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Therefore, I see a mix of approaches and ways of studying the cell in the future, with investigator-initiated research being in the majority. The need for other approaches, however, is becoming increasingly important. While investigator-driven research has evolved to work well to address specific hypotheses and problems, the evolution of large-scale team science is still a relatively new frontier and successful models for its effective implementation are still being developed.

Resources

[i] www.genome.gov/10001772/all-about-the–human-genome-project-hgp/

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Special Issue: Future of Cell Biology

Forum

3D Biomimetic Cultures: The Next Platform for Cell Biology

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Advances in engineering of cells and culture formats have led to the development of a new generation of 3D cultures that can recapitulate a variety of multicell-type, morphogenetic behaviors that were previously largely observable only in *in vivo* settings. Ultimately, these systems are likely to be assimilated into and forever change the landscape of biomedical research.

As with all sciences, biology advances through our ability to experiment, in this case with living systems. Modern biomedical research essentially relies on two experimental test beds: animals and cultured cells. The knowledge revolution of the past half century that we know as cell biology largely rests on the dissemination of cultured cells – HeLa cells first, then other immortalized lines, and now a wide array of primary cells and stem-like cells – as accepted experimental systems to understand cell structure and function. As an apt adjunct to animal systems, which capture the full complexity of biology but with limited ability to quickly isolate detailed mechanisms, experimental manipulation of cells in culture is straightforward and has revolutionized our molecular understanding of cells. However, despite amazing advances our ability to translate cell biological insights has been mitigated because culture on plastic dishes is so different from the *in vivo* microenvironment. Cells not only change their behavior in this non-physiologic environment but are also unable to remodel the matrix and reorganize freely as they would during development and homeostasis. Thus, many key functions are lost or unobservable in cell culture. These limitations compel us to consider whether innovative platforms that allow us to examine cells cultured in more biomimetic contexts can be developed to bridge the gap between traditional cell culture and the whole organism and what impact would such systems have on our biomedical research enterprise (Figure 1).

In vivo, local tissue structure defines the cellular environment, constraining how cells interact with the surrounding extracellular matrix (ECM), neighboring cells, soluble growth factors, and physical forces. These 'microenvironmental' cues cooperate to regulate cell behavior. Thus, while it is no surprise that culture on plastic dishes results in decompensated cell signaling, gene expression, phenotype, and function, attempting to fully reconstruct a tissue environment for *in vitro* applications would be excessive. The real challenge is in identifying which factors to incorporate to appropriately model different *in vivo* processes in cell culture and then establishing what such systems would and would not be able to recapitulate.

In recent years, several *ex vivo* experimental models have been developed to capture various higher-level behaviors that historically were largely reserved for animal models. Some of these models are methodologically 'simple', natural extensions of classical 3D cultures that have been used to generate mammary acini, hanging-drop embryoid bodies, or spheroid cultures, although with remarkable new morphogenetic capabilities. For example, single intestinal stem cells embedded within ECM gels have been shown to give rise to self-organizing structures characteristic of the crypt–villus of the intestine [1]. Similarly, spheroid cultures of neuronal stem cells have been developed to recapitulate the layering and morphogenesis of the developing

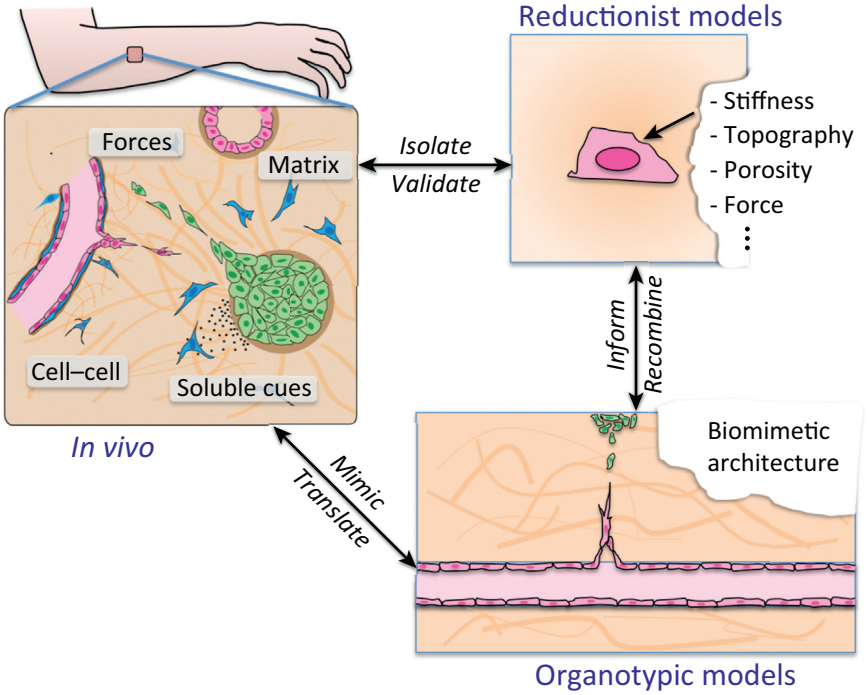

Figure 1. *In Vivo* and *In Vitro* Models Have Coevolved Synergistically to Provide Distinct Approaches to Understanding Living Systems. New biomimetic models offer the potential to provide a third approach to the ecosystem, reconstituting more complex behaviors in culture.

brain [2]. Other systems, by contrast, involve substantial engineering and incorporation of synthetic materials, prefabricated architectures, and/or microfluidics to model specific biological processes. For example, using a device containing two microfluidic channels separated by a porous elastic membrane Ingber and colleagues were able to model the interface between lung alveolar air, epithelium, capillary endothelium, and blood [3]. Using pumps to control air and blood flow and mechanical actuators to mimic the stretching forces of breathing on the epithelial–endothelial interface, the model has been used to recapitulate injury and inflammation and has inspired a cadre of organ-on-chip efforts from cardiac muscle to liver tissue [4,5]. Incorporation of human cells and human iPS-derived cell types into some of these systems has suggested the possibility that these biomimetic systems have the potential to close the gap between traditional animal models and human physiology and disease [6,7]. DARPA, the NIH and NCATS, and the popular press have embraced the idea that these systems will ultimately replace preclinical testing of therapeutics in animals [8–10]. How can the research community come together to realize such high expectations, separate reality from hype, and ultimately benefit with a bevy of experimentally tractable systems that model human physiology and disease?

Classically, biological experimental systems were used not as models to predict the behavior of other systems but as an end in themselves. Knowledge of anatomical structures in HeLa cells or wing formation in *Drosophila* was valued for its own sake. By contrast, the 3D biomimetic systems that are now being developed are explicitly valued for their ability to model specific processes, mostly in human biology. It stands to reason that a large part of establishing such models will be defining what the models can or cannot recapitulate. It is important to note that, unlike *in vivo* systems, these models are necessarily and intentionally simplifications to capture a narrow range of behavior, physiology, or time. For example, while intravital recordings of the developing vasculature of the avian ovum or zebrafish can capture vasculogenesis (when endothelial cells assemble to form networks spontaneously), angiogenesis (when existing vessels sprout and branch to form new vessels), or tumor cell trafficking, different biomimetic culture systems have been established to capture each of these events separately (for example, see [11–13]). Thus, a key feature of these models in their current state of evolution is that they are best adopted when fit for a specific purpose, and expectations that such models would have universal applicability would be unrealistic.

Thus, key questions remain about how and when different models can or should be used. If minibrains can recapitulate some aspects of neuronal organization, will they show aberrations with known genetically caused brain malformations? Will they respond predictively to neurochemical modulators? Will they predict the neurological side effects of test compounds? Can they model aging? If the lung on chip can model inflammation, can it also recapitulate effects of cystic fibrosis? Will it respond similarly to biomechanical injury? If we take the lessons learned from cell culture and animal models, the key to answering these questions is not to wait for the group that first described these models to test all of these conditions. The only path to establishing these models, continually improving them, or deciding to abandon them is to make the models widely accessible to as many scientists as are willing to study them. There are many reasons why only a handful of cell lines and animal models dominated the research community, but perhaps the foremost were ease of adoption and the ability to share insights and advances among scientists. This poses a major challenge for many of these

engineered organotypic models: they do not reproduce themselves; many of the systems are assembled as artisan pieces with many parameters that can affect the model so it can be difficult to teach; many different biomimetic systems or variations would be expected to emerge to highlight different biological events and this customization inherently may limit wider adoption of each specific system; and it remains unclear which models scientists should congregate around versus leave under-investigated.

Despite these hurdles, the eventual incorporation of these synthetic biomimetic culture systems into biomedical research laboratories is inevitable. The confluence of technological advances in the engineering and biological communities appears to be a virtual perfect storm that will push us to continue establishing engineered 3D organotypic cultures. On the biological side, iPSC technologies and stem cell biology are coming together to advance access to human cell types and the application of genomic editing technologies offers the possibility of both modeling human genetic diseases and mechanistically implicating molecular players in these culture systems. On the engineering side, a suite of technologies have been established that can be used to build various types of system for organ-on-chip applications, including the development of biomaterials that can begin to mimic and decouple aspects of the ECM, the application of microfabrication and nanofabrication tools such as microfluidics to support cell-based systems, advances of 3D printing and other technologies to organize cells in three dimensions, microscopy advances to observe living cells in 3D contexts, and the use of insights gained by tissue engineers to assemble cells and ECM. The dire need for better models of human physiology and disease than either traditional cell culture or animals also provides a pull to advance these systems. Last, while ultimately these systems may become a primary platform for preclinical testing, their development will play a major role in our basic understanding of life's design principles. Analogous to the *in vitro* reconstitution of subcellular processes, the iterative effort that leads to the synthetic reconstitution of multicell-type morphogenetic events will reveal the key components and subsystems necessary to generate such behaviors. Thus, one can only presume that these efforts will lead to a more complete understanding of how cells organize and stabilize within their surroundings and will at a minimum become a mainstay approach alongside standard reductionist and animal models to deepen our understanding of life.

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Forum

Compositional Dynamics: Defining the Fuzzy Cell

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Proteomic studies find many proteins in unexpected cellular locations. Can functional components of organelles be distinguished from biochemical artefacts or misguided cellular sorting? The clue might reside in compositional changes that follow biological challenges and that can be decoded by machine learning.

The Fuzzy Cell

Textbook views of cellular components, from protein complexes to organelles, follow the paradigm 'localization = function'. If a protein is found at a cellular location it also functions there. Consequently, the focus of organelle proteomics has been to get the localization right. For decades this was attempted by subcellular fractionation and by sorting out assumed contaminants. However, protein location may have other reasons than function: cellular components possess an intrinsic, compositional 'fuzziness'.

An often overlooked feature of subcellular organization is that it results from affinities and equilibria, in other words is quantitative and not qualitative. Membranes act as barriers but also need to be permeable. The nuclear envelope, for example, is permeable to proteins smaller than ~40 kDa. However, larger proteins might also make an uncontrolled entry into the nucleus, for example by having some affinity to the nuclear import machinery or at the end of mitosis, when the endoplasmic