from the dust that is supposed to accompany the cold H_2 was not observed in the Milky Way (*5*), and because the extra mass should make disks too unstable and too thin if it is all inside the gas layer (the thin disk in the figure) (*6*). Moreover, Kuijken and Gilmore (*7*) and others previously showed that the vertical motions of stars in the disk do not require the presence of dark matter. Crézé *et al.* (*8*) found the same result more recently using densities and velocities of A-type stars within 125 pc (1 pc = 3.26 light-years) of the Sun. The thickness of any dynamically significant component of dark matter has to exceed ~4 kpc (*9*, *10*), which is thicker than the visible gas disk by a factor of 8. Revaz and Pfenniger (*11*) found bending instabilities for massive thin disks that are in nice agreement with observed galaxy warps, but they did not consider other observational constraints.

The dark matter in the dwarfs studied by Bournaud *et al*. is not excessive—a factor of 2, not 10—and some H_2 can certainly be hidden when only CO emission is used as a proxy. However, the mass of hidden H_2 has to be large, three times that of the atomic hydrogen plus the molecular hydrogen already inferred from CO emission. Observations might be expected to show this much H_2 or its associated dust in emission. So far, only warm H_2 (400 to 460 K) has been seen in these dwarfs and its total mass is low, 5×10^{-4} of the H_2 traced by CO (*12*). The hidden H_2 has to be much colder to be invisible, only a few kelvin. Dust-related polycyclic aromatic hydrocarbon emission has also been observed, but it is warm too (140 K versus the more usual 50 K in starbursts) (*12*). The stars in the Bournaud *et al*. dwarfs are unusual as well: No evolved stellar population has been detected at 1.6 µm, so most of the stars are young (*12*). This is to be expected if the dwarf stars formed because of the interaction, but it is an anomaly for normal dwarf galaxies.

The most famous interacting system is the Antenna, which is composed of two spiral galaxies merging in a dense core and two long tails that extend for 120 kpc. A clump in the larger tail has about the same mass as the dwarfs studied by Bournaud *et al*., and it also needs a factor of ~2 more matter to be gravitationally bound than is visible (*13*). This clump is not a dense galaxy yet and it does not rotate like the objects studied by Bournaud *et al*. Still, it could be a younger version of these objects, and perhaps more clues to disk dark matter can be found there. In any case, the dwarfs in NGC 5291 are unique at the present time, and they appear to be telling us something important about the nature of dark matter in the universe and its existence in galaxies.

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MATERIALS SCIENCE

Hydrogel Cell Cultures

Three-dimensional synthetic gels that mimic the extracellular matrix provide a promising tool for studying cell interactions.

Melinda C. Cushing and Kristi S. Anseth

Cells often behave differently when they are isolated from the complex architecture of their native tissues and constrained to Petri dishes. For example, human breast epithelial cells proliferate abnormally (like tumor cells) when cultured as a two-dimensional monolayer, but display normal cell growth behavior and form structures typical of breast tissue when cultured in three-dimensional membranes that resemble their native environment (*1*). Embryonic stem cells differentiate more efficiently to blood-forming stem cells when cultured in three-dimensional scaffolds compared to cells cultured in two dimensions (*2*).

This difference in cell behavior has constituted a major obstacle for tissue engineers. But in the past 10 years, the field has made progress in creating successful three-dimensional cellular microenvironments with hydrogels—networks of interacting polymer chains that are highly hydrated, with elasticity similar to that of natural tissues. The structure and composition of these gels can be tailored to bear the appropriate chemical, biological, and physical cues that encourage the development of tissuelike structures in vitro. However, it remains uncertain which endogenous factors of a tissue must be recapitulated in a gel, and better strategies must be developed for delivering those factors to the right place, at the right time, and in the right context within the gel.

Regardless of the nature of the hydrogel, challenges must be overcome related to the general approach of three-dimensional cell culture. First, even in two-dimensional culture, heterogeneities exist in the cellular microenvironment, and these will only be further exaggerated in three-dimensional gels. Second, engineering functional tissue equivalents requires careful attention to oxygen availability, because no cell in a metabolically active tissue is further than 100 µm from a high-oxygen source. Oxygen-sensing transcription systems, such as hypoxia-inducible factors, play an important role in regulating the differentiation of stem cells (*3*), as does the distribution of diffusing growth factors. Finally, many standard techniques for analyzing proteins and protein distributions are more difficult to perform, because they require isolation of the cells from the matrix. Thus, new fluorescent probes coupled with noninvasive live-imaging and real-time analyses will be critical to examine the cause and effect of stratification on cellular functions in three dimensions.

Physiological processes are guided by interactions between cells and their extracellular matrix, the proteins and polysaccharides that cells secrete into their environment to support tissue structure and survival. "Naturally based" hydrogels such as Matrigel (made of native extracellular matrix proteins) and collagen (the major extracellular matrix

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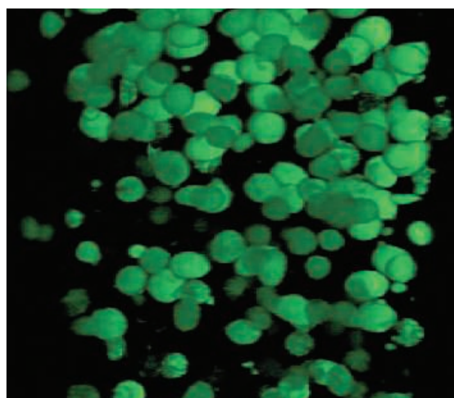
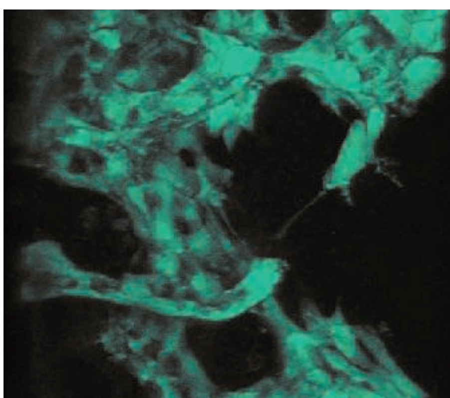
protein) have been explored extensively for applications in three-dimensional tissue culture and regenerative medicine. Natural gels provide a milieu of endogenous signals that promote the cellular interactions that underlie tissue formation. However, the complexity, variability, and ill-defined nature of these interactions make it difficult to understand the proliferation, differentiation, and migration of cells embedded within these natural gels.

Hydrogels can also be created from inert synthetic molecules such as poly(ethylene glycol). The advantages of synthetic gels include their consistent composition and predictable manipulation of properties, but they lack functional sites to interact with soluble or cell-surface proteins. Thus, synthetic gels provide little more than a blank slate to permit undirected cell function (*4*). Researchers are now bridging the gap between natural and synthetic gels by combining well-characterized synthetic materials with biomimetic cues to support physiologically relevant cell-gel interactions (see the figure).

Sophisticated synthetic hydrogels can be created through highly controlled, selective, and orthogonal reaction schemes—such as Click reactions that efficiently link small molecular subunits (*5*). Further, Click reactions allow cross-linking of biologic and synthetic precursors under physiological conditions. For example, light-initiated reactions that result in chemical cross-linking between functionalized poly(ethylene glycols) and biomimetic peptides have been used to encapsulate human mesenchymal stem cells (*6*). This process promotes their survival by facilitating specific cellular interactions with the covalently bound peptides. The peptide concentration, peptide conformation, and degradation behavior of such a system can be controlled through simple manipulation of reactant stoichiometry and functionality. Gels with this type of regulated chemistry and molecular structure will help to decouple the complex effects of structural (or mechanical) signals from biochemical ones on cellular activities in a three-dimensional environment (*7*).

Improved artificial hydrogels can also be generated through physical cross-linking (such as hydrogen bonding). For example, protein folding and protein-protein interactions can be used to create well-ordered and modular networks. This approach has been used to create a synthetic hydrogel from the self-assembly of leucine zipper domains—a protein motif that facilitates protein-protein interactions; the rate of gel degradation and mass loss can be precisely controlled in this gel (*8*).

The power of synthetic hydrogels. In this example, degradable poly(ethylene glycol) gels were modified with signals to promote the function of human mesenchymal stem cells and their evolution into a tissuelike structure. Scanning confocal microscopy of fluorescently labeled cells was used to visualize changes in cell morphology in the gel microenvironment. At the start of the experiment, the cell-laden gel contains rounded cells with few interactions (**left**). Over the course of 2 weeks, the gel forms a dynamic system with multiple cell-gel interactions that promote attachment, migration, and ultimately, differentiation of the encapsulated cells to bone-forming osteoblasts (**right**).

In addition to controlling the structure and chemistry of synthetic hydrogels, advances in gel materials that respond to some form of stimulation allow manipulation of the temporal and spatial availability of bioactive moieties within the cellular microenvironment. Cell-initiated proteolysis of chemical cross-links in a gel allows cell migration by mimicking proteolytic processes that occur in native tissues and has been shown to facilitate bone tissue regeneration (*9*); it may also be useful as a model for studying cell metastasis in tumors.

Some gel networks undergo abrupt conformational changes in response to a stimulus. These changes allow control of gel swelling, which in turn dictates the release of encapsulated biomacromolecules. For example, gels made from calcium-sensitive protein building blocks (*10*) or single-stranded DNA components (*11*) can expand or contract in response to the addition of calcium or single-stranded DNA, respectively, to sense, gate, and transport biomolecules within hydrogels. Photolabile linkers (*12*) and photosensitive reactions provide a means to spatially pattern gel environments with biological signals. Target molecules might include chemotactic agents to direct cell movement or orientation, tissue morphogens to influence cell fate, and/or physical structures to control cell morphology and interactions over multiple size scales.

These hydrogels may prove useful in controlling the distribution of biological signals in the three-dimensional environment (*13*). For example, photopatterning has been used to create latticelike gels that minimize diffusion distances, thereby facilitating the three-dimensional regeneration of hepatic tissue (*14*). Bending and folding of materials that undergo temperature-dependent shrinkage are enabling gels to assume complex surface topologies and macroscopic structures (*15*). These advances will be important for tissue engineers aiming to recapitulate the shapes of small physiologic structures, such as aortic heart valves.

Tissue-engineering strategies are also focused on generating dynamic gels that allow the presentation of multiple biological factors to cells that vary in space and time. Gels that selectively bind cell-secreted factors are providing glimpses of the cell–extracellular matrix feedback that occurs during wound healing and normal tissue homeostasis. These sophisticated advances in gel design are creating new tools for hypothesis testing in cell biology and advancing cell-based approaches to repair and regenerate tissues.

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