
This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of February 4, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/339/6118/402.full.html>

This article **cites 20 articles**, 7 of which can be accessed free:

<http://www.sciencemag.org/content/339/6118/402.full.html#ref-list-1>

This article appears in the following **subject collections**:

Cell Biology

http://www.sciencemag.org/cgi/collection/cell_biol

its high spontaneous polarization (the polarization present with no electric field applied), high dielectric constant, and high polarization saturation (the maximum that can be achieved with an applied field), as well as stability at room temperature. The limitation of these materials is that they are expensive to produce in the forms necessary for current and next-generation devices, and they often contain environmentally unfriendly elements such as lead.

It has been nearly a century since ferroelectricity was discovered in organic compounds, but during the intervening years, none of these materials exhibited the properties or the stability of the order of those of oxides. Being guided by the principles of atomic structure and symmetry, Fu *et al.* considered molecular structures that met the structural requirements for the presence of ferroelectricity and imposed a further constraint of a high melting point. They came upon diisopropylammonium bromide (DIPAB), which has the additional advantage that it can be processed from aqueous solutions. Using comprehensive crystallographic analysis and theory, they showed that the compound occurs in two polymorphs, and that one of these, the α phase, is ferroelectric and stable over a wide temperature range (77 to 416 K) of interest for many applications (see the figure).

The authors used several approaches to characterize the ferroelectric properties of DIPAB. The spontaneous polarization measured directly by electric field cycling

was higher than that of other molecular compounds by a factor of 4 and, remarkably, matched that of BaTiO₃. The dielectric constant for DIPAB (measured at 400 Hz) was higher than those of polymer ferroelectric compounds by a factor of 10 and remained relatively high even at 1 MHz. Finally, the coercive field for DIPAB, which dictates the amount of voltage required to reorient the electric dipoles (and which should be low for energy-efficient applications), was one-hundredth that for polymers and half that for BaTiO₃. The properties of DIPAB considerably surpass those observed for other organic materials and approach or match those of the ferroelectric oxides.

The dipole coupling that is the basis of ferroelectricity is an intrinsically local phenomenon. The dipoles are separated by and interact across distances of 0.4 to 1 nm, depending on the compound; the domains of aligned dipoles have sizes ranging from nanometers to micrometers. Thus, spatially localized measurements of ferroelectric behavior are essential. Fortunately, a toolbox of local-property probes is now available to characterize properties at these length scales (9). Fu *et al.* used a variant, piezoresponse force microscopy, to map the domain orientations and determine the ferroelectric properties of individual domains, illustrating the insight gained from local analysis.

This combination of ferroelectric phase stability and outstanding properties sug-

gests that DIPAB may replace oxides in some applications, with benefits in terms of ease of processing and sustainability. Further, the surprisingly strong ferroelectric behavior in this organic compound suggests a reconsideration of the generality of charge interactions that underlie related properties. Other properties, notably piezoelectricity and electrostriction, are manifestations of charge interactions in structures and are well known in solids. Recently, evidence of all three of these properties has been observed on local scales in components of biological or physiological materials (10, 11), so perhaps ferroelectric coupling is an important part of some biological processes. In that case, the highly functional DIPAB may be the bridge in understanding between coupling in oxides and that in complex soft materials.

References

1. H. D. Megaw, *Nature* **155**, 484 (1945).
2. J. F. Scott, *Science* **315**, 954 (2007).
3. D.-W. Fu *et al.*, *Science* **339**, 425 (2013).
4. J. Wang *et al.*, *Science* **299**, 1719 (2003).
5. D. Schlom *et al.*, *Annu. Rev. Mater. Res.* **37**, 589 (2007).
6. D. Li, D. A. Bonnell, *Annu. Rev. Mater. Res.* **38**, 351 (2008).
7. S. Kalinin *et al.*, *Adv. Mater.* **16**, 795 (2004).
8. J. F. Scott, C. A. Paz de Araujo, *Science* **246**, 1400 (1989).
9. D. A. Bonnell *et al.*, *Rev. Mod. Phys.* **84**, 1343 (2012).
10. S. V. Kalinin *et al.*, *Annu. Rev. Mater. Res.* **37**, 189 (2007).
11. Y. Liu, Y. Zhang, M. J. Chow, Q. N. Chen, J. Li, *Phys. Rev. Lett.* **108**, 078103 (2012).

10.1126/science.1232939

CELL BIOLOGY

Deconstructing Dimensionality

Martin A. Schwartz¹ and Christopher S. Chen²

Cells were first cultured in glass dishes over a century ago (1, 2), a technology still used with only minimal changes. Yet, cells on glass or tissue culture plastic, so-called two-dimensional (2D) culture, often fail to reflect *in vivo* function. The ability to grow cells within extracellular matrix (ECM) gels (3D culture) was a major advance that recapitulated *in vivo* cellular behaviors, ranging from differen-

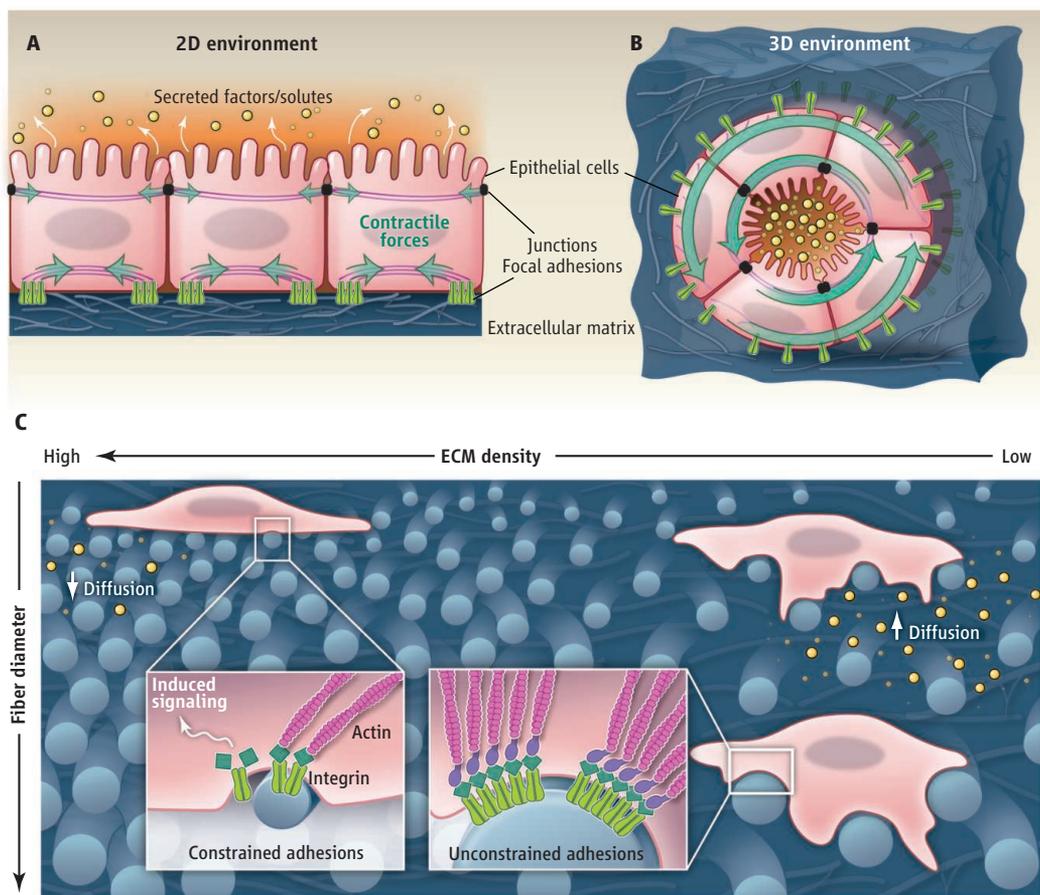
tiated function to maintenance of stem cell niches (3–5). The question of how cells distinguish between 2D and 3D environments to determine gene expression, cell behavior, and morphogenesis is of much interest to cell biologists and tissue engineers. However, cells do not sense “dimensionality” directly as an independent variable; rather, it is ascertained through its effects on various cell processes.

Epithelial cells inside ECM gels typically form tubes or spheres with hollow cores (5, 6), and thus differ topologically from monolayer cultures. One key consequence of this reorganization is the size of the compart-

How do cells sense a three-dimensional environment?

ments (see the figure). Cells in a 3D environment usually polarize with a basal compartment facing the gel and an enclosed apical compartment that is quite small, often comparable to the combined volume of the cells themselves (7). Apically secreted factors are therefore highly concentrated compared to those secreted in 2D cultures, and paracrine effects are magnified. Ions can also be secreted in a polarized manner—in kidney tubule cells, for example, this leads to differences in electrolyte composition. As membrane receptor localization is often polarized, accessibility of autocrine factors to receptors can modulate cell responses as well.

¹Cardiovascular Research Center, Yale School of Medicine, 300 George Street, New Haven, CT 06511, USA. ²Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, USA. E-mail: martin.schwartz@yale.edu; chrischen@seas.upenn.edu



Independent variables. (A) Cells on a rigid planar surface organize focal adhesions and actin stress fibers at the basal surface of the cell and transfer contractile forces to the surface and to other cells. Apical domains face the medium where secreted factors are diluted; basal domain faces the ECM-coated surface. (B) Cells in 3D culture form a spherical acinus or tube with a curved surface. Curvature and soft matrix materials limit formation of actin stress fibers. The apical domain faces a small interior compartment where secreted factors can be highly concentrated. Mechanical tension can be transmitted through cell-cell junctions to generate hoop stresses around the whole structure. (C) ECM fibers control cell behavior through differences in density and fiber diameter. These variables influence cell protrusions, diffusion of solutes, and the local clustering and force transmission by integrins that bind to the ECM.

Topographical differences in ECM proteins also can modulate cell responses. In either 2D or 3D environments, ECM constituents can be arranged into fibrils that are thick, thin, long, or short (or uniformly coated on a solid support) (8, 9). This organization can affect curvature of the plasma membrane and the clustering and organization of membrane receptors, as well as alter mechanical forces at the cell-ECM interface. Additionally, the spacing between fibers, determined by ECM density, can influence whether a cell forms a relatively planar interface with a fiber mat, crawls inside the ECM and is surrounded with fibers, or adopts some intermediate state in which it extends processes into the pores of the matrix (8).

The difference in curvature between cells in flat monolayers versus spheroids or tubules also affects cell functions. Although

the radius of curvature is too large to affect cell structures at the nanoscale [for example, the binding of Bin/amphiphysin/Rvs (BAR) domain proteins to membranes (10)], changes in curvature at the micrometer scale can control the assembly of large cytoskeletal structures such as focal adhesions (which link actin filaments to the adhesions) or microtubules. Curvature may also influence cell-cell and cell-matrix interactions through changes in the size of the domains and the distribution of mechanical forces. For example, cells in a tube or acinus can undergo apical contraction to decrease the structure's radius. They can also experience "hoop" stresses around the entire structure. This distribution of forces throughout the structure creates opportunities for multicellular morphogenetic movements such as sprouting or invagination (11). Moreover, the size and curvature of the apical and basal

domains are also variable in a 3D ECM, which influence contractility and cytoskeletal organization within these regions. By contrast, forces experienced by cells in 2D are ultimately resisted in only one domain (basal) by the stiffness of the substratum, thereby limiting such effects.

The degree to which the ECM resists deformation from cell-generated tractions (ECM rigidity or stiffness) modifies the structure and dynamics of the cell-ECM adhesions, which subsequently influences cell signaling and behavior (12). Although this is true for both 2D and 3D cultures, sensing ECM rigidity differs depending on how the ECM is presented. In 2D, cells experience tractions primarily as shear stresses on their basal surface, whereas cells in a 3D environment experience both planar stresses and stresses that are perpendicular to the cells' basal surface. There are also substantial differences in the mechanics of synthetic gels used to model stiffness effects in 2D settings as compared to natural ECMs (13). Such gels exhibit linear elastic behavior, whereas the fibrillar nature of natural ECMs give rise to complex nonlinear elastic behavior with appreciable viscous components. These physical differences could influence how cells respond to their environment.

For cells in 2D culture, apical-basal polarity is essentially fixed by having the solid support below and the medium above. Cells in 3D gels initially have ECM on all sides. Epithelial and endothelial cells create their polarity, reorganizing into structures that generally have the basal domain facing the ECM and a fluid-filled apical compartment inside (6, 7). This arrangement can be reversed by suitable manipulations (14), indicating that polarity is a regulated variable. Mesenchymal cells also organize differently. In 2D they are forced into an epithelial state where apical and basal domains differ. However, in 3D culture, they contact ECM on all sides without evident apical-basal polarity (15). Binding ECM on the apical domain triggers different responses than at the basal domain—for example, there are distinct effects on gene expression, cytoskeletal organization, and even cell survival (16–18).

Transport of soluble components differs between 2D and 3D environments. In 2D, solutes undergo free diffusion through the medium and convective mixing, leading to their rapid distribution. Three-dimensional matrices eliminate convection and restrict the diffusion of large molecules (19). ECM components also bind many soluble molecules, including most growth factors, which further slows their transport but can also concentrate them within the microenvironment. All of these factors reduce transport rates such that some solutes could take days to reach embedded cells.

Cells can freely spread, migrate, and rearrange on a 2D surface because there is no constraint in the plane of the substrate, whereas in a 3D matrix, cells have to either squeeze through pores or degrade the matrix to spread or migrate (8). Such constraints affect the speed of migration in 3D, which could alter cell signaling triggered by the assembly of new cell-matrix adhesions. Furthermore,

such constraints can lead to an apparent paradox in which increasing cross-linking of a matrix enhances cell spreading and motility by increasing substrate stiffness; cross-linking cells into a 3D matrix is likely to retard spreading and motility by making it harder for cells to degrade ECM components and move through the environment.

Cells can only sense their surroundings over a limited distance, essentially a single cell length. Thus, “dimensionality” per se is not a single variable that can be sensed on a cellular scale. Identifying the mechanisms by which cells assess the nature of their environment will advance basic cell biology and facilitate the development of synthetic matrices (20) for specific tissue engineering applications.

References

1. A. Carrel, *J. Exp. Med.* **15**, 516 (1912).
2. R. G. Harrison, *J. Exp. Zool.* **17**, 521 (1914).
3. E. Santos, R. M. Hernández, J. L. Pedraz, G. Orive, *Trends Biotechnol.* **30**, 331 (2012).
4. G. Benton, J. George, H. K. Kleinman, I. P. Arnaoutova,

J. Cell. Physiol. **221**, 18 (2009).

5. B. Weigelt, M. J. Bissell, *Semin. Cancer Biol.* **18**, 311 (2008).
6. G. E. Davis *et al.*, *Cell. Mol. Biol.* **288**, 101 (2011).
7. L. M. McCaffrey, I. G. Macara, *Trends Cell Biol.* **21**, 727 (2011).
8. K. Wolf, P. Friedl, *Trends Cell Biol.* **21**, 736 (2011).
9. J. S. Harunaga, K. M. Yamada, *Matrix Biol.* **30**, 363 (2011).
10. B. J. Peter *et al.*, *Science* **303**, 495 (2004).
11. G. M. Odell, G. Oster, P. Alberch, B. Burnside, *Dev. Biol.* **85**, 446 (1981).
12. D. E. Discher, P. Janmey, Y. L. Wang, *Science* **310**, 1139 (2005).
13. C. Storm, J. J. Pastore, F. C. MacKintosh, T. C. Lubensky, P. A. Janmey, *Nature* **435**, 191 (2005).
14. K. D. Liu *et al.*, *Am. J. Physiol. Renal Physiol.* **293**, F1633 (2007).
15. E. Cukierman, R. Pankov, D. R. Stevens, K. M. Yamada, *Science* **294**, 1708 (2001).
16. X. Chen, S. L. Thibault, *Tissue Eng. Part A* **18**, 2528 (2012).
17. Y. K. Wang, H. H. Lin, M. J. Tang, *Am. J. Physiol. Cell Physiol.* **280**, C1440 (2001).
18. R. Eisen, S. Walid, D. R. Ratcliffe, G. K. Ojakian, *Am. J. Physiol. Cell Physiol.* **290**, C1297 (2006).
19. A. S. Hoffman, *Adv. Drug Deliv. Rev.* **54**, 3 (2002).
20. M. P. Lutolf, J. A. Hubbell, *Nat. Biotechnol.* **23**, 47 (2005).

10.1126/science.1233814

CHEMISTRY

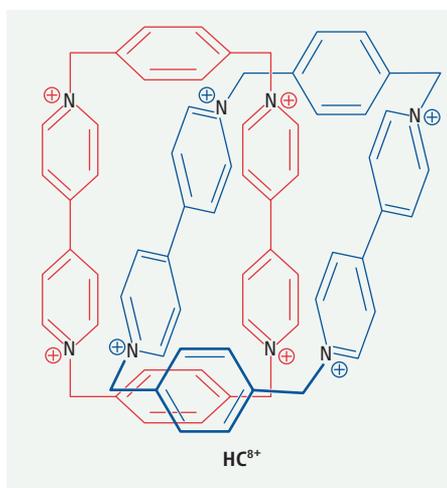
Corralling Positively Charged Molecular Radicals

Andrew Benniston

At the outset, the idea of forcing together multiple positive charges onto an organic molecule and ending up with a stable interlocked supramolecular structure seems destined for failure. Columbic repulsion forces usually extract an energetic penalty on adding an additional charge onto a highly charged molecule, and the organic radicals that would form with stepwise charge addition are likely to be unstable. On page 429 of this issue, Barnes *et al.* (1) show that counterintuitive thinking about this problem can reap rewards. They show that a [2]catenane molecule, in which two identical rings interlock noncovalently like links of a chain (2), displays a rich vein of electrochemical responses. This homocatenane (HC) can stabilize organic radicals and bear up to eight positive charges.

[2]Catenanes have been viewed as curiosity structures of interest mainly for their topology; like the links of a chain, the components can only be separated by breaking

one of the rings. Over many years, examples of molecular-based [2]catenane structures have emerged based on neutral components, using either hydrogen bonding (3), anion recognition (4), or cation binding (5) to template their formation. Previous work by Stoddart's



Charging ahead. The molecular structure is shown of the octacationic [2]catenane produced by Barnes *et al.*, which was characterized by proton nuclear magnetic resonance spectroscopy.

Linear organic rings can form highly charged molecular and radical ions with unusual stability.

group focused on the tetracationic bipyridinium (blue box), as shown in the figure, to form interlocked structures with highly electron-donating crown ethers (6).

In essence, the approach of Barnes *et al.* forces together the two “blue box” structures depicted in the figure, and a total of eight positive charges can be localized on the structure when it is oxidized electrochemically in solution. Because HC⁸⁺ contains such a high charge and suffers high Coulombic repulsion, it is not surprising that it was not isolated as a solid. However, it was fully characterized in solution by proton nuclear magnetic resonance spectroscopy. The as-prepared state of the [2]catenane is actually an equilibrium mixture containing HC⁶⁺ (diradical) and HC⁷⁺ (monoradical).

Two points are noteworthy. One is that the electrons are spin-paired in the diradical and form a singlet state. The other is that the radical HC⁷⁺ is stable and was isolated as a single crystal for x-ray structural analysis. Organic radicals are usually highly energetic and short-lived because they can readily react with one another to form dimers. Thus, the capture of HC⁷⁺ in the solid state is very

School of Chemistry, Newcastle University, Newcastle upon Tyne, NE1 7RU UK. E-mail: andrew.benniston@newcastle.ac.uk