responsive with a single copy of the antibiotic resistance gene and work with the transposon excision-and-repair method. This results in a streamlined single-copy, integrated transgene system that benefits from the strict conditionality and flexibility of antibiotic selection.

The benefits of the described antibiotic selection systems extend beyond the single-copy transgene application. Indeed, when the systems are used as the markers to maintain traditional extrachromosomal transgenes, they result in populations of worms that retain the transgene at essentially 100% frequency, improving the reproducibility and consistency of experiments carried out with these traditional transgenes. They can also be used with other nematode species that lack the full genetic toolbox of *C. elegans*. Both groups show the selection system works in the related *C. briggsae*, and Giordano-Santini *et al.*[2] demonstrate G-418 sensitivity in various nematode species.

An exciting prospect for the work is its definition of a methodological approach that might be applied to many invertebrate experimental animals (**Fig. 1**). The use of an antibiotic resistance system means that a detailed genetic analysis is not required before developing transgenesis methods for an experimental species. Consequently, the work uncovers a long-term opportunity for pan-species transgenesis in comparative studies of gene function and gene evolution.

COMPETING FINANCIAL INTERESTS
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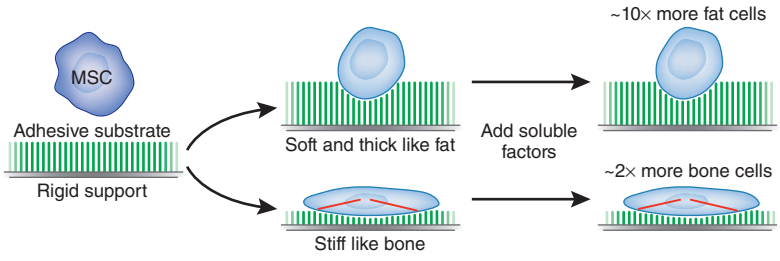
Stem cells feel the difference

Amnon Buxboim & Dennis E Discher

Arrays of microposts of different heights generate substrates with different flexibility, on which cells can be grown.

Fat is softer than bone, and mimicking this difference with flexible substrates has been observed to influence the differentiation of adult stem cells. In this issue of *Nature Methods*, Chen and co-workers have made dense arrays of various-flexibility pillars for cells to adhere on top of and show that the potency of soluble factors that induce either fat or bone depends on pillar flexibility[1].

Cells lack eyes to see differences in substrates, but they do possess tactile mechanisms that allow them to feel differences. Using a silicone rubber material commonly used to caulk the gaps between tile and tub and using methods for micromolding widely adapted from the computer industry for lab-on-a-chip microfluidics, Chen and co-workers made arrays of short or long, micrometer-diameter pillars spaced by a few micrometers so that mesenchymal stem cells (MSCs)—which are about 10 micrometers in diameter in suspension—could adhere to the tops of many pillars all at once.



Katie Vicari

Figure 1 | Adherent stem cells need to attach to a solid substrate to survive, but whether the cells attach to a substrate that is soft or one that is stiff can influence differentiation.

MSCs are adult stem cells that can be obtained from many human tissues and can be induced to differentiate into cells that express markers for various solid mesenchymal tissues such as fat, muscle, cartilage and bone. Empirically established cocktails of soluble factors, when added to cultured MSCs, will stimulate them to differentiate over days to weeks into select lineages. Adhesion to plastic culture dishes is the usual means by which MSCs are separated from tissues, and these cells must adhere in order to survive and differentiate in culture. However, MSCs will certainly attach to other substrates, and some physical properties of the substrate seem to affect how the cells respond, with wider implications for how cells in general respond to soluble factors, including drugs.

In their work, Chen and colleagues adsorbed the extracellular matrix molecule fibronectin to the flat tops of the pillars so that cell membrane adhesion receptors could engage the pillars, but such cell attachment does not stop at the cell membrane. Receptors that mediate adhesion are quickly linked to the actin cytoskeleton, which also contains highly active myosin similar to that found in muscle. What this means is that cells adhere and pull in a manner similar to someone grabbing and lifting a barbell. If the pillars are long, a cell pulls with relative ease and deflects the tops of posts by many micrometers, whereas if the pillars are short, then the cell cannot deflect the tops of posts very much at all. For insight, grab a plastic ruler with a hand at either end; you will find it rather easy to bend, but if you bring your hands closer together (say by a factor of ten) then the ruler will be harder to bend to the same extent (in fact, 1,000-fold more force is needed). Arrays of long pillars are therefore effectively equivalent to a soft substrate, perhaps as soft as fat, whereas arrays of short pillars might be perceived by cells as effectively stiff or rigid like bone (**Fig. 1**).

Past work with various gels of different elasticity had indeed shown that matrix elasticity can direct lineage specification of stem cells. Very soft gels that mimic brain tissue tend to be inductive for neuronal genes[2,3], whereas an intermediate elasticity

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typical of muscle tends to turn on a muscle program[2,4], and very stiff gels are inductive for bone[2,5]. The present work with pillars of a single material but different lengths[1] points in the same direction and provides many additional opportunities for insight into mechanisms and applications. For example, pillar arrays permit relatively rapid and frequent determinations of traction forces that cells exert as they bend the pillars. High-throughput testing of drugs that affect both differentiation and cell contractility therefore seems more feasible with such arrays. Furthermore, the molecular-scale porosity of hydrogels can limit access to the basal surface of a cell, so that delivery of soluble factors, particularly protein growth factors, to any basal receptors should be optimal on the pillar arrays.

It has been clear for about a dozen years[6] that the adhesion and pulling of a cell on a stiff or rigid substrate promotes more pulling, more spreading and more actin-myosin 'stress fibers' in comparison to a cell on a soft or flexible substrate. In other words, cells simply do not need to pull very hard to deflect flexible posts, so they remain relatively relaxed and rounded on arrays of long, bent pillars. Within about a day, very different cell forces and cell structures thus emerge on stiff versus soft pillar substrates and likewise on gels[7], and these somehow set up very different signaling pathways that strongly influence differentiation processes over subsequent days to weeks. Chen and colleagues report that the density of integrin-rich focal adhesion structures is nearly the same on all substrates[1], so it seems that the number of such complexes is key. With the pillar substrates, the most dramatic impact is on fat development, which occurs in almost 20% of cells on the long soft pillars but only in 1–2% of cells grown on the short stiff pillars or on glass[1]. Whether even softer substrates magnify the difference is a matter for further study.

Past work with micropillar arrays of varying geometry has suggested that for any such array there is an equivalent gel substrate with an elasticity that is inversely proportional to the flexibility of the pillars[8]. Past estimates of the effective pillar elasticity[8] encompass the range that has been noted previously[9] for normal fat tissue (~3 kilopascals) and for premineralized bone matrix (~30 kilopascals). In comparing to tissues, it should be noted that tissues seem mechanically more homogeneous[9] than micrometer-diameter pillars, and as long as sufficient extracellular matrix is attached to homogeneous gels and therefore not limiting, cells exhibit distinct responses to substrate elasticity over the relevant tissue elasticity range of ~0.1 to 100 kilopascals[10]. In other words, synthetic substrates might thereby be quantitatively related in their elasticities to tissues. Deflections of the pillars can be visualized, and the forces that cells generate can then be calculated and divided by cell area to estimate an average traction stress under the cell.

Chen and colleagues observed that average traction stress is sustained at nearly 1 kilopascal for the osteogenically directed MSCs on stiff pillars, whereas the adipogenic cells appear to be only about half as strong[1]. What is more, the stress of adipogenically directed MSCs increases for only a day after the initiation of differentiation and then decays to a low, basal stress typical of undifferentiated MSCs. The transient nature of this cell-generated stress in an adipogenic medium is surprising in being sufficient to turn on fat genes and emphasizes the need to make frequent observations of cell forces. With gel substrates rather than pillars, a similar tenfold increase in elasticity has been found previously[2] to increase the cell stress by about threefold in the low-kilopascal range, and so the observations with MSCs seem reproducible with pillars even if the triggering of expression owing to transient mechanical stress remains a mystery.

What is increasingly clear is that stem cells feel the difference between soft and hard, integrating this cue with other signals that can direct differentiation.

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Oxygen maps in the brain

Ulrich Dirnagl

Oxygen concentrations in the rodent brain are revealed at a microscopic scale by measuring the lifetime of two photon–excited phosphorescence.

The human brain consumes more than 70 liters of oxygen every day, which it uses to maintain its high basic cellular metabolism, as well as to support the energetic demands of neuronal signaling. In this issue, Sakadžić *et al.*[1] take on the exact mechanisms by which blood flow in the brain is regulated to provide oxygen (and glucose) to different brain areas with a spatial resolution of several hundred micrometers, a phenomenon that has puzzled scientists for more than a hundred years[2].

Most modern neuroimaging methods, notably functional magnetic resonance imaging, rely on combinations of vascular and metabolic signals to localize areas of change in neuronal activity. In synchrony with the enormous popularity of these neuroimaging techniques, interest in their physiological underpinnings has greatly increased over the last decade.

The use of confocal microscopy[3] and more recently its variant, two-photon microscopy[4], have made it possible to study the brain vasculature through cranial windows of experimental animals with high temporal and spatial resolution. Neuronal activity can be optically monitored with millisecond resolution with the use of voltage-sensitive dyes. However,

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