STEM CELL DIFFERENTIATION

Multipotency retained

Stem cells that are cultured in the laboratory differentiate in response to the mechanical properties of the substrate and its topography. It is now shown that mesenchymal stem cell multipotency is prolonged when the cells are cultured on a surface patterned with an ordered arrangement of nanoscale pits.

Milan Mrksich

Stem cells that are multipotent — that is, they can give rise to diverse cell types — offer the possibility of restoring damaged or diseased tissues. For instance, mesenchymal stem cells (MSCs) — a type of stem cell that can be isolated from adults — can be cultured in the laboratory to expand their numbers and transplanted to restore tissues. However, MSCs grown in the laboratory have the propensity to spontaneously differentiate into other cell types and to lose their multipotency. Significant effort has led to the identification of biological strategies to prevent or direct the differentiation of stem cells, including the addition of growth factors and drug-like molecules to the cultures. However, these approaches are often empirical in nature, and are far from satisfying clinical needs. Writing in *Nature Materials*, Matthew Dalby and colleagues describe a materials solution to this biological problem. They show that MSCs cultured on a nanostructured substrate maintain their multipotency for up to eight weeks[1].

A series of recent publications have shown how material-based approaches can be used to control the fates of MSCs. One study demonstrated that cells sense and respond to the stiffness of the substrate they are cultured on[2]: MSCs differentiated into bone cells when cultured on stiff substrates, but gave rise to neuronal cells under identical conditions when soft substrates were used. Another study used patterned substrates to show that cells that were allowed to spread differentiated into bone cells, whereas those maintained in a rounded shape became fat cells[3]. Further work with patterned substrates determined that MSCs responded to geometric aspects of their shape: on a star-shaped island the cells preferred a bone-cell fate, whereas those on flower-shaped islands of the same size differentiated into fat cells[4]. These earlier examples suggested that the physical properties of the substrate represented a promising strategy to engineer the fate of stem cells.

Building on that strategy, Dalby and collaborators hot-embossed polycaprolactone substrates to pattern square-shaped pits

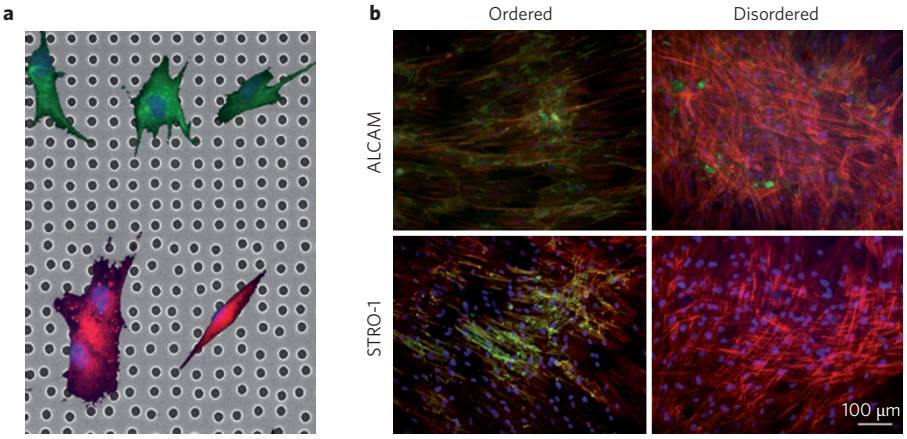

Figure 1 | Substrate topographies used by Dalby and colleagues, and expression of protein markers in mesenchymal stem cells cultured on the substrates. **a**, Polycaprolactone surfaces with an array of nanoscale pits organized in either an ordered square lattice with lattice spacing of 300 nm (upper half) or a disordered arrangement in which the pits are offset with respect to the square-lattice positions by 50 nm in both axes of the surface plane (lower half). The adhered MSCs, which are of the order of tens of micrometres in size, are not shown to scale. Image courtesy of Matthew Dalby and co-authors. **b**, Expression of ALCAM and STRO-1 — proteins that are expressed in multipotent cells but are lost on differentiation — after culture of MSCs on the ordered and disordered lattices for eight weeks. ALCAM and STRO-1 proteins are shown in green, cell nuclei in blue and cell cytoskeletons in red. The cells cultured on the ordered array of pits (left) retained expression of the multipotent cells, whereas MSCs cultured on the disordered arrangement (right) did not express these proteins as a result of differentiation. Images reproduced from ref. 1.

120 nm in size, arranged in a square lattice with a separation of 180 nm between the pits. The authors then cultured MSCs on the nanopatterned substrates as well as on planar ones. They observed that MSCs cultured on the pitted substrate maintained an undifferentiated state for up to eight weeks, whereas cells on the control (planar) substrates rapidly differentiated into various cell types (mostly bone cells), thus demonstrating that the topography of the substrate has a profound effect on the preservation of multipotency. Interestingly, because the authors had found that a substrate embossed with pits having the same size and spacing, but in a staggered arrangement, failed to maintain the cells in an undifferentiated state and directed them towards a bone-cell fate[5], they concluded that the ability of the substrate's nanoscale topographical features to maintain stem-cell multipotency does not depend only on the structure and density of the features, but on their arrangement on the substrate (Fig. 1).

How do MSCs sense these subtle differences in the arrangement of features on the substrates? The cell's cytoskeleton, which influences a broad range of cellular activities in a tension-dependent manner, interacts with the substrate through focal adhesions — multiprotein structures through which regulatory signals (among them, mechanical signals) are transmitted. For example, a tense cytoskeleton favours differentiation of MSCs into bone cells. The organization and signalling properties of the cytoskeleton can therefore be engineered with nanopatterned substrates — which define the positions, shapes and sizes of the focal adhesions — and with three-dimensional culture matrices having nanoscale structure[6,7]. What is unexpected, and exciting, is that cells can respond to the spatial arrangement of nanoscale features in their environments.

The authors also studied the mechanisms allowing MSCs to respond to the pitted substrates. They used DNA arrays to profile gene expression in cells, and found that several transcription factors involved in maintaining multipotency were not significantly affected by the nanoscale topography. Instead, they identified a group of small, untranslated RNAs that are up-regulated in response to the substrate. These findings are consistent with the idea that the small RNAs override the normal cues that direct differentiation by maintaining cells in a low metabolic state and by suppressing signalling pathways.

The work of Dalby and colleagues has two noteworthy implications. First, it identifies a cellular activity that is strongly influenced by the nanoscale structure of the substrate and characterizes the cellular response. This early example should serve as a model system for revealing mechanisms by which the cytoskeleton responds to the substrate's physical features. Second, the work shows that to control the fate of stem cell cultures in the laboratory, material-based strategies can be complementary to biological and chemical approaches. In view of the availability of inexpensive and scalable nanofabrication techniques, nanostructured surfaces may actually find practical use in stem cell therapies and regenerative medicine. ❐

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PLASMONICS

Hydrogen caught red-faced

A single nanodevice that detects the presence of a single molecule would perhaps be the ultimate sensor. The demonstration of hydrogen sensing based on a single gold nanoaerial brings that possibility nearer.

Roy Sambles

How does a dog do it? Detect scent I mean; specifically in a complex environment with a multitude of volatile components. Indeed a variety of animals are extraordinarily sensitive to low levels of some specific molecular species. One of the dreams of those working in technologies seeking to develop the equivalent chemical-sensing capability is the ultimate single-atom detector. Writing in *Nature Materials*, Na Liu and colleagues present results on the use of a single gold nanoaerial to sense hydrogen when it binds to a nearby palladium island[1]. Although this does not provide the dreamt for single-atom sensor, it does go some way towards that possibility using relatively standard optical technology.

The core of the idea presented goes all the way back to James Clerk Maxwell's equations of electromagnetism, which dictate that near the pointed end of a good conductor there may be highly localized and substantially enhanced electromagnetic fields. Even at optical frequencies this is true, particularly when the metal object is electromagnetically resonant, that is, an aerial. The smaller the aerial and, in general, the higher the excitation frequency (provided it is below the plasma frequency of the metal), the more tightly confined the electromagnetic field will be. For a metallic nanoparticle the electromagnetic field can be strongly concentrated in a volume of only a few hundred nm^3 or less. This optical nanofocus is ideal for plasmonic sensing[2,3]. In Liu and colleagues' work the aerial is a 70-nm-sided equilateral triangle of gold some 40 nm thick. This has a localized surface plasmon resonance in the visible region of the spectrum that may be readily visualized using dark-field microscopy — the scattering from the gold particle giving a strong peak in the red region of the spectrum. The properties of such a scattering antenna are sensitive to nearby objects within the near-field of the light, and as such may be used to detect small changes in the adjacent electromagnetic environment. Therefore, by precisely placing a small palladium particle of 60 nm radius only 10 nm away from one of the tips of the gold triangle, as illustrated in Fig. 1, the authors have been able to readily detect the absorption of hydrogen by the palladium particle. On introduction of hydrogen, the dielectric properties of the palladium island change, as the authors suggest, owing to the incorporation of free electrons from the adsorbed hydrogen. This then results in a change in the resonant frequency of the gold island through the interaction of its fields with the palladium particle, which shows itself as a redshift of some 8 nm in the scattered peak wavelength.

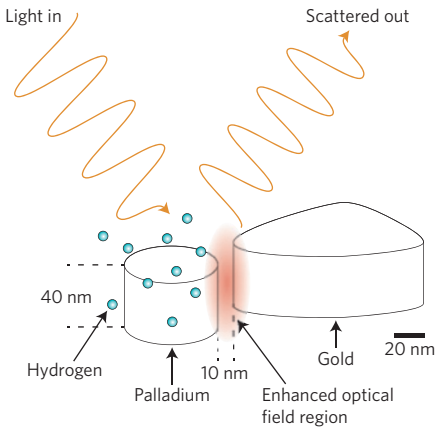

Figure 1 | Schematic of resonant gold nanoaerial. Incident radiation is resonantly scattered by the gold nanoaerial and when hydrogen is absorbed by the palladium nanoparticle it changes the peak scattering wavelength through changes in the palladium, altering the near-field interaction between palladium and gold.

The sensitivity to hydrogen of this arrangement is not particularly impressive as the pressure of the hydrogen introduced is 8 Torr, and the concentration of hydrogen in the nitrogen carrier gas is 1%. One may estimate the number of hydrogen atoms absorbed by the palladium island is perhaps as much as ten thousand if one hydrogen atom is absorbed for each surface palladium atom or ten million if one is absorbed for each bulk palladium atom. However, as the 8 nm wavelength shift is orders of magnitude greater than the shifts one could measure, there is substantial room here for