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
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

Cultured lung myofibroblast, wrinkling a deformable silicone substrate
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
Costameres Are Sites of Force Transmission to the Substratum in Adult Rat Cardiomyocytes

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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.

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

Transfect cells with cDNA constructs
Caldesmon inhibits calmodulin, actin, and myosin activity
CD$_{445}$B is truncated caldesmon without actin, calmodulin, & myosin binding sites
GFP construct used as control

Myofibroblast Differentiation

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

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)