. Additionally, agonists such as histamine, ATP, and bradykinin are potent releasers of intracellular calcium in endothelial cells (26). Although second messenger pathways and calcium regulation are well investigated for agonist stimulation of endothelial cells, the membrane behavior and associated biomechanical properties are not as well studied.

The repeated demonstration by imaging of poke-induced calcium mobilization in HUVEC in the micropipette experiments is consistent with calcium mobilization measured photometrically in endothelial cells (K. Naruse and M. Sokabe, personal communication) and smooth muscle cells (7, 8) using cell-stretching assays. Consistent with earlier reports that low shear stresses do not enhance PGI_2 production (15) or calcium mobilization (12, 16, 22, 30), we found that locally applied flows (<0.1 dyn/cm^2) had no effect. Because the flow streams are so narrow, the spatial shear stress gradients may actually be quite high (on the order of 1,000 dyn/cm^2 per cm) yet were not capable of increasing intracellular calcium.

We modeled the calcium activation process in these cells using the transient diffusion equation for one-dimensional radial diffusion (6) of calcium from a center zone of elevated calcium outward to the periphery of the cell. In this model, the transient spatial distribution of calcium, $C(r,t)$ (where C is calcium concentration, r is radius, and t is time), is governed by *Eq. 1* with associated boundary and initial conditions. The diffusion coefficient for calcium within cellular cytoplasm ($D_{Ca,\,cyto}$) was taken as 0.6×10^{-5} cm^2/s (14)

$$\frac{\partial C}{\partial t} = D_{Ca,\,cyto}\left\{\left(\frac{1}{r}\right)\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right)\right\} \qquad (1)$$

Boundary condition I

C is finite at $r = 0$ (at center of cell) for all time (t)

Boundary condition II

$$\frac{\partial C}{\partial r} = 0 \quad \text{at } r = R \text{ (outer radius of cell)}, \qquad \text{for all } t$$

Initial condition

$$C(r) = \begin{cases} 1{,}000 \text{ nM} & \text{at } 0 < r_{crit} \\ 100 \text{ nM} & \text{at } r_{crit} < r < R \end{cases} \qquad \text{at } t = 0$$

Using this model for high initial activated levels of calcium with r_{crit} at 0.2 and R at 15 μm, we predicted that a calcium wave would move with a velocity of several hundred micrometers per second, depending on radial position. Near the center of the cell, where calcium gradients were highest, the wave velocity exceeded 750 μm/s. Moving from the center of the cell toward the periphery, where smaller gradients existed, the velocity decreased from ~500 to ~200 μm/s. In this model, the initial calcium distribution equilibrated to a constant value over the entire cell within ~100 ms. In our measurements of the spread of calcium from the center of the endothelial cell outward, we typically found that a time of several hundred milliseconds (Figs. 4*A* and 5*A*) was required for the calcium to move across the cell. In our system, the observed wave velocity for calcium spreading in endothelial cells was found to be ~50 μm/s, which corresponds to earlier reported values (19) for endothelial cells.

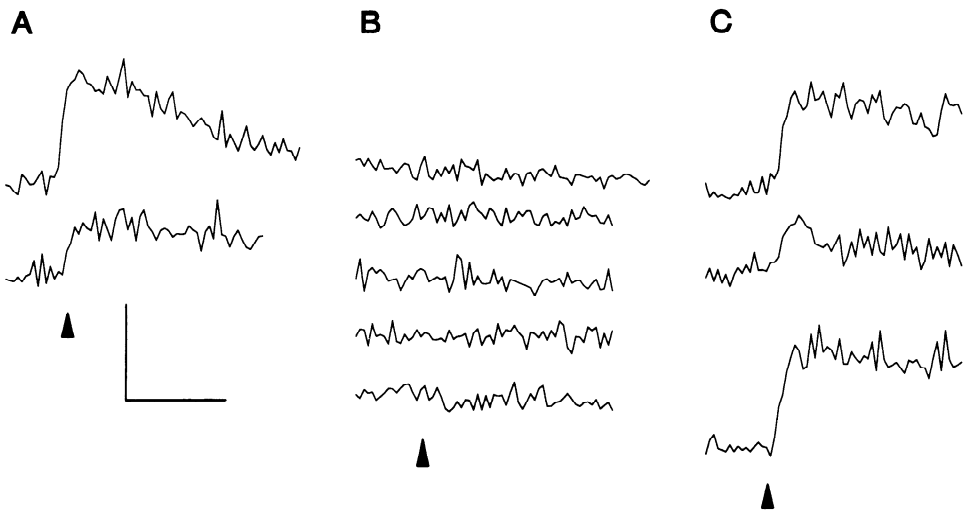

Fig. 6. Extracellular calcium ($[Ca^{2+}]_o$) was required for the endothelial response to mechanical perturbation. *A*: 2 cells were established to be responsive under normal $[Ca^{2+}]_o$. *B*: when bathing medium was replaced with EGTA-buffered medium, none of the 5 cells tested responded to mechanical stimulation. *C*: addition of $[Ca^{2+}]_o$ restored the cellular response to stimulation in 3 different cells. All traces were normalized to initial fluorescence intensity. Arrowheads indicate times of contact with pipette. Scale, 6 s and 50% change in normalized fluorescence.

Thus the observed spreading of calcium likely required a "reaction" step that was rate limiting, since the spreading of calcium required a significantly longer time than would be predicted by a purely diffusional process. For example, $InsP_3$-mediated or calcium-induced calcium release have time constants slower than that of diffusion. Additionally, using the diffusion model, we predict that the relatively large rise of calcium (over 1.5-fold increase in fluo-3 fluorescence) seen at the periphery of the cell could not be produced solely by the redistribution of a large central "bolus" of calcium ions, since a large dilution effect occurs as the calcium spreads radially.

Although we have not considered the cell's ability to pump out calcium, the experimental data and diffusion modeling suggest that the spreading of calcium we observed was not due to diffusion from a point or zone source (either a "finite bolus" or "fed bolus"), which would occur with a physical hole in the membrane. The relatively slow nature of the calcium spreading when compared with diffusion would suggest that the phenomenon is governed dynamically by some reaction/diffusion process. The initiating event in the mechanical activation of endothelial cells requires extracellular calcium, but the spread of calcium across the cell is likely due to internal release. Cells in zero external calcium were incapable of responding to the stimulus, yet when low calcium levels were present, only a single response was observed. It is possible that at low levels of extracellular calcium, enough calcium was still present in some critical compartment to mediate one response but that the compartment could not be refilled to trigger later responses.

In considering the increase of intracellular calcium in cells neighboring the mechanically activated cell, it is unlikely that diffusion of a released activator could cause the wave of activation away from the stimulated cell. Gap-junctional communication via a calcium-gated channel opening likely mediates the spread through cells that are in contact with each other.

The presence of mechanosensitive (MS) ion channels in many cell types (27) has made these channels attractive candidates as the cellular transducers of mechanical forces acting on cells. Whereas MS channels were found in porcine aortic endothelial cells (21), we have observed MS nonselective cation channels in only a single patch in >10 patch recordings using HUVEC (Sigurdson, data not shown). This low success rate has prevented a determination of whether the MS channel blocker gadolinium (Gd^{3+}) can block this channel. During the prodding of endothelial cells in the presence of Gd^{3+}, a calcium response can be achieved on the first mechanical stimulation if the deformation is large enough. Subsequent calcium responses appear attenuated, but not blocked. This is comparable with the results obtained with embryonic heart cells (31), in which Gd^{3+} inhibited a mechanically induced calcium increase and blocked a cation-selective MS channel. However, the heart cell preparation permitted an unequivocal determination of whether the stimulus was so excessive that irreversible cell damage would result. Without a more precise calibration of the forces applied to the endothelial cells, it remains difficult to establish the effects of Gd^{3+} and the role of MS channels as mechanotransducers. In addition to MS channels, the role of membrane-cytoskeletal interactions, cytoskeletal-induced signaling, or membrane "tearing" at the microscopic level (as in scrape-loading techniques) remain important areas under investigation. The biphasic nature of the calcium response, its repeatability, and the dynamics of the calcium spreading would suggest a mechanism other than membrane tearing.

It is possible that some degree of cellular activation occurs during normal electrophysiological measurements of endothelial membrane channels, even when the cell-blebbing phenomenon is not observed. Additionally, the use of micropipettes to measure passive mechanical/viscoelastic properties of living endothelial cells should take into account the acute response of the cells to deformation with subsequent calcium-activated protease degradation of membrane-cytoskeletal interactions.

Intracellular calcium is likely to be an important second messenger of endothelial response to mechanical deformation. This pathway may play a role in vessel responses that occur *1*) normally in large arterial vessels with pulsatile flow, *2*) chronically under conditions of hypertension, *3*) pathologically in venous distension during postoperative deep vein thrombosis (4), or *4*) clinically during cardiovascular interventions such as balloon

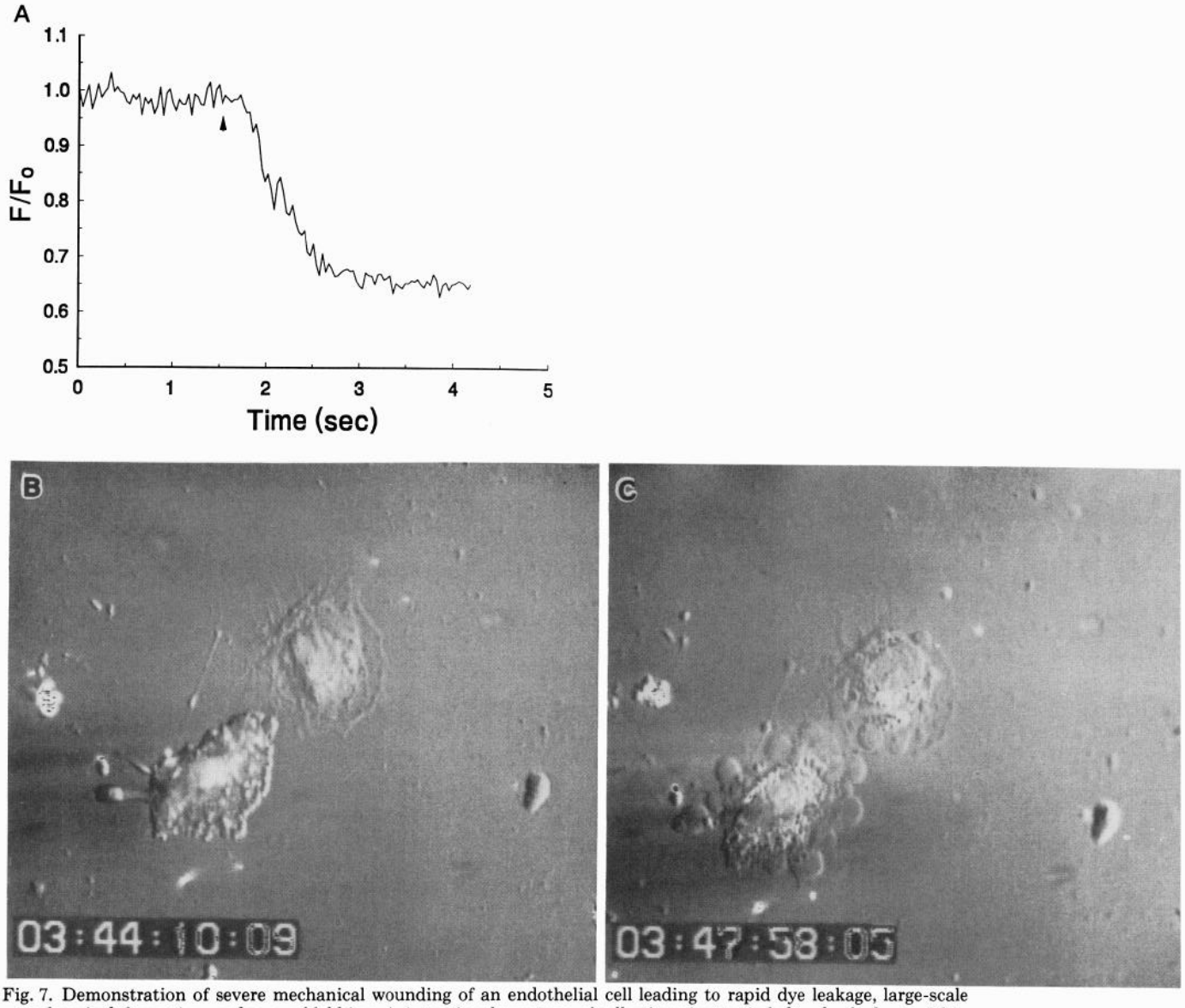

Fig. 7. Demonstration of severe mechanical wounding of an endothelial cell leading to rapid dye leakage, large-scale cytoplasmic deformation, and severe blebbing. *A*: intentional puncture of cell using pipette tip led to dye leakage within a few seconds with concomitant complete loss of cellular fluorescence. Endothelial cell wounding could also be achieved by large deformations of nucleus or cytoplasm, after which the endothelial cell was easily detachable from glass substrate. Arrowhead indicates time of contact with pipette. *B* and *C*: DIC images of a pair of intentionally punctured endothelial cells showing cells before (*B*) and after (*C*) puncture. Note extensive blebbing and swelling in cell periphery. Length of time code bar represents 66 μm.

angioplasty or angioscopic procedures. In the first and second cases, small forces imposed by transmural pressures may facilitate endothelium-mediated dilation via calcium-dependent mechanisms such as endothelium-dependent relaxing factor release. In the third and forth cases, large forces imposed on the vessel intima could lead to endothelial responses similar to those described in this work. Finally, our system may provide a very sensitive system for screening pharmacological compounds that enhance endothelial resistance to mechanical wounding.

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