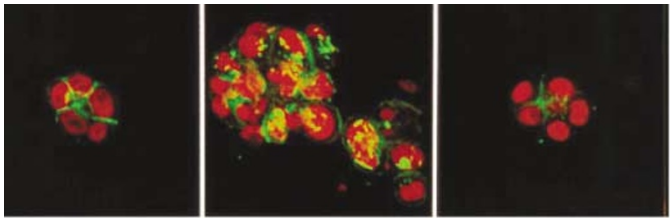
Biology's new dimension

There's a big difference between a flat layer of cells and a complex, three-dimensional tissue. But until recently, many biologists have glossed over this fact. Alison Abbott discovers what they've been missing.

Let's hear it for the humble petri dish! Many of the seminal findings in cell and molecular biology have come from cultures of cells grown cheaply and conveniently in these familiar, flat receptacles. But the limitations of considering biology in, effectively, just two dimensions are now becoming clear.



M. BISSELL

Role reversal: unlike in 2-D cultures, breast tumour cells in 3-D culture (left) that become malignant (centre) can be made to revert to their original state (right) when an antibody against β-integrin is added to the system.

Led by cancer researchers, biologists are increasingly turning to three-dimensional cell cultures, where they are discovering patterns of gene expression and other biological activities that more closely mirror what happens in living organisms. "Scientists are starting to realize just how much a cell's context matters," says Mina Bissell, a pioneer of 3-D cell culture at the Lawrence Berkeley National Laboratory in California.

In mammalian tissues, cells connect not only to each other, but also to a support structure called the extracellular matrix (ECM). This contains proteins, such as collagen, elastin and laminin, that give tissues their mechanical properties and help to organize communication between cells embedded within the matrix. Receptors on the surface of the cells, in particular a family of proteins called the integrins, anchor their bearers to the ECM, and also determine how the cells interpret biochemical cues from their immediate surroundings.

Given this complex mechanical and biochemical interplay, it is perhaps no surprise that researchers will miss biological subtleties if the cells they are studying grow only in flat layers. But providing an appropriate environment in which to culture cells in three dimensions is no easy matter (see 'The matrix, reinvented', below). Some researchers use simple gels consisting of collagen, whereas others make their own gels by extracting ECM material from relevant tissues. Another popular option is the commercially available Matrigel, which consists of structural proteins such as laminin and collagen, plus growth factors and enzymes, all taken from mouse tumours[1,2].

Culture shock

Bissell has been experimenting with 3-D culture systems for some three decades. But for years, critics argued that her methods were expensive, cumbersome and unnecessary. Their views changed after the publication of a landmark paper in 1997, in which Bissell's group showed that antibodies against a cell-

The matrix, reinvented

Interest in culturing cells in three dimensions has taken off in the past few years — but when it comes to the basic tool of the trade, most researchers are still using 1980s technology.

To grow in 3-D culture, cells need to be embedded in a structure that mimics the extracellular matrix (ECM) of structural proteins and other biological molecules found in real, living tissues. Many researchers use a material called Matrigel, a cocktail of substances extracted from the ECM of a type of mouse tumour and first described some two decades ago[1,2]. Matrigel is a liquid below 4 °C — so cells can easily be mixed in it. Gently warming the culture then embeds the cells in the newly solidified gel.

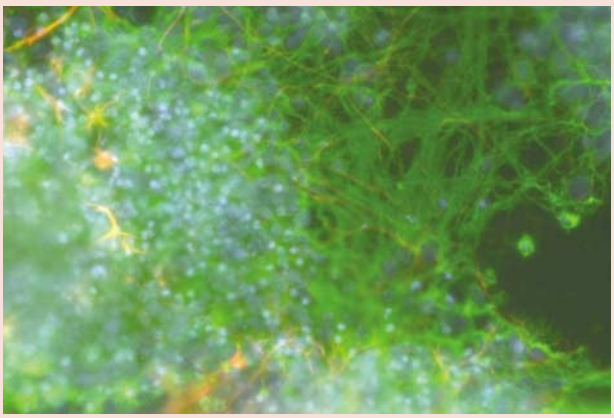
Although Matrigel has proved effective, its inventors admit that they are surprised that it hasn't yet been superseded. "I'm absolutely shocked that there isn't really anything better," says Hynda Kleinman of the National Institute of Dental and Craniofacial Research in Bethesda, Maryland, lead author of the papers that introduced Matrigel to the world.

In many cases, Kleinman argues, it would be more appropriate to extract material from tissues directly relevant to the cells being studied, to provide a more suitable environment for their growth. "People should be able to find tissue-specific matrices that would further fine-tune these investigations," she says. Indeed, some experts in 3-D tissue culture do make their own custom matrices in this way.

But in the long run, many researchers would like to get away from using materials derived from living tissues — which may vary from batch to batch and are difficult to customize for the demands of a particular experiment. Tissue engineers who eventually want to use 3-D matrices to grow implants to graft into human patients are particularly keen on finding an alternative to materials derived from animal tissues.

Many experts predict that the future will lie in synthetic materials that can be tailor-made for specific studies. But progress has so far been slow. "It is taking us a long time to make systems that are representative and that biologists can use easily," admits Jeffrey Hubbell, a biomedical engineer at the Swiss Federal Institute of Technology in Zurich.

Most work to date has used synthetic polymers that form 3-D matrices with micrometre-scale pores. For instance, David Mooney, a bioengineer at the University of Michigan in Ann Arbor who is interested in developing materials for biomedical tissue engineering, uses poly(lactide-



G. SILVA, NORTHWESTERN UNIV.

Growing up: neural stem cells (blue) in a 3-D protein-fibre scaffold differentiating into neurons (green) and glial cells (orange).

SCOTT LEWIS PHOTO/SILICON VALLEY BIZ INK

The third way: Mina Bissell says cells can behave very differently in 3-D rather than 2-D cultures.

surface receptor called β1-integrin completely changed the behaviour of cancerous breast cells grown in 3-D culture: they seemed to become non-cancerous, losing their abnormal shapes and patterns of growth[3]. This result had never been observed in 2-D cultures. Just changing the way a cell interacts with its 3-D environment, Bissell had shown, can radically alter its behaviour.

Since then, Bissell has demonstrated further important differences in the behaviour of cells grown in 2-D and 3-D cultures. For example, in the same breast-cancer system, she has shown that antibodies against β1-integrin also decrease signalling by receptors for epidermal growth factor (EGF); antibodies against EGF receptors similarly depress the activity of β1-integrin[4]. Again, this reciprocal interaction does not happen in 2-D cultures.

Receptors for growth factors play a key role in the initial development of tumours. But this isn't the only aspect of cancer research to have benefited from the new 3-D perspective. Peter Friedl, a cell biologist at the University of Würzburg in Germany, studies metastasis — the migration of cells away from primary tumours to cause secondary cancers around the body. Over the past couple of years, 3-D studies by Friedl's group and others have revealed unexpected subtleties in the mechanisms that cancer cells use to break out from primary tumours — clues that may help to explain the disappointing clinical performance of a promising class of cancer drugs.

Cancer cells undergoing metastasis normally cut themselves free from a tumour's ECM using protein-digesting enzymes. Yet in clinical trials, drugs that inhibit these enzymes have done little to slow the progress of cancer[5]. In his 3-D culture system, Friedl blocked the activity of the protein-chopping enzymes in two types of cancer cell, and found that the cells changed into an amoeba-like form, which could squeeze through gaps in the matrix[6]. "3-D tissue culture is really challenging our assumptions," says Friedl.

Chris Marshall, a cell biologist at the Institute of Cancer Research in London, has extended this finding, showing that the formation of amoeba-like cells depends on a particular signalling pathway in a range of different tumour cell lines. When this pathway is blocked, drugs that inhibit the protein-digesting enzymes stop the cells from moving through Matrigel[7]. This result suggests that a combination of drugs might work where inhibitors of the protein-digesting enzymes alone failed.

In another recent paper, 3-D cell culture has improved the prospect of treating cancer with gene therapy. Researchers led by Michael Korn of the University of California, San

co-glycolide), which gives a sponge-like structure with pores 100–200 μm in diameter[11].

But some researchers are now turning to systems based on amino acids that assemble into protein fibres of their own accord. When mixed with water, these fibres form gels with a nanoscale structure that, the researchers argue, more closely matches that of a living tissue. "Self-assembly yields nanofibres that mimic the architecture of fibrils in the ECM," enthuses materials scientist Samuel Stupp of Northwestern University in Chicago. In unpublished work, for instance, his group has used a matrix constructed from self-assembling nanofibres to coax neural stem cells into becoming neurons.

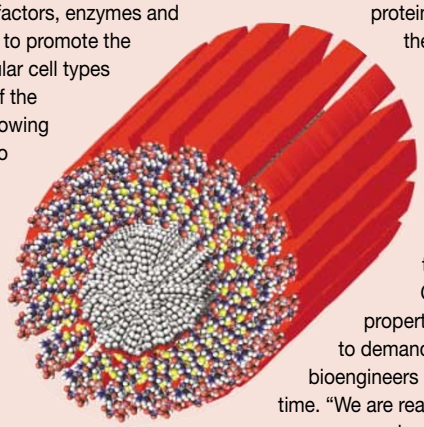
Similarly, Shuguang Zhang and his colleagues at the Massachusetts Institute of Technology's Center for Biomedical Engineering have weaved nanofibres of self-assembling peptides into a mesh with just the right porosity to slowly distribute nutrients and other necessary biological molecules to embedded liver stem cells. In this environment, the stem cells both continued to divide to reproduce themselves, and differentiated into mature liver cells[12].

Several research groups are now investigating ways to adjust the shape and surface chemistry of the self-assembling fibres in the hope of producing gels that are better able to support cell growth. Earlier this year, for instance, Maxim Ryadnov and Derek Woolfson of the University of Sussex in Brighton, UK, tinkered with a self-assembling system to produce protein fibres that were kinked, waved or branched[13].

But perfecting the structure of the material is only part of the battle. Doping synthetic matrices with the correct growth factors, enzymes and other molecules needed to promote the normal growth of particular cell types is far from trivial. "One of the biggest challenges is knowing what biology you need to build into your system," says Mooney.

Researchers are slowly beginning to manipulate their materials to control the biology of the cells contained within. Using different manufacturing processes to embed two different growth factors into his matrix, for example, Mooney has succeeded in controlling the rate at which they are released by the structure[14]. "Most biological systems are driven by a complex combination of signals present in a defined sequence," he says.

Hubbell, meanwhile, has equipped his polymer-based matrix with 'sacrificial' peptides that make it possible for cells to migrate. Cells moving through a natural ECM release protein-digesting enzymes to cut themselves a path. Using a range of peptides in the matrix that differ in their sensitivity to degradation by these enzymes, Hubbell found that he could control the degree of movement of skin cells through his matrix[15].



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Do-it-yourself: self-assembling nanofibres can be used to form an artificial matrix.

Given the vast range of properties that biologists are likely to demand of 3-D culture systems, bioengineers should be in for a busy time. "We are really talking about doing hundreds, if not thousands, of different things," says Mooney.

David Cyranoski

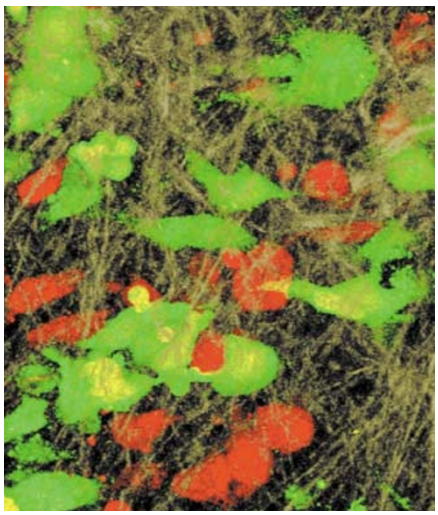
Francisco, studied the cell-surface receptors to which adenoviruses bind. In 2-D cultures, both normal and malignant breast cells had similar, high levels of the receptors. But in 3-D cultures, only malignant cells carried large numbers of the receptors[8]. Adenoviruses have been used as 'vectors' to introduce therapeutic genes into target cells, and Korn's findings suggest that they may be particularly suitable for targeting cancerous cells.

Developmental biologists are also getting in on the 3-D act. In 2001, for instance, a team led by Kenneth Yamada of the National Institute of Dental and Craniofacial Research in Bethesda, Maryland, directly compared the growth and development of fibroblasts, collagen-secreting cells that are found in many tissues, in 2-D and 3-D cultures. In three dimensions, the cells moved and divided more quickly, and assumed the characteristic asymmetric shape that fibroblasts have in living tissues[9]. "At the very least, developmental biologists who have worked with normal tissue culture will have to seriously consider comparing their results to those obtained in 3-D culture," says Yamada.

Imitating life

Some researchers are now trying to make systematic comparisons of gene activity in 2-D and 3-D cultures. In unpublished work, Linda Griffith, a bioengineer at the Massachusetts Institute of Technology, has used DNA microarrays to look at profiles of gene expression in liver cells. "Our preliminary analysis shows that the expression profile in 3-D is much closer to *in vivo* expression profiles than the profile we've seen in 2-D," she says.

If 3-D culture can provide a better model for what happens in the body, it might allow researchers to reduce their use of experimental animals — although experts stress that it is far from a complete alternative. "3-D culture will allow a lot of basic questions to be answered before having to turn to whole-animal research," says Friedl, whose work has been supported in part by a German research-ministry programme dedicated to reducing animal use. Encouragingly, when Friedl transplanted metastasizing cells into mice and used imaging techniques to track their development, they underwent the same amoeba-like morphological changes seen in 3-D culture[6].



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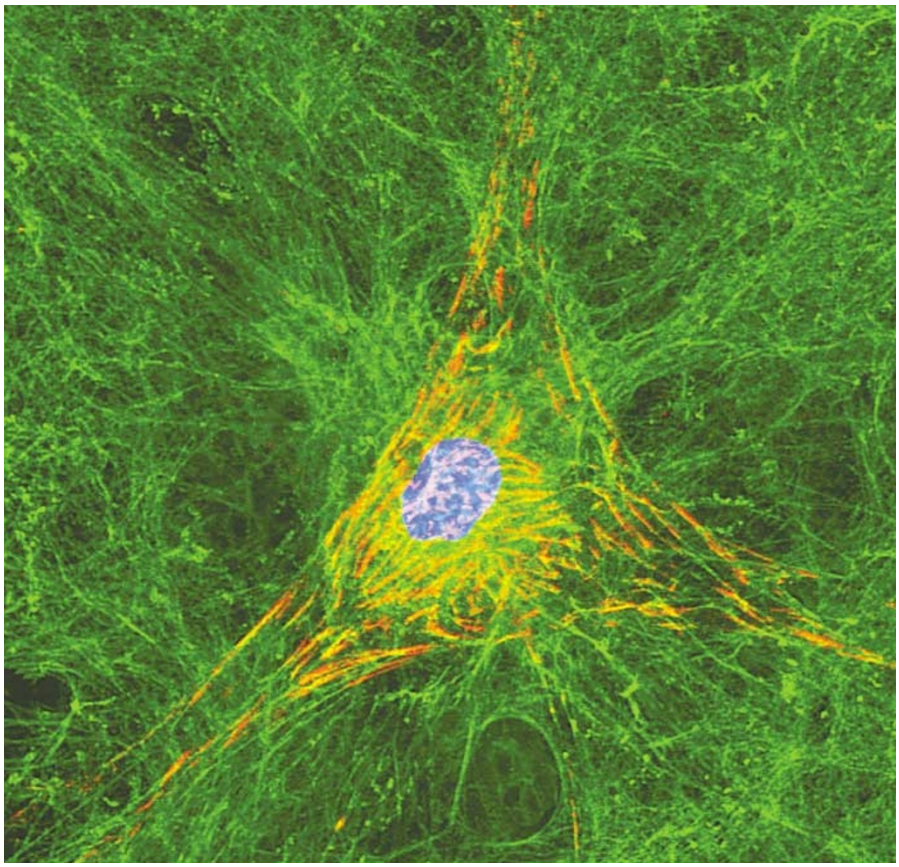
Analyses using live animals have confirmed that cancer cells (green) can escape from their location by becoming amoeba-like (red), an observation first made using a 3-D tissue culture.



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Joined up: a cell in a 3-D culture forming links by means of β-integrin (orange) with the scaffolding.

In October, 3-D cell culture will receive an important boost when the National Cancer Institute (NCI) in Bethesda, Maryland, launches a new section on the cellular micro-environment, which will rely heavily on 3-D studies. This programme will have an annual budget of some US$40 million, and will include specific funding to spur the development of 3-D culturing techniques.

For Robert Weinberg of the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, the new NCI programme is a welcome development. In the 1970s and 1980s, he pioneered the study of cancer-causing genes and their associated cell-signalling pathways, mostly using 2-D cultures. "There is a whole dimension of signalling that we purposefully didn't deal with, for simplicity's sake," says Weinberg. "But now we are ready to move onto the next stage — the more complex level that 3-D culture allows."

In an article late last year, Weinberg went so far as to describe the study of cancer cells in two dimensions as "quaint, if not archaic"[10]. And where cancer researchers have led, he predicts, other biologists will follow.

Influential players in industry are already thinking along 3-D lines, says Mihael Polymeropoulos, chief scientific officer of Vanda Pharmaceuticals in Rockville, Maryland, and formerly head of pharmacogenetics at the Swiss-based drugs giant Novartis. "In 10 years, anyone trying to use 2-D analyses to get relevant and novel biological information will find it difficult to get funded," he predicts. ■

Alison Abbott is *Nature*'s senior European correspondent; additional reporting from David Cyranoski, *Nature*'s Asian-Pacific correspondent.

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