

## SUBSTRATE COMPLIANCE VS LIGAND DENSITY IN CELL ON GEL RESPONSES

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**INTRODUCTION:** Substrate stiffness is emerging as an important physical factor in the response of many cell types. Like other anchorage dependent cells, smooth muscle cells derived from rat aorta (A7R5 line) are found to spread more and organize their cytoskeleton and focal adhesions much more so on rigid glass and 'stiff' substrates than on 'soft' gels. Collagen density also factors into cell on gel responses, with minimal spreading on very low collagen and a weak maximum in cell spreading on intermediate collagen densities. Bell-shaped curves are readily modeled to highlight the coupling between ligand density and substrate stiffness. Most surprising, however, spreading on soft gels is found to be almost independent of adhesive ligand density: even with high collagen densities, the minimal spreading of cells cannot be over-ridden. On soft gels, however, a fraction of GFP-actin (over)expressing cells do spread. Such cells invariably show an organized cytoskeleton of stress fibers, which suggests that the cytoskeleton is at least one structural node in a signaling network that can override spreading limits typically dictated by soft gels.

**METHODS:** Gel substrates consisted of polyacrylamide (PA) supported on glass by a method modified from Wang and Pelham [1]. Briefly, coverslips were treated for attaching a 70-100  $\mu\text{m}$  thick gel of PA polymerized from 3-10% acrylamide monomer plus bis-acrylamide (0.03-0.3%). The %ages dictate the final elastic modulus as measured by AFM. Collagen (plus fluorescent collagen) at a desired concentration was chemically crosslinked to the gel surface with a heterobifunctional crosslinker, Sulfo-SANPAH, at 37 °C overnight.

The A7r5 SMC line is generally known to maintain differentiation markers for  $\alpha$ -actinin and myosins. SMC were cultured in polystyrene flasks with DME Medium supplemented by 10% fetal bovine serum. Some cells were pre-transfected in a hypo-serum medium with GFP-paxillin (for focal adhesions) or GFP-actin plasmids using standard Lipofectamine transfection protocol (Invitrogen). Cells were plated on the PA substrates of controlled stiffness and collagen density and observed up to 24 hours.

**RESULTS:** Varying the collagen density on PA gels and glass was widely found to modulate cell spreading to different degrees (Fig. 1). On soft substrates, however, collagen density could not override the minimal cell spreading; instead it seemed to pin cells down and give a bell-shaped

response. The biphasic behavior here is reminiscent of that seen in cell motility versus ligand density [2], which had also been explained by over-attachment at high ligand.

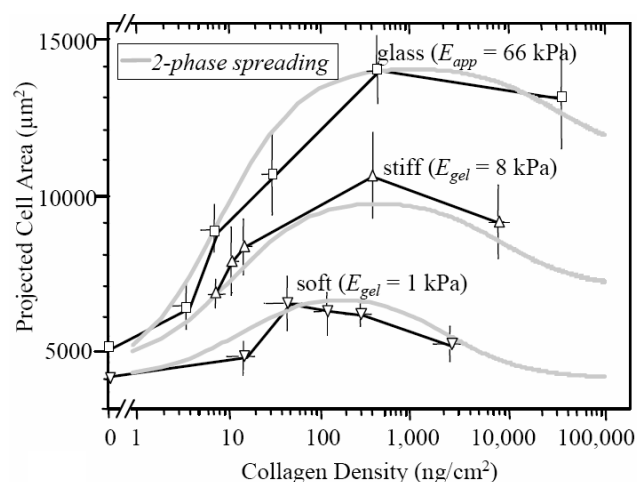


Fig. 1: Cell spreading on substrates of various stiffness and various ligand density.

To make the coupling above explicit, the two phases of cell spreading are modeled as a single function for *Area* dependent on  $E$  or  $E_{\text{eff}}$  (units of kPa) and  $\text{coll} = [\text{collagen}]$ . The collagen dependence is expressed in the sum of two hyperbolic terms typical of saturable equilibrium associations. The area-promoting association of cell-substrate binding is modeled by the first term of Eq. 1 below (with constant  $K_1$ ) while the second term models the fractional dissociation in a separate, area-inhibiting reaction (with constant  $K_2$ ). Importantly, a power law fit of *Area* ( $\mu\text{m}^2$ ) versus  $E$  (kPa) has been used to scale the area-promoting reaction obtained from cell spreading over a 24-hour time course. Cells cultured on glass see  $E = E_{\text{eff}}$  based on the power law fit. The baseline area response of a cell in solution appears as a constant,  $C$ , and the association constants  $K_1$  and  $K_2$  are taken to be power laws in  $E$ .

$$\text{Area} = C + 3000E_{\text{eff}}^{0.29} \left[ \frac{K_1 * \text{coll}}{1 + K_1 * \text{coll}} \right] + 3000 \left[ \frac{1}{1 + K_2 * \text{coll}} \right] \quad (\text{Eq. 1})$$

with  $C = 1000$ ,  $K_1 = 0.07 * E_{\text{eff}}^{0.13}$ , and  $K_2 = 0.0005 / E_{\text{eff}}^{0.66}$ . A kinetic approach to biphasic cell motility has been described by a ratio of dissociation rate constants, which correspond here to a ratio of  $K_1$  and  $K_2$ . The correspondence supports the notion that similar phenomena may underlie the behavior in cell spreading.

Labeling of the actin cytoskeleton with rhodamine phalloidin invariably showed that the most spread cells had a well-organized cytoskeleton of stress

fibers that appeared lacking in the unspread cells (Fig. 2).

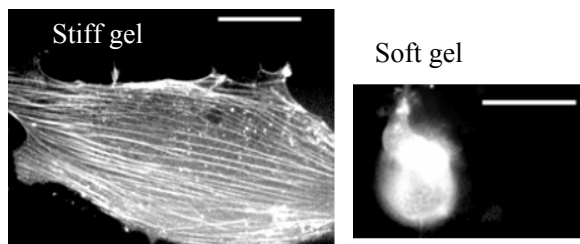


Fig. 2: Range of cytoskeletal organization typically seen on different substrates.

Transfected GFP-actin and GFP-paxillin cells helped to elucidate the coupling of substrate elasticity to cytoskeletal and adhesion expression and structure. On both stiff PA gels and rigid substrates pretreated with collagen, structured actin stress fibers and paxillin focal adhesions were again typically observed as well-spread cells predominate. On the softest PA gel with optimal collagen, the GFP-actin cells were remarkably well-spread as opposed to rounded. Conversely, without collagen, all substrates showed just a small sub-population of highly spread cells with organized actin stress fibers. The results collectively illustrate an emergent rule: spreading has a basis in or at least correlates well with cytoskeletal assembly. GFP-paxillin expression, in contrast, had little ability to over-ride the inadequate signal from a given substrate.

**CONCLUSIONS:** Overall, cell spreading and cyto-organization are suggested to be a function of a

substrate's mechanical as well as chemical properties. Of course, additional physical variables such as substrate topography can be equally important. However, one must remember that cells are highly active, their filaments are incessantly turning over, their stress fibers are constantly pulling on the matrix. Hence, the idea that these stresses on the underlying substrate will strain it and feedback on cell activity is as intuitive as grabbing a small rubber ball in one's hand and successfully squeezing it in approximate relation to its compliance. In a simplistic way, an active cell is as much like your hand - highly structured and responsive to feedback - as a liquid drop that passively wets a surface. It is thus understandable that cells display "durotaxis" [3] as well as haptotaxis and chemotaxis.

**REFERENCES:** <sup>1</sup>Wang, Y-L. and Pelham, R., 1998, "Preparation of a Flexible, Porous Polyacrylamide Substrate for Mechanical Studies of Cultured Cells." *Meth Enzym*, 298, 489-496. <sup>2</sup>Lauffenburger, D. and Lindermann, J., 1996, *Receptors: Models for Binding, Trafficking, and Signaling*. Oxford University Press, London. <sup>3</sup>Lo, C. M., Wang, H. B., Dembo, M., and Wang, Y. L., 2000, "Cell movement is guided by the rigidity of the substrate." *Biophys J*, Vol 79, pp. 144-52.

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