Beyond the Petri dish

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When he invented the ubiquitous dish that bears his name, Julius Petri, a technician in Robert Koch's laboratory, fundamentally transformed our ability to culture, manipulate and analyze cells. Since then, over a century ago, the Petri dish has become a staple in laboratory work on both prokaryotic and eukaryotic cells. But the time has come to move on from two-dimensional dishes to culture systems that better represent the natural context of cells in tissues and organs.

3D or not 3D?

The *in situ* environment of a cell in a living organism has a three-dimensional architecture. Cells are surrounded by other cells. They are held in a complex network of extracellular matrix (ECM) nanoscale fibers that allows the establishment of various local microenvironments. Their extracellular ligands (*e.g.*, collagens, laminins and other matrix proteins) mediate not only attachment to the basal membrane, but also access to a variety of vascular and lymphatic vessels. Oxygen, hormones and nutrients are ferried to cells, and waste products are carried away.

This contrasts starkly with a cell's environment in two-dimensional culture. First, in a whole organism, the movements of cells typically follow a chemical signal or molecular gradient in all three dimensions. Molecular gradients play a key role in biological differentiation, determination of cell fate, organ development, signal transduction, neural information transmission and countless other biological processes. Such gradients cannot be replicated in two dimensions.

Second, the metabolism and gene expression patterns of cells isolated directly from higher organisms are frequently altered during growth in two-dimensional culture. Key metabolic pathways are influenced by protein-protein signaling and interactions at the cell surface, which is disrupted by the adaptation of cells to a two-dimensional Petri dish. Such adaptation requires significant adjustment of the surviving cell population not only to changes in oxygen, nutrients and extracellular matrix interactions, but also to the accumulation of waste products.

Third, cells growing in a two-dimensional environment can significantly reduce production of ECM proteins and often undergo morphological changes (*e.g.*, resulting in increased spreading). It is likely that cell surface receptors preferentially cluster on parts of the cell directly exposed to culture media rich in nutrients, growth factors and other extracellular ligands; in contrast, cell receptors attached to the surface may have less opportunity for clustering. Nonoptimal orientation or clustering of receptors is likely to affect communication between cells.

Figure 1 Architecture that mimics three-dimensional cellular architecture? The San Simeon Piccolo Dome in Venice, Italy. Each of the metal rods has a diameter of ~4 cm, 500 times smaller than the size of the dome, a diameter of ~20 meters. Each rod also serves as a construction scaffold for building or repairing the dome that is truly embodied in three dimensions.

Just how realistic is a picture of cell behavior that doesn't take account of cellular communication, the transport of oxygen, nutrients and toxins, and cellular metabolism in the context of all three dimensions?

Adding an extra dimension

Attempts have been made to culture cells in three dimensions using synthetic polymers/copolymers. However, processed synthetic polymers consisting of microfibers ~10–50 μm in diameter are similar in size to most cells (~10–30 μm in diameter). Thus, cells attached on microfibers are still in a two-dimensional environment with a curvature dependent on the diameter of the microfibers[1]. Furthermore, the pores (~10–200 μm) between the fibers are often ~1,000 to 10,000 times larger than biomolecules, which as a consequence can diffuse quickly away[2]. For a true three-dimensional environment, a scaffold's fibers and pores must be much smaller than the cells.

Biomaterials derived from animals (*e.g.*, collagen gels, poly-glycosaminoglycan and Matrigel) have been used as an alternative to synthetic scaffolds[2–4]. Although they do have the right scale, residual growth factors, undefined constituents or non-quantified impurities are frequently present. It is thus very difficult to conduct a completely controlled study using such biomaterials because they vary from lot to lot. This not only makes it difficult to conduct a well-controlled study, but also would pose problems if such scaffolds were ever used to grow tissues for human therapies.

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What is needed is a three-dimensional culture system that could be fabricated from a synthetic biological material with defined constituents at the nanoscale. In this respect, molecular-designed self-assembling peptide scaffolds might provide the answer.

A new mode of cell culture

Work in my laboratory has demonstrated that peptides, made from natural amino acids, undergo self-assembly into well-ordered nanofibers and scaffolds, often 10–20 nm in diameter with pores between 5 and 200 nm[5]. These peptides can be chemically synthesized, tailor-made to incorporate specific ligands such as ECM ligands for cell receptors, purified to homogeneity and manufactured readily in large quantities. Their assembly into nanofibers can be controlled at physiological pH simply by altering NaCl or KCl concentration. Because the resulting nanofibers are 1,000 times smaller than synthetic polymer microfibers, they surround cells in a manner similar to extracellular matrix. Moreover, biomolecules in such a nanoscale environment diffuse slowly and are likely to establish a local molecular gradient.

Using the nanofiber system, every ingredient of the scaffold can be defined, just as in a two-dimensional Petri dish; the only difference is that cells now reside in a three-dimensional environment where the extracellular matrix receptors on the cell surface can bind to the ligands on the peptide scaffold. Cells now behave and migrate as they would in a truly three-dimensional environment. Ultimately, higher tissue architectures with multiple cell types, rather than monolayers, may also be constructed using these three-dimensional self-assembling peptide scaffolds.

It's time to move away from technology that predates the past century. Quantitative biology requires *in vitro* culture systems that more authentically represent a cell's environment in a living organism. In doing so, *in vitro* experimentation can truly become more predictive of *in vivo* systems.

As David Housman of the Massachusetts Institute of Technology (Cambridge, MA, USA) aptly puts it: "What we need is a three-dimensional culture system—something between a Petri dish and a mouse." It's time to move away from technology that predates the past century. Quantitative biology requires *in vitro* culture systems that more authentically represent a cell's environment in a living organism. In doing so, *in vitro* experimentation can truly become more predictive of *in vivo* systems.

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