Session 19

SILICONE WRINKLING MEMBRANES

Traction Forces

- Previously Paradigm
  - First cells cultured inside plasma clots (a/k/a fibrin gels)
  - Widely held belief that cells cause gels to shrink by dehydration
- Albert Harris worked under Michael Abercrombie
  - Cell-gel distortions were “side-effect” of propulsion forces
  - Sought to develop flexible gels to map cell forces
  - Difficult to get funding

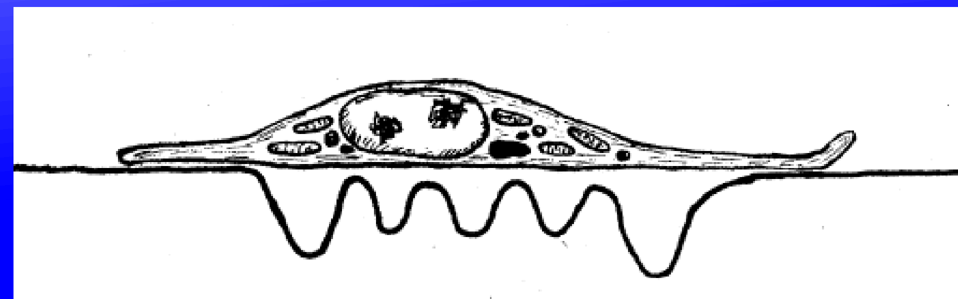
Albert Harris

Flexible Substrata

- Particle movement
  - Carbon black particles (soot) mixed in thin layer of plasma clot
  - Centripetal movement of carbon particles
  - Difficult to maintain uniform Young's modulus
  - Many grant applications rejected
- Cross-linked silicone fluids
  - Flame-cured flexible skin on silicone fluid covered coverslip glass
  - Silicone impervious to hydration/dehydration effects
  - Received tenure on year before *Science* break-through

Wrinkling

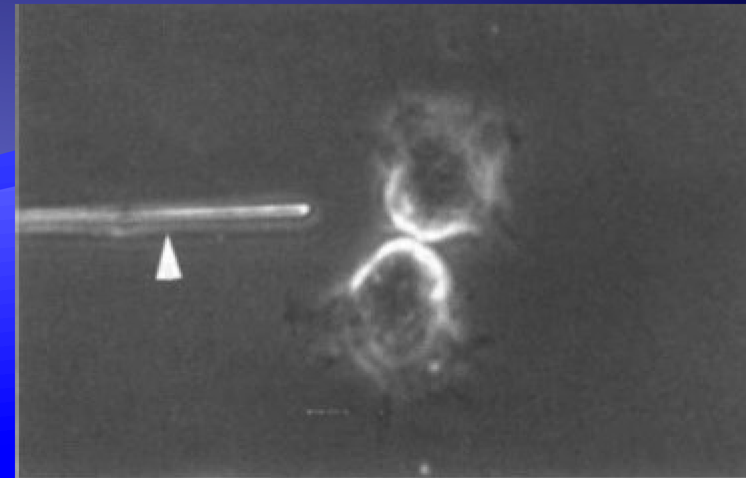
- Compression folds underneath cell
- Tension wrinkles radiate outward
- UV treatment increases wrinkling by weakening cross-links in silicone film

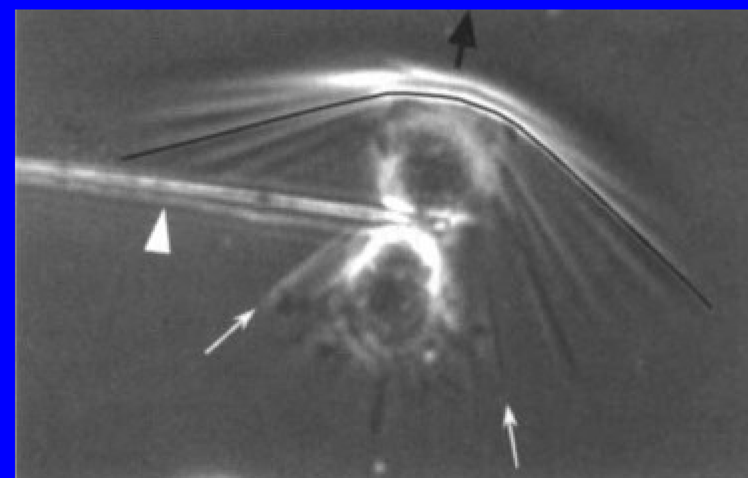


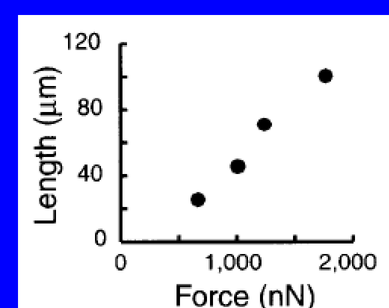
Wrinkling Video

Calibration

- Pulled glass needle
  - Spring constant of needle calibrated with hanging weights
  - Pushing force applied to fixed cells on sheet
  - Force causes reversible wrinkles
  - Linear relationship between wrinkle length and applied force







Force thru Costameres

- Costamere are dense plaques b/w Z-disc and cell membrane

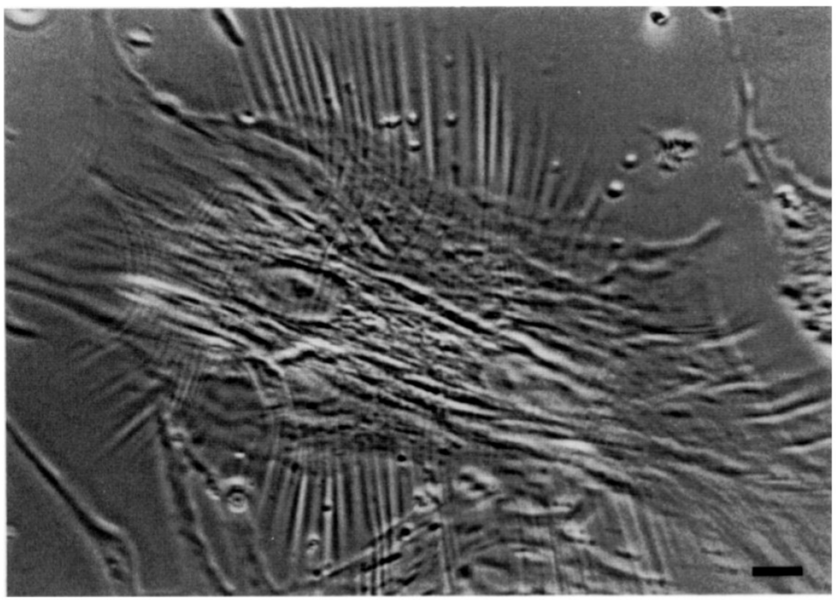


Figure 1. A living, contracting adult rat cardiac myocyte cultured on the flexible silicone rubber substratum for 9 d. Note the closely spaced, pleat-like wrinkles in the rubber substratum. Bar, 10 μm.

Costameres Are Sites of Force Transmission to the Substratum in Adult Rat Cardiomyocytes

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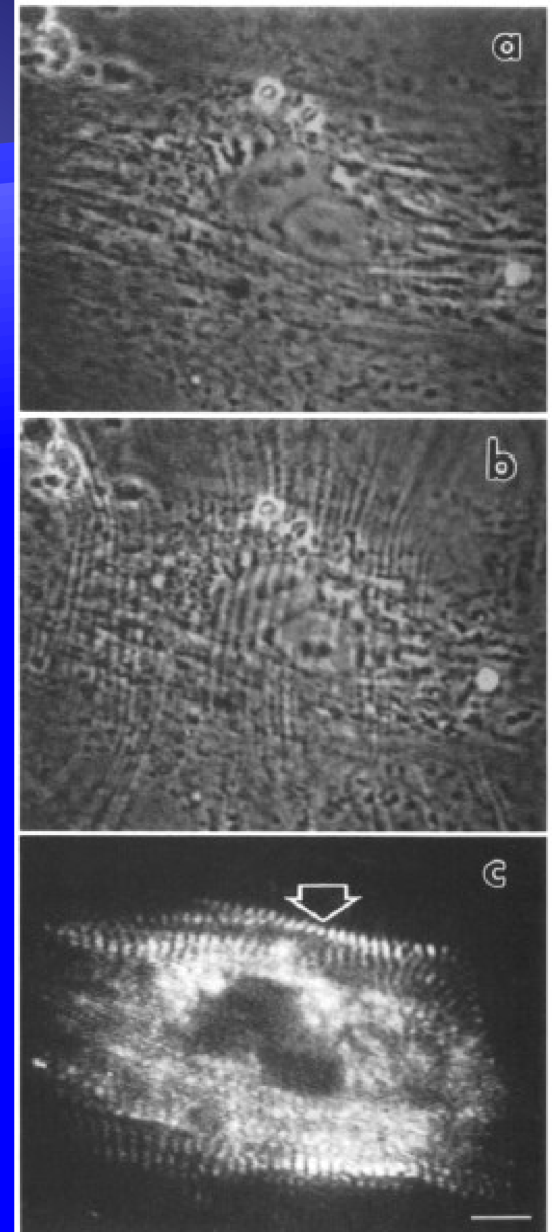
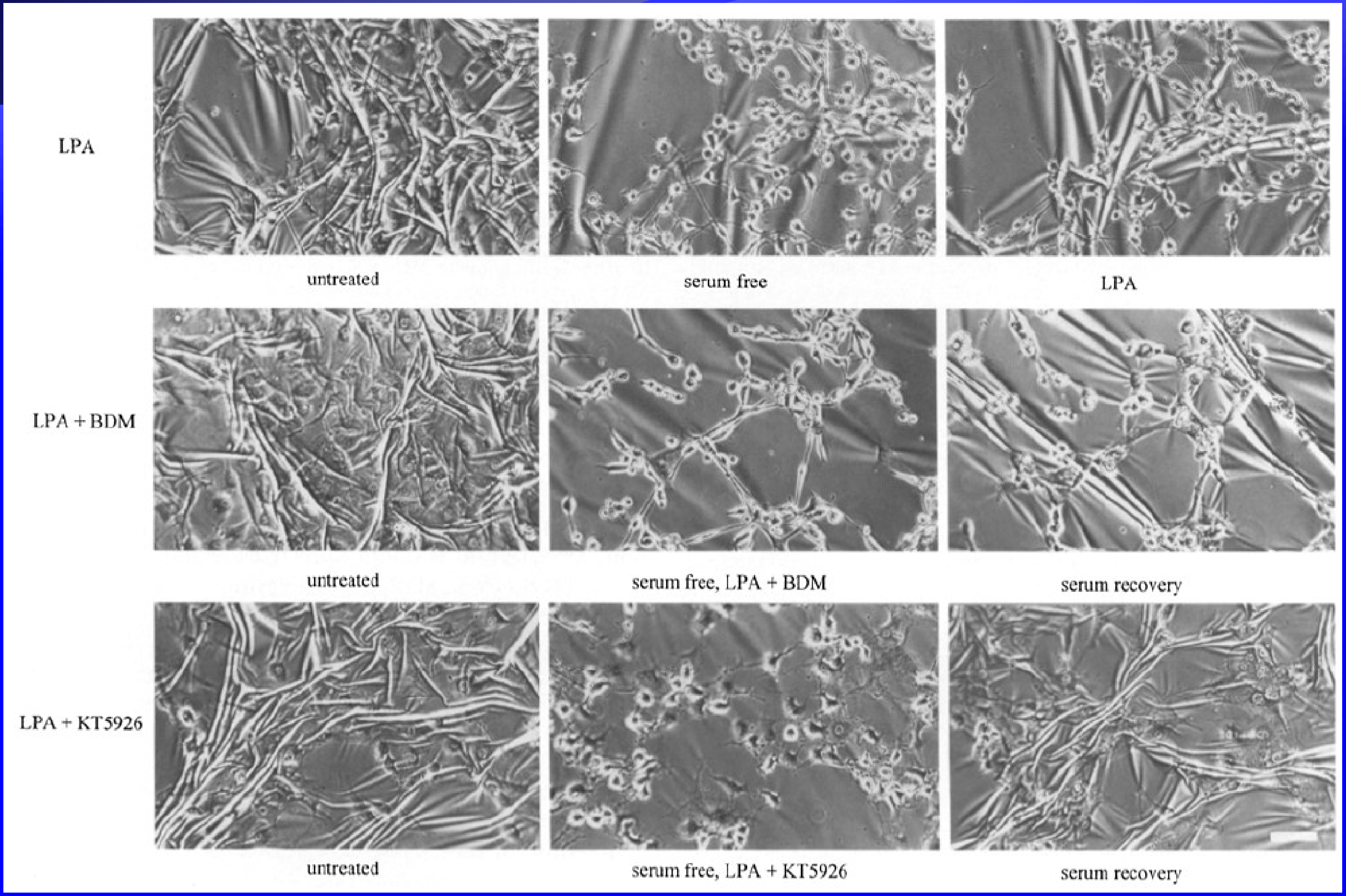


Figure 3. Distribution of IATR-a-actinin in a 10-d cultured adult heart cell, which is producing pleat-like wrinkles upon contraction. (*a*) Relaxed; (*b*) contracted; (*c*) fluorescent image showing the distribution of the a-actinin–containing Z-lines. Arrow indicates the area of enlargement in Fig. 4. Bar, 10 μm.

Confirmed Rho/Myosin involvement



LPA activates Rho

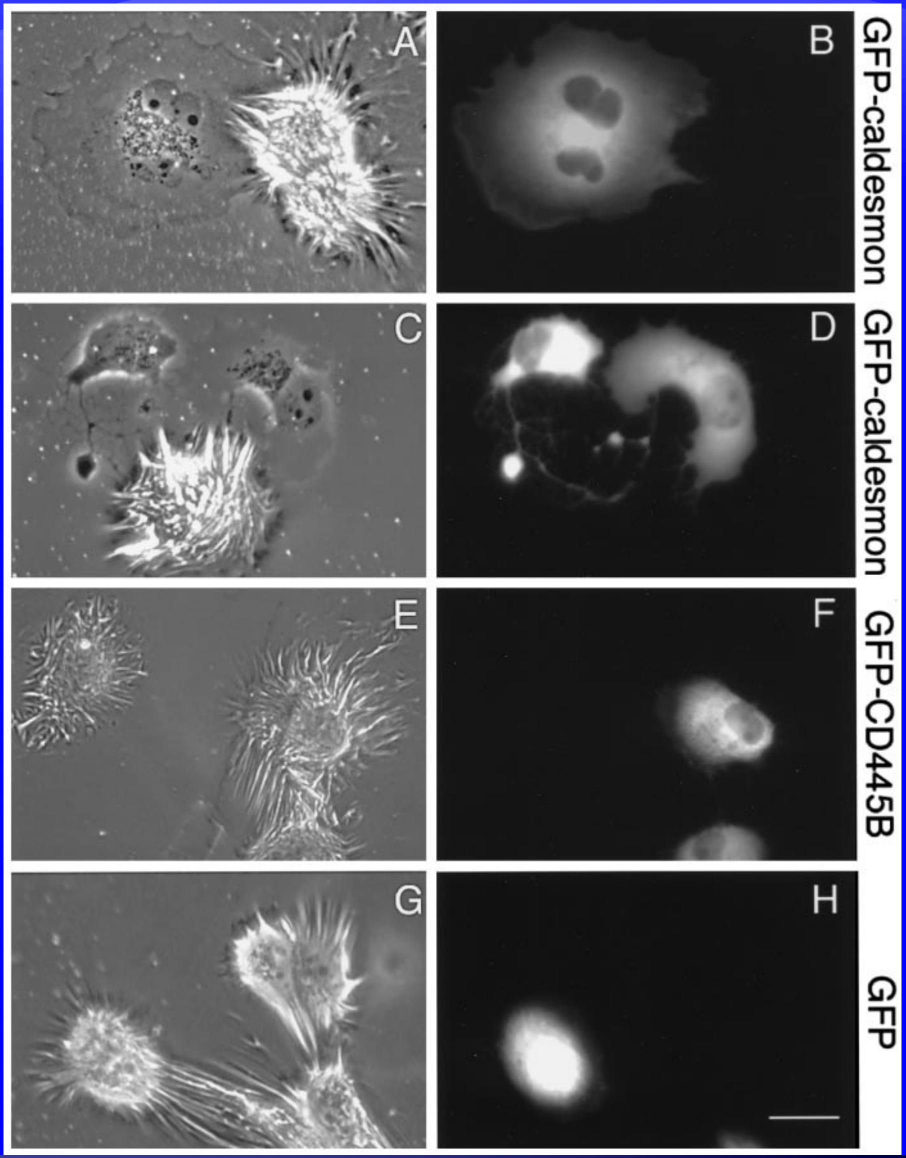
BDM inhibits myosin ATPase activity

KT5926 inhibits MLCK

Chrzanowska-Wodnicka M., *et al.* (1996) *J Cell Biol*, 133(6):1403

Calmodulin/Myosin Involvement

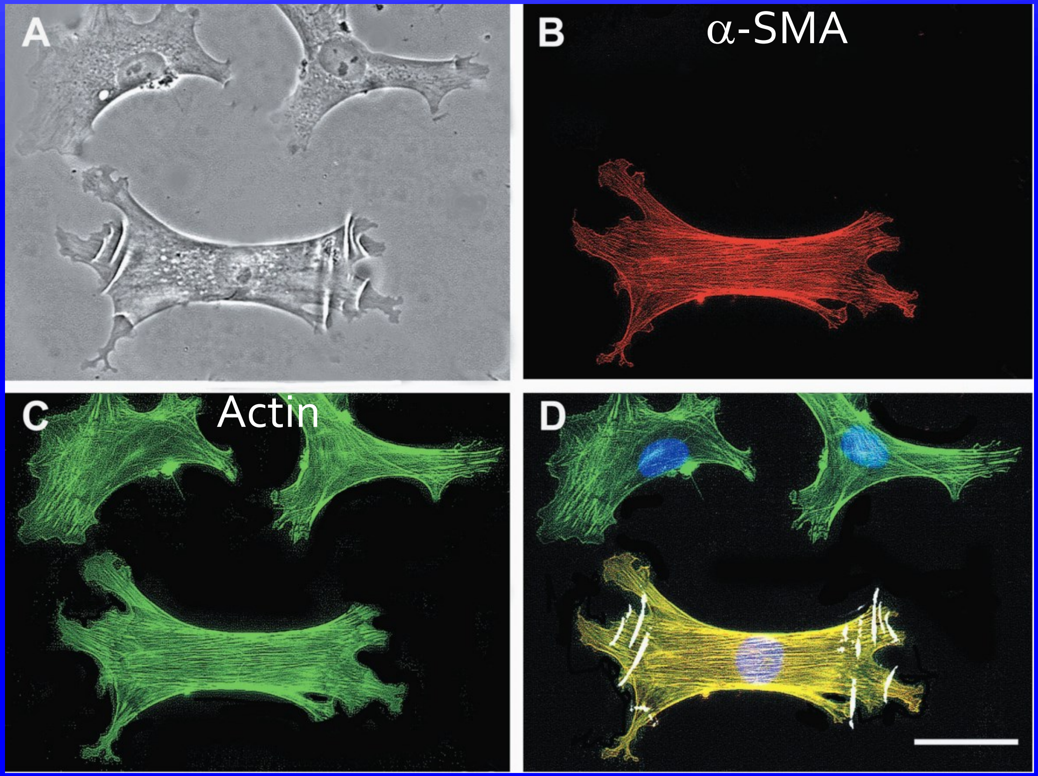
- Transfect cells with cDNA constructs
- Caldesmon inhibits calmodulin, actin, and myosin activity
- CD445B is truncated caldesmon without actin, calmodulin, & myosin binding sites
- GFP construct used as control



Helfman, *et al*. (1999) *Mol Biol Cell*, 10:3097

Myofibroblast Differentiation

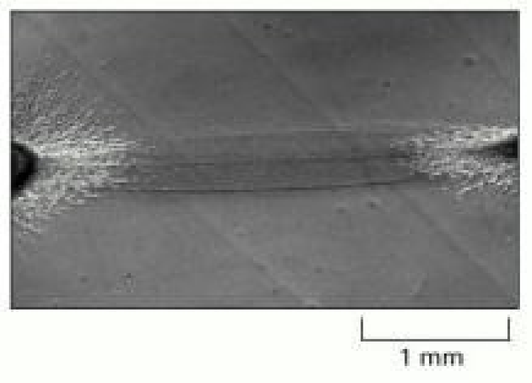
- Fibroblasts expressing α-smooth muscle actin generate large traction forces
- Contractile differentiation important for wound healing



Hinz, B., *et al*. (2001) *Mol Biol Cell*, 12:2730

Impact of Harris' work

- Direct observation of small, weak forces not possible before (and strange to some)
- Technique is not easily reproduced
- Not a direct quantitative approach
- Cell force techniques improve on reproducibility and quantification



(MBOC) Figure 19-50. The shaping of the extracellular matrix by cells. This micrograph shows a region between two pieces of embryonic chick heart (rich in fibroblasts as well as heart muscle cells) that were cultured on a collagen gel for 4 days. A dense tract of aligned collagen fibers has formed between the explants, presumably as a result of the fibroblasts in the explants tugging on the collagen. (From D. Stopak and A.K. Harris, *Dev. Biol.* 90:383–398, 1982)