© 2017. Published by The Company of Biologists Ltd | Journal of Cell Science (2017) 130, 3-5 doi:10.1242/jcs.200550

FOREWORD

Goodbye flat biology – time for the 3rd and the 4th dimensions

Mina J. Bissell[1,2,*]

It is now more than a decade since Allison Abbot of Nature wrote an editorial using the first part of the above title (Abbott, 2003a) and, inside the same issue, under the title of 'Nature's Third Dimension'(Abbott, 2003b), described the efforts of a number of laboratories that had come to terms with using three-dimensional (3D) strategies, and described in more detail our laboratory's development of 3D assays, which involved growing cells inside laminin-rich gels. These assays could be used for both functional studies of tissue-specific gene expression in rodents (Barcellos-Hoff et al., 1989) and, in collaboration with Ole Petersen, to develop a simple and robust assay to distinguish normal and/or non malignant breast cells from those that were premalignant and malignant (Petersen et al., 1992).

The assays we developed are already more than 25 years old, and the procedures were described further in Nature Methods in 2007 (Lee et. Al., 2007). These were the beginnings of the 3D cell biology field, although they followed a respectable body of literature that had been published years earlier describing many attempts to cultivate cells to restore function in culture using everything from weighing papers floated on top of the media to floating collagen gels from rat tails (for a timeline of some such attempts, please see Simian and Bissell, 2017).

The defining difference between the use of laminin-rich gels and the previous attempts to culture cells was that, with time, the laminin-rich cultures developed the foot prints of both functional and morphological characteristics of the tissue of origin. In the case of the mammary gland, such cultures could make copious amounts of milk and also form acini that were similar to the unit structure of mammary glands of pregnant mice. Appreciable numbers of laboratories worldwide have begun to switch from flat substrata to laminin-rich cultures to test drug behavior with promising data. Many companies not only provide different kinds of scaffolds but are rushing also to provide different high-throughput techniques and pre-fabricated substrata, and different disciplines have joined the game of who can provide better 'designer microenvironments'(Stoker et al., 1990)!

I must admit, however, that in my many invited travels across the USA and other countries to lecture on our work, I am often astonished at how many people in my audience have either never heard of 3D cultures or have not given it a serious thought, and thus are startled to see how profoundly results differ with changes in context. There are many differences in the integration of signaling pathways and in the conclusions reached between 2D and 3D assays, with the latter providing results that are much closer to the *in vivo* situation. In fact, quite a few scientists still don't see any need to change the ease of culturing on tissue culture plastic, and they don't want to deal with what they perceive to be the complexities and expense of using 3D cultures, and so continue to look under the light. But I think that the time is now to make the switch to 3D if functional integrity is the end point.

I attribute this lack of knowledge and interest to the real gaps in our teachings and our textbooks in the classrooms. Of course we all know some organisms make excellent 3D models. We would not be anywhere close to where we are in our understanding of biology without bacteria, worms, *Drosophila*, zebrafish and rodents, and many of us use one or more of these organisms as well as 3D cultures. The animal models have provided rich genetic and functional data, and have contributed immensely to our understanding of biological systems through high-throughput mutational analysis, physiological studies and useful screens. Yet the 3D culture systems are unique in their ability to allow us to study human cells and tissues, and also to unravel regulatory circuits and pathways that differ between other species and humans (e.g. for example, see Bissell et al., 1987).

An offshoot of thinking in three-dimensions is the past few years' excitement and the literature of what is being referred to as 'organoids'. Organoids are great 3D models, but treating them as if it is a new field is like calling 'a rose by any other name'! What others and we have developed and reported in the literature in the past three decades is nothing if not organoids. Anything that can be cultivated on a substratum that is not tissue culture plastic or any other flat surface, and that would allow imitation of form and, hopefully, function in an organ- and tissue-specific manner fits under the umbrella of 3D culture and/or organoids. Thus, it is with pleasure that I accepted the invitation to write a short foreword to this impressive volume, conceived by the Journal of Cell Science editorial team and expertly edited by Andrew Ewald. He has also penned the introduction to the volume, in which he pays specific attention to the contributed articles.

The past five years have produced a bonanza of reviews and articles from a number of other laboratories, including ours, and in other journals in addition to this volume (Shamir and Ewald, 2014; Lancaster and Knoblich, 2014; Clevers, 2016; Fatehullah et al., 2016). So my intent here is to simply acknowledge the past and raise a few questions and concepts for the future. I see little gain in repeating and re-embellishing 3D and organoid culture history, and so look forward to where we need to go.

How to define a unit of function?

How do we move beyond our current gains in '3D Omics' and maximize our ability to understand biological complexity, especially in humans? Much of the recent literature on organoids and related aspects deals exclusively with tumor cells and tissues. I have always argued that cancer is an organ-specific disease and thus, to understand cancer, one must understand what is the definition of the normal organ of interest. Consequently, to understand disease, including cancer, we must deeply comprehend the normal organ in detail in terms of form and function as well as aging! But in the final analysis, the unit of function is the organism itself (Bissell and Hall, 1987). Organs communicate with many other organs and with their

[1]Distinguished Scientist, Division Biological Systems and Engineering, Lawrence Berkeley National Laboratory, One Cyclotron Road, MS 977–225A, Berkeley, CA 94720, USA. [2]Graduate Groups in Comparative Biochemistry, Endocrinology, Molecular Toxicology and Bioengineering, University of California, Berkeley, CA 94708, USA.

*Author for correspondence (mjbissell@lbl.gov)

Journal of Cell Science (2017) 130, 3-5 doi:10.1242/jcs.200550

Mina Bissell with the steady-state machine. As reported in Bassham et al., 1974.

microenvironment. We now know that even if one were to study a single organ, such as the mammary gland, a lifetime is barely enough to scratch the surface (another reason not to be arrogant and to keep an open mind!). We still know so little about mammary cycles – how the gland involutes and remodels to allow feeding of the next baby, how neurons communicate with mammary glands and what exactly is the function of the associated mast cells, etc. It has taken a scientific lifetime and I still know so little. Others have written on many of these topics, but they too have barely scratched the surface of the understanding of mammary glands or other organs.

My laboratory has managed to model only a few snapshots of the stages in the life of a mammary gland of mice or women: how an acinus is formed, the branching morphogenesis of a few stages of mammary glands of virgin mice (Simian et al., 2001) and highly intricate alveogenesis resembling the gland of the lactating animal (Fata et al., 2007). We would start with either a single or group of mammary cells, or explant tiny pieces of tissue, and culture them inside different kinds of gels or on top of gels, and then take simple pictures or movies of how they grew and took shape – making organoids! We would compare the movement of normal and malignant cells within a laminin-rich gel using high-resolution microscopy. We would then marvel at the movement generated within the laminin-rich gel and at how the interaction of cells would give rise to the shape of the mammary organoid or mammary tumor (Tanner et al., 2012). But each snapshot has a rich history of signaling *in vivo*, and the advantage of 3D cultures is that we can slow down the rapid development *in vivo* by choosing just a little piece of the scenario, and ablate or introduce different bits of it, to understand signaling. It is important to commit to studying an organ, but unlike the process of describing single molecules, it takes a lifetime. But even single molecules could take a lifetime to understand. In this context, it makes sense to me that pathologists typically specialize in a single organ. My awareness of the complexity of each and every organ, and my fascination with the mystery of the emergent phenomena in each experiment never gets old. We have stayed with the mammary gland for most of my scientific career. There is so much to see and learn from a single organ.

The 4th dimension

With the advent and excitement of molecular biology, and because we have the tools to use more complex animal systems and the ability to obtain better images, as well as to solve more and more complex molecular structures, the fields of biochemistry, metabolism and bacteriology were gradually disappearing from the pool of knowledge of our graduate students. I am glad, however, to see that there are exciting returns, especially with microbiology and metabolism. As discussed above, we have also been playing with the 4th dimension (time), which has not generally been considered in most cell and molecular biology experiments. It is time to rethink how to obtain the correct 3D architecture in cultures and the resulting behaviors of the tissue as a function of time. The understanding of kinetics can clarify much of the confusion in signaling.

When I first joined the virology and cell biology fields as a postdoctoral fellow in the Berkeley campus, coming from a background of chemistry and bacterial genetics, I was amazed at how we performed our culture studies. To begin with, there was almost no attention to kinetics, nor was there any attention to changes in pH and temperature. Incubator doors would open and remain open until the trays had been pulled out and the plates had been removed. We then would proceed to take the plate to the microscope, take a look, then take it to the hood and scrape the cells; by then many things had changed, including whatever it was we were trying to measure.

One of the first things I did when I went to the laboratory of Chemical Biodynamics, where Melvin Calvin had done his Nobel-Prize-winning work, was to elucidate the path of carbon during photosynthesis. One of my goals was to look at how normal fibroblasts and Rous sarcoma virus (RSV)-transformed cells could differ in their rate of aerobic glycolysis. Being in the nation's first National Laboratory had a number of advantages (and of course also disadvantages!). Together with Al Bassham, a former graduate student of Calvin, we designed what we referred to as a steady-state machine (Bassham et al., 1974) (Fig. 1). This had 30 tissue-culture plates and two ports covered with rubber tops through which radioactivity could be introduced, and a kinetic experiment could be performed by then removing the plates. The apparatus could be kept closed to the outside environment by isolating one plate from the rest through one of the ports; the plate of interest could then be rapidly removed and treated on the spot with our solvent. A series of papers, of which I am quite proud, chronicled the kinetics of the path of carbon in glucose metabolism (Bissell et al., 1972; Bissell et al., 1973; Bissell, 1976; Bissell et al., 1976; Bissell et al., 1977). The 3rd and the 4th dimensions do not and should not finish our quest to understand how the human body can do what it does. For every tissue in the body, we need to add all of the other ingredients to make the unit structure and function of the organ. To make a complete model of an organ, we need to put in the stroma and other cell types, learn what to add from the immune system to make it tissue specific, imitate the blood flow and, lest I forget, the lymph system, as well as the organ- and tissue-specific functions of neurons, and so on. There is no reason to be timid: while we are at it, how about we simulate different interactions between and among organs? I rest my case; there is much to find and thus do! Even though we may we believe we know what appears to be a lot, we are ignorant of so much still.

Journal of Cell Science (2017) 130, 3-5 doi:10.1242/jcs.200550

There is no time for arrogance and getting full of ourselves. What is needed is a bit of humility, and a lot of curiosity and passion. Achieving a close to complete understanding of human organs and their interactions is by no means hopeless, and should not be a source of despair – biology's exquisite complexity is also its beauty and a joyful source of fascination and curiosity, which we can have fun exploring.

References

Abbott, A. (2003a). Goodbye, flat biology? *Nature.* **424**, 861.

Abbott, A. (2003b). Biology's new dimension. *Nature.* **424**, 870-872.

Barcellos-Hoff, M. H., Aggeler, J., Ram, T. G. and Bissell, M. J. (1989). Functional differentiation and alveolar morphogenesis of primary mammary cultures on reconstituted basement membrane. *Development.* **105**, 223-235.

Bassham, J. A., Bissell, M. J. and White, R. C. (1974). Quantitative tracer studies of metabolic dynamics of animal cells growing in tissue culture. *Anal. Biochem.* **61**, 479-491.

Bissell, M. J., Hatié, C. and Rubin, H. (1972). Patterns of glucose metabolism in normal and virus–transformed chick cells in tissue culture. *J. Natl. Cancer Inst.* **49**, 555-565.

Bissell, M. J., White, R. C., Hatié, C. and Bassham, J. A. (1973). Dynamics of metabolism of normal and virus–transformed chick cells in culture. *Proc. Natl. Acad. Sci. USA.* **70**, 2951-2955.

Bissell, M. J. (1976). Transport as a rate limiting step in glucose metabolism in virus–transformed cells: studies with cytochalasin B. *J. Cell Physiol.* **89**, 701-709.

Bissell, M. J., Rambeck, W.A., White, R.C. and Bassham, J. A. (1976). Glycerol phosphate shuttle in virus–transformed cells in culture. *Science.* **191**, 856-858.

Bissell, D. M., Areson, D. M., Maher, J. J. and Roll, F. J. (1977). Support of cultured hepatocytes by a laminin-rich gel. Evidence for a functionally significant subendothelial matrix in normal rat liver. *J. Clin. Invest.* **79**, 801-812.

Bissell, M. J. and Hall, H.G. (1987). Form and function in the mammary gland: The role of extracellular matrix. In: Nevell MC and Neville CWD, eds., *The Mammary Gland: Development, Regulation and Function*, pp. 97-146. New York: Plenum Publishing Corp.

Bissell, D. M., Arenson, D. M., Maher, J. J. and Roll, F. J. (1987). Support of cultured hepatocytes by a laminin-rich gel. Evidence for a functionally significant subendothelial matrix in normal rat liver. *J. Clin. Invest.* **79**, 801-812.

Clevers, H. (2016). Modeling Development and Disease with Organoids. *Cell.* **165**, 1586-1597.

Fata, J. E., Mori, H., Ewald, A. J., Zhang, H., Yao, E., Werb, Z. and Bissell, M. J. (2007). The MAPK(ERK–1,2) pathway integrates distinct and antagonistic signals from TGFα and FGF7 in morphogenesis of mouse mammary epithelium. *Dev. Biol.* **306**, 193-207.

Fatehullah, A., Tan, S. H. and Barker, N. (2016). Organoids as an in vitro model of human development and disease. *Nat. Cell Biol.* **18**, 246-254.

Lancaster, M. A. and Knoblich, J. A. (2014). Organogenesis in a dish: modeling development and disease using organoid technologies. *Science.* **345**, 1247125.

Lee, G. Y., Kenny, P. A., Lee, E. H. and Bissell, M. J. (2007). Three–dimensional culture models of normal and malignant breast epithelial cells. *Nat. Methods.* **4**, 359-365.

Petersen, O. W., Ronnov-Jessen, L., Howlett, A. R. and Bissell, M. J. (1992). Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. *Proc. Natl. Acad Sci. USA.* **89**, 9064-9068.

Shamir, E. R. and Ewald, A. J. (2014). Three-dimensional organotypic culture: experimental models of mammalian biology and disease. *Nat. Rev. Mol. Cell Biol.* **15**, 647-664.

Simian, M., Hirai, Y., Navre, M., Werb, Z., Lochter, A. and Bissell, M. J. (2001). The interplay of matrix metalloproteinases, morphogens and growth factors is necessary for branching of mammary epithelial cells. *Development.* **128**, 3117-3131.

Simian, M. and Bissell, M. J. (2017). Organoids: A historical perspective of thinking in 3-Dimension. *J. Cell Biol.* (In press).

Stoker, A. W., Streuli, C. H., Martins–Green, M. and Bissell, M. J. (1990). Designer microenvironments for the analysis of cell and tissue function. *Curr. Opin. Cell Biol.* **2**, 864-874.

Tanner, K., Mori, H., Mroue, R., Bruni-Cardoso, A. and Bissell, M. J. (2012). Coherent angular motion in the establishment of multicellular architecture of glandular tissues. *Proc. Natl. Acad. Sci. USA.* **109**, 1973-1978.