Three-Dimensional Cell Culture Assays: Are They More Predictive of *In Vivo* Efficacy than 2D Monolayer Cell-Based Assays?

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Sitta Sittampalam: *Welcome to this roundtable discussion. I am a senior scientist and senior advisor to the director of the National Center for Advancing Translational Sciences (NCATS) at the National Institutes of Health (NIH). Previously, I was a faculty member at the University of Kansas Medical Center for 4 years, where I was doing research on circulating tumor cells and their relation to cancer stem cells.*

Before that, I was at Eli Lilly & Company for almost 24 years, where I held a variety of different positions, ending up as head of the lead optimization and screening lab at Lilly. I started a program in stem cell-based screening and became very interested in 3D cocultures and culturing of tissues.

Richard Eglen: I am the general manager at Corning Life Sciences. My background has been in drug discovery, in which I worked for 20 years at Roche, managing both the neuroscience drug discovery group and some of the high-throughput screening (HTS) activities. I then moved into businesses that were developing technologies for screening and for imaging. I worked for companies such as DiscoveRx and PerkinElmer, and now Corning Life Sciences.

Throughout that time, an emerging theme of my work has been cell-based assays, and particularly those that are used in HTS; more recently, the emphasis has been on primary cells and cells that are used in different sorts of cell culture, including 3D.

Jason Maynes: I am at the Hospital for Sick Children in Toronto, Canada. Clinically, I am a pediatric anesthesiologist and my doctorate is in physics. My research involves drug design, drug screening, and devising new HTS models, specifically around heart failure and cardiac disease, ensuring translation to patient care.

Kenneth Olden: My research has been in the area of cancer, and specifically development of antimetastatic agents. For several years, I was pharma director at the National Institute for Environmental Health Sciences at the NIH, and there we were very interested in developing toxicogenomic approaches to improve drug development and toxicity testing. I am now director of the National Center for Environmental Assessment at the Environmental Protection Agency.

Laura Schrader: I am president and CEO of 3D Biomatrix, and we make 3D hanging drop cell culture well plates in 96- and 384-well formats.

I joined the company just over 4 years ago to launch this technology from the University of Michigan and bring it to market. We have been very active on the 3D cell culture front working with researchers that focus on cancer, toxicity testing, and stem cell research in the drug discovery world, and also working with automation companies to integrate those technologies with 3D cell culture. Previously, I was a medical device consultant and worked with a lot of different equipment manufacturers in the medical device space.

Todd Shelper: I am a research fellow working in Prof. Avery's Discovery Biology lab at Griffith University in Brisbane, Australia. Our lab is primarily an HTS drug discovery lab, and recently we have been doing a lot of work with 3D cell cultures and trying to introduce this technology into our HTS campaigns.

Stephen Ferguson: I am a scientist with the National Toxicology Program at the NIEHS. My primary role is to incorporate more physiologically relevant *in vitro* models (in both 2D and 3D configurations) into our Tox21 Program to evaluate and, if possible, predict human responses to chemical exposure. Formerly, I led the ADME/tox R&D program at Life Technologies, where we focused on the development of primary liver cell models, HepaRG cell models, drug metabolism, drug–drug interaction, and *in vitro* toxicology research.

Marc Ferrer: I work at NCATS, NIH, and my career of 15 years has mostly been in HTS. I spent 10 years at an HTS site at Merck Research Laboratories in North Wales, and then did small molecule screening. In the last 5 years, I have been at NCATS doing HTS development and small molecule screening. Here we have developed an interest in 3D models, multicell types of models, and use of stem cells for drug development primarily.

Sitta Sittampalam: *I would like to begin our discussion with your impressions on the general usage of 3D cultures, as opposed to 2D in industry and academia, and various other settings in which you work.*

Marc Ferrer: Speaking for those of us at NCATS, our 3D work is, at this moment, very investigational. We do not use it for routine screening yet, and we are focusing mostly on tumor and cancer models. We have spent the last 2–3 years learning how to generate spheroid organoid cultures, trying to characterize them as much as we can, not only pharmacologically using a set of control compounds, but also morphologically in a mixed type of validation, and investigating which cells use spheroids and which do not, and why.

I would also like to point out that not all 3D models are spheroid models. We also had a project that involved a 3D layer type of model for studying cell adhesion in cancer that we were able to miniaturize to a 1536 HTS format for large-scale screening. So 3D models are not necessarily only organoid spheroid types of models, but could also be layers, sort of bioprinting types of models as well.

Richard Eglen: From our perspective, as we develop technologies in this area, and from the kind of laboratories we interface with, both in pharma and academia, the majority

of HTS assays are probably still undertaken in 2D. However, we are seeing a fairly rapid acceleration in terms of assays being done in 3D, particularly in the area of oncology screening.

I think if you could broaden the view of 3D cell culture to include the use of extracellular matrices, as well as synthetic polymers and spheroids, then the usage would be greater.

We have also seen several academic labs that are excited about this area because of the ability to do cellular assays in patient-specific culture systems. They are starting to see differential effects of compounds depending on the sourcing of the cells from patients. Taken together, I would say it is certainly increasing, and it is making rapid inroads, but it is nowhere near as widespread as 2D culture.

Sitta Sittampalam: *Would you say it has advanced more rapidly in the last 3–4 years?*

Richard Eglen: Yes, I would certainly say that the field has grown exponentially. From 2014 to 2019, the use of 3D culture is projected to triple, if not quadruple, based on industry estimates.

Kenneth Olden: My impression is that 3D culture models offer considerable promise, although I have not personally worked with them. The issue is, "Are they biologically relevant?" I am confident that they are going to have broad applications in drug development and toxicity testing. A couple of things still need to be worked out, such as scalability; they do not yet have the capacity to be put on high-throughput platforms.

Another concern is the cost of developing these 3D models versus the cost to do the studies in animal models.

Sitta Sittampalam: *Those are definitely important concerns, the accessibility, the cost, and the scalability. Also, something we are going to discuss a bit later is the validity of 3D models and how well they represent disease pathology.*

Laura Schrader: Regarding Ken's comments about scalability and use on a HTS platform, I think that is one of the challenges—and opportunities—that relate to the many different technologies emerging on the market right now for 3D; they all have different places along the process to fit in.

But there are products—ours being one of them—that were built specifically for creating 3D cell culture spheroids and cocultures that can be used in HTS applications, with 96- and 384-well plates. So they do exist, and the world needs to know about this. There has to be a plan to set up the right experiments using the right 3D path as they go forward through their process. What are the endpoints they want to answer, and those sorts of things?

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These types of products do exist, and they do not necessarily have to cost a lot. There are different levels of products available to achieve 3D with controllability. On the assay front, we have seen the market develop, and I would say that the majority of our scientists use assays on the spheroids that they grow on our plates and agree that this market is growing rather rapidly, and has grown since we launched over 4 years ago. If assays work in 2D, they can be optimized and tested for repeatability to achieve things in 3D.

Stephen Ferguson: Adding to what Dr. Olden said, I absolutely agree that one of the main drivers for moving toward 3D is an ability or a hope that these models will improve the physiological relevance, and support a broader biological space that better mimics tissue/organ function.

We are particularly interested in evaluating these models, developing them, and assessing the extent of "normal" biology modeled. For example, in our case, we are interested in models that, in the near term, improve the physiological relevance of *in vitro* liver models to support xenobiotic metabolism to evaluate chemicals and mechanisms associated with metabolically activated toxicity.

I also want to comment on the idea of cost. The general dogma is that 3D is going to be more expensive than 2D. I think that can be true, especially in a situation in which you are looking at cancer cells that oftentimes grow and proliferate without additional costs (except for the media/flasks). However, in my field, employing primary liver cells or HepaRG cells, these are quite expensive. Therefore, the opportunity to miniaturize with 3D (e.g., spheroid configurations) could actually improve their compatibility with and costs for screening.

Sitta Sittampalam: That is an interesting point because the primary cells are so expensive.

Stephen Ferguson: Yes.

Sitta Sittampalam: What Steve just said about the physiological relevance takes us directly into the second question about the predictive value of this compared to 2D versus in vivo. *It is a crucial question for* in vivo *efficacy and toxicity and is one of the biggest technical challenges, which is one of the other main topics we are going to discuss.*

Jason Maynes: I think I am the only physician on the panel, and I really have significant doubts as to the physiological relevance of these in the near term. I think you have to consider this in terms of two groups: oncology and then everything else.

Whereas the usefulness of these 3D models has been shown mostly in oncology-type assays, which makes sense because most cancers do not actually have a strong anatomy *per se*, and the toxicology or the drug efficacy in certain cancers is often associated with whether the drug can penetrate the cancer. So it makes sense then to look at the many sorts of disorganized spheroids or organoids, or even a monolayer with an endothelial cell underneath. But in terms of a more organized tissue and discovering therapies for preventive medicine or organ function, I do not think this is something that is going to happen in the next 10 years. It may be accelerating, but in terms of getting it to a point where it is used in drug screening, that is pretty tough.

We have collaborations with engineers where we are trying to develop a higher throughput way to measure cardiomyocyte contraction on a nanotube. In terms of scar formation or cardiomyocytes or any contractile cell, you can grow them on a 3D wire or tube and measure contractions.

But even with those examples, you are not really mimicking what the organ is doing. There is minimal cell–cell cooperation, and you are ignoring the different types of tissues that may be involved, or different types of cells that exist within a single tissue. So I have real doubts as to the short- to medium-term ability of any of these approaches to mimic true physiology and make a difference.

I think it is an important thing to evaluate, and certainly there is a lot of movement toward it. But I just do not see the relevance of it right now outside of oncology.

Sitta Sittampalam: When you say cell-to-cell contact, are you talking about extracellular matrices and the whole tissue context, and whether this can be generated outside the oncology platform in a relevant way?

Jason Maynes: Yes, in a way that adds something that we do not already have the ability to mimic in some other way. If you are going to develop a new technology, there certainly has to be a reason to develop it.

Sitta Sittampalam: Todd, do you have an opinion on whether 3D culture is any better than 2D and whether it is any more predictive of what occurs on the in vivo *side, even outside the area of oncology?*

Todd Shelper: Most of our screening, our routine screening, is still done using monolayer culture methods. But over the last 4–5 years, we have definitely been doing most of our assay development in 3D cell culture with cancer cell lines—either basic mono- or coculture 3D formation using Matrigel. Some of the technical challenges are miniaturization, trying to bring the costs down by moving to 384- and 1536-well plates, and a lot of work has gone into characterizing these types of assays. Once you get down to the 1536 level, the costs are actually

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significantly reduced and are comparable to the monolayer assays.

Sitta Sittampalam: Are you also doing 3D culture in 1536-well plates?

Todd Shelper: Yes, absolutely. With the cost of Matrigel, which we use for our 3D culture generation, at the 384-well level, the cost per data point or the cost per compound tested is significantly higher.

Stephen Ferguson: I think that the general concept that these sorts of 3D models will be able to fully mimic tissues and organs is probably, as previously stated, quite a long way away from being a reality. However, we do know that the 2D models can predict more apical endpoints related to drug metabolism and drug–drug interaction potential. The early evidence indicates that 3D and flow models more closely resemble *in vivo* function. For example, numerous reports have shown improved functionality with 3D cultures and flow cultures for xenobiotic metabolism competence more closely mimicking *in vivo* levels.

So I think that some properties are definitely improved by using these models. However, mimicking comprehensive tissue or organ function with these systems is definitely some time away.

Marc Ferrer: I would like to emphasize what Jason said about oncology versus nononcology. For oncology we can generate spheroids in 384 wells quite well now, and quite inexpensively. We can use the typical kinds of cell data Glo [CellTiter-Glo; Promega] types of readouts and measure the size of the spheroids quite well.

What I have noticed is that when we start screening compounds we see differences between 2D and 3D culture systems. But we do not really understand why, because we do not know whether the compound is not penetrating or whether there are changes in the signaling or the metabolism when you go into 3D, and whether that could be responsible. Are these differences what really happens *in vivo*, and is that why you lose the efficacy of the compound?

So that is one aspect we are focusing on, understanding the differences, why we see differences for 2D versus 3D. Investigating the biology of these spheres is one of our priorities.

One of the main technical challenges involves how you visualize these spheroids: with confocal, with nonconfocal, what penetration you get, what size spheres do you need to have relevance?

These are all questions that we still have and need to investigate before putting this into large-scale screening, because these factors will impact the predictability. You do not

want to be using a system for screening that is not going to be predictable. So how much do we need in a sphere to make it predictable? That is what we are trying to answer.

Richard Eglen: In terms of the use of 3D culture for metabolic liability testing, I think emerging data suggest that, in terms of compound screening and compound optimization in the liver field, the physiological relevance is improving. So this may be one area in which 3D cultures will be useful in the nearer term.

There is actually very little reported evidence on real differences in pharmacology when you compare 2D and 3D culture, although by the nature of 3D culture you get different wrapped assemblies of surface receptors. Consequently, you can get homodimerization versus heterodimerization, particularly in some cancer targets. And that will give different pharmacologies.

I also want to mention that in terms of providing disease models, there are now data being published showing that 3D cultures may make good models for Alzheimer's disease. So it may be that the field is starting to broaden in applicability beyond the oncology area. In fact, there is one group, as reported, that is now using 3D neuronal cultures to look at tau phosphorylation and inhibitors of that process.

Therefore, while it may be early days, my feeling is that it is on its way and is broadening beyond the bridgehead, if you like, of oncology screening.

Sitta Sittampalam: Okay, that is good to know. I think I remember somebody publishing something on 3D cultures/ neuronal cultures for Alzheimer's disease. I do not recall whether they are spheres, but I do remember something on the news.

Laura, based on your experience with customers, what technical challenges and issues related to validity and predictive capabilities are they looking at?

Laura Schrader: When I look at this question of whether 3D cell cultures are better predictors than what they are using now in large-scale 2D screens, a lot of our customers are still in the exploratory stages. We have large pharma customers that have said that it takes a little more time to set up 3D cultures, but you get much more robust and specific data that reveal a lot more information that may be missed in 2D because of the quantity and the quality of the data obtained from 3D cultures.

3D culture is never going to be the same as a human, but the whole goal is to make preclinical research as physiologically relevant as early as possible. At first we thought it would narrow down the number of hits in secondary screening. We learned it also makes the hits stand out more because of the

additional data. So those are the targets that are beginning to move forward, and the companies are moving them forward with greater confidence, just based on the greater depth of data attained.

While it may still be a few years before the use of 3D cultures in HTS becomes common practice, in terms of 3D being more predictable than 2D, we are getting good indications that this is the case.

Sitta Sittampalam: *Do you see it being applied mostly in oncology?*

Laura Schrader: It is largely oncology, yes, and toxicology. It is also growing more and more in stem cell differentiation. Because there is no plastic for the stem cells to adhere to, they like that.

Kenneth Olden: With the fact that it is mostly applied in oncology now, is that simply a reflection of the community's interest in developing these models for cancer systems? Diseases, all of them, are organ-specific, and these 3D cultures maintain differentiated states. So I do not see why they would not have relevance to other diseases as well. And one could certainly monitor the biomarkers to make sure that the differentiated state is either maintained or induced.

Marc Ferrer: I think for other diseases it is technically more challenging. I think it is easier to make a spheroid; you just need a round-bottomed ULA [ultra-low attachment] plate. But for other diseases, the geometry of the culture and how the cells are organized is more critical. For that you go into sort of the bioprinting world, and it is very early, technically challenging days for that, but is another direction for 3D cultures in the future. Not all 3D cultures are spheres.

Richard Eglen: Just to pick up on what Laura mentioned about the increasing use of stem cells. They appear to grow better and differentiate best in a 3D environment. If you think about the increasing adoption of stem cells in drug discovery, then maybe its adoption is coinciding with the increasing adoption of 3D culture as well. As those two technologies come together in lead optimization and disease modeling, they probably will ultimately find their way into HTS as well.

Sitta Sittampalam: *Before I go on to the next questions, I want to ask any or all of you whether you have looked at penetration of molecules, small molecules, into 3D cultures in a systematic way. Promega, for example, is putting out assays for 3D cultures that have very special types of Glo detergents so the dyes can penetrate.*

This is an issue on the detection end of the business. I saw an article in Cell *recently in which the researchers incubated so-called tumor organoids with a drug for 7 days. Have any of you studied drug penetration and diffusion into these tumors? That would be one of the big technical challenges.*

Laura Schrader: From a commercial standpoint and based on what we hear from our customers, being able to control the size of the spheroids is an important factor in being able to read inside of them. The technology exists to be able to read inside of them with high-content analysis, but we have customers that are able to create spheroids that are 200 microns, or grow them even bigger to get a necrotic core on purpose.

And they use a lot of different kinds of assays. CellTiter *Glo* works, and they improved it for 3D, as does Alamar Blue. There are a lot of existing assays that have been optimized and tested for spheroid reading and they work as well.

Stephen Ferguson: I would say that, in general, even 2D cultures have been underserved with regard to understanding the amount of compound accumulating inside the cells. But for 3D cultures, many factors, including increased surface area, ratios of compound to cellular biomass, and other factors, may play important roles in our ability to relate *in vitro* responses to *in vivo*. Our lab is interested in exploring these approaches to add context to *in vitro* toxicology data.

In the near term we have begun looking at high-content imaging approaches such as cholyl-lysyl-fluorescein (CLF), which actually is reported to be a BSEP [bile salt export pump] substrate in liver, an efflux transporter on the canalicular membrane. What we see is that the spheroids take up the CLF and transport it to canalicular networks that formed over time in culture within the spheroids. I think there may be other articles in the last few years that have shown similar data.

I believe there is sufficient evidence to show that high-quality 2D and 3D liver models are not cholestatic, as some have suggested, but actually have a form of cellular circulation including uptake transport and biliary efflux into canalicular pockets. However, the kinetics, resulting accumulations/disposition, and dependence on size and media composition need to be further explored with 3D models.

Sitta Sittampalam: *How big are your 3D structures?*

Stephen Ferguson: We have used different sizes, but the ones that we are most intrigued by so far actually are quite small. They are only 1000 cells, so probably 100–150 microns.

Todd Shelper: We have performed studies looking at standard chemotherapy agents and penetration through some of our

pancreatic cancer and breast cancer 3D cultures. Looking at doxorubicin with high-content imaging, you can see the levels of penetration through different-sized spheroids.

Sitta Sittampalam: *How big were the spheroids, the biggest?*

Todd Shelper: They ranged from 100 to 500 microns, but with confocal microscopy you start to reach the limits of what you can actually penetrate through with the dyes and the lasers we are using.

Marc Ferrer: Did you see a difference in the types of cancer? We have seen that some types of cancer form very tight spheres, whereas others do not form as tight spheres, regardless of how much ECM they secrete. That is something we are exploring further because you might have penetration for one cell type but not for the other ones.

Todd Shelper: With the tightly packed 3D structures that have a lot of cell-to-cell contact, we saw fairly similar penetration levels as those of the more loosely packed cell types. It seemed to be independent of where the cells originated from.

Richard Eglen: Several authors have looked at the pharmacology of compounds in hepatocytes cultured in 2D versus 3D, and here you can see a frame shift to the right in 3D, which probably more accurately reflects the potency of compounds. That may be a penetration issue, but it may also be that the cells are performing as they do *in vivo.*

I would also mention cell migration assays, and the fact that they can be done with these models as well. As you start to image the cells migrating into the organoid or the spheroid, then you can get really appropriate pharmacology compared to what occurs *in vivo.* Those are surrogate measures, and not exact measures of compound penetration, but there are pharmacological outcomes that can be measured.

Sitta Sittampalam: *Okay, thank you. I am now going to move on and talk about 3D multicellular cocultures—multiple cell types in a 3D structure versus organoids, which are essentially miniature organs.*

In the same article in Cell *that I mentioned earlier, the researchers were taking human biopsies from colon cancer and from normal colon about 10 cm away from the same patient, and they were growing the samples and calling it an organoid and doing drug testing.*

The two questions I would like us to discuss, which are related in many ways, are as follows: Are validated multicellular 3D cocultures representing disease pathology readily available? And is growing 3D organoids from diseased tissue a superior approach, compared to 3D cocultures developed from cell lines or primary cells?

Laura Schrader: I can discuss this from a more commercial standpoint and some of the current ideas around 3D cell culture. First, cocultures are easier to attain and use than are organoids. But the main point is that they are both relevant.

Organoids are probably more costly, especially if they are coming directly from a human biopsy. They are comprised of more costly and valuable cells, and are very intricate, valuable technology. But this is a good example of how there is not one "silver bullet" 3D platform.

Various 3D methods can help you accomplish different results at different stages. So, if we are working to discover therapies in 3D human tissue, then research should start as early as possible using more simple 3D platforms, like our Hanging Drop Plates, then moving those promising targets forward for greater scrutiny in more sophisticated 3D environments like organoids. All of this, of course, precedes moving into more extensive and expensive animal studies.

The exploratory work some of our customers in biotech and pharma companies are doing is focused on trying to figure out the best path to achieve physiological relevance as early as possible. I think all of the 3D technologies have a certain place, depending on the type of research, and organoids are certainly sophisticated tools to use.

Sitta Sittampalam: *Jason, what do you think? You had some concerns about how long this is all going to take?*

Jason Maynes: I think a lot of this depends on what disease you want to look at and what system you want to model. As Laura said, there is not going to be one solution.

I do worry a little bit about the comment that the 3D cultures give better results. I think they currently give different results compared with 2D cultures, but is that better or just different? And that is a challenge you need to be aware of, because you may work with organoids or with a coculture and you may get two different results. There is no evidence that one is right and one is wrong. They are just different.

Marc Ferrer: I agree, Jason.

Jason Maynes: Until you go back and validate in an *in vivo* system and determine which worked and which did not, then you cannot be sure that either of them is relevant. They are just different. The closer you get to the organ, the better, but you are limited by thickness and nutrient flow and other factors; at this particular time, the main issue is validity.

Whichever of those two methods ends up giving you the better model of your *in vivo* system, then that is the right answer to pick. But you have to validate it. You cannot say it is

better just because it gives different results. What it will take is a few of these systems to be developed, utilized, discovered, and validated before we will really know which of them should be used more broadly.

Sitta Sittampalam: Yes, I agree.

Marc Ferrer: I agree with Jason, and I think one of the things that is hard to find is the sort of positive indicators—the compounds or the treatments that you can use to validate these models. Ideally, those would be compounds that work in the clinic, or maybe they did not work in the clinic but they worked in 2D.

What profile of compound do you use to validate and benchmark these models? What sort of compound will you use to say, "Yeah, now this is a predictive model." You have to have the clinical data or *in vivo* data to use as a benchmark, and sometimes getting that data is not easy.

Sitta Sittampalam: It is not easy, but then you can take your existing drugs and try to validate them and go into an in vivo *model and try to see whether all of them correlate.*

Marc Ferrer: But you have to go into the clinic and say, "Okay, this compound worked in a xenograft, but failed in the clinic," or "This compound, where did it fail, why did it fail?," or "This one worked, and why did it work?" And we have compounds that actually work in the clinic, but did not work in a 2D culture. Can we use those to validate or develop a 3D culture, and will that 3D culture be predictive?

Stephen Ferguson: I agree with what Jason said as well on a lot of fronts. We know that as you remove primary hepatocytes from the tissue they can rapidly dedifferentiate in terms of xenobiotic metabolism competence. When you put these cells in 3D and/or dynamic flow contexts, they often far exceed their 2D thresholds of metabolic competence and regain levels more comparable to their initial *in vivo* levels.

The way we view it, the closer an *in vitro* liver model can mimic the metabolic competence found in cells directly derived from liver, the better chance we are going to have to model normal liver metabolism.

Sitta Sittampalam: Do any of you think that this is in the context of tumor 3D versus other organs? Are there some organs that are much more difficult than other organs? I think, Steve, the liver is one of the organs that some companies are going after right now to make 3D tissue.

Stephen Ferguson: At this point my perception is that for tissues with more independent functionality, they are going to

have a better chance. However, when you try to model organs that are more interactively dependent on other organs/systems for "normal" function, it may limit our ability to effectively model these organs/tissues without integrating these systems in some sort of systemic flow.

Richard Eglen: To add to what Steve was saying, you can make organoids from a range of tissues: intestinal, retinal, all the way through to even neuronal tissue. But they require the characteristics of the tissue, not just colocalization of the cells, but also flow, removal of waste products, delivery of metabolic products, etc. That is likely the reason why the liver physiology has advanced.

Sitta Sittampalam: In that context, Richard, most of you probably know about the tissue chip program that NCAT funds at multiple sites. DARPA [Defense Advanced Research Projects Agency], FDA [Food and Drug Administration], and the NIH also fund this program, in which they are going to put various 3D organs onto a tissue chip and integrate them as a human-on-a-chip. Essentially, it is a 10- or 15-year project, if it can be done at all. But it is really a moon shot. I just wanted to mention that in the context of this discussion.

Before we conclude, do any of you have questions for each other?

Kenneth Olden: There is one point I would like to bring up and that is getting regulatory bodies to adopt these systems. What effort has been made to inform regulatory bodies of the pros and cons and strengths and weaknesses of these systems as they are being developed? I think that is going to be very important. We do not want to develop a technology and have regulatory bodies be reluctant or slow in accepting them.

Sitta Sittampalam: I think that is a very, very important point.

Kenneth Olden: When we developed the toxicogenomic center in NIEHS, we got the National Academy of Sciences to develop a roundtable and bring industry and environmental groups and regulatory bodies together to discuss the science so they would understand it and there would not be rejections out of ignorance..

Laura Schrader: There is also the Center for Responsible Science, or CRS. They are a group that focuses on streamlining drug and device development to get safer and more effective products out to patients faster in a less costly manner. I know that they are working on some ways to approach the FDA to include emerging technologies as a part of the regulations, including for preclinical stages. This is more for validation. So I think there is such an effort underway.

Jason Maynes: I think the FDA is certainly open to technology like this. We interface with the CIPA [Comprehensive In Vitro Proarrhythmia Assay] Initiative, which is the cardiac safety committee at the FDA, and HESI [Health and Environmental Sciences Institute], and so forth. They are certainly willing to look at new models for preclinical cardiac toxicity, and the FDA has shown an ability to apply that evidence for phase I, and to be open to looking at other modalities.

Richard Eglen: Marc mentioned bioprinting a couple of times, and I was wondering what people on the line think of that and how close the field is to being used in these kinds of areas?

Stephen Ferguson: We have a small collaboration going on with a bioprinting group. I think there is a lot of excitement around the way that they can essentially print vasculature. The challenge that I see now is combining cells to more closely mimic tissue architecture (e.g., liver lobular structures). This should drive these models toward more physiologically relevant function.

Jason Maynes: We have a couple of collaborations with biomedical engineering groups in which we do both Inkjet, spin printing, and also, as I said, the biowires, where we spit out the cells onto wires. I agree that the architecture is not there yet, but at least we can generate defined layers of cells that seem to interact with each other and maintain that architecture. So the technology is getting there, but it's a long way from high throughput.

Richard Eglen: Yes, I agree.

Sitta Sittampalam: Marc and I are working on bioprinting as well. We are focusing on retina and skin with the National Eye Institute and also with the National Institute of Arthritis and Musculoskeletal and Skin Diseases.

These structures have very special architecture, very special layers, and we are experimenting with bioprinting those structures. Although the technology is very early right now, in my opinion, it is worth looking at.

Laura Schrader: There is a question I would like to throw out to this dynamic group. As we have been going through this over the last few years, there have been questions about what it is going to take to make researchers think that 3D works. Jason touched on the fact that you absolutely have to have a model and validate that it has the expressions that are present

in vivo. Does this group have any comments or thoughts about what it will take for 3D to become the norm?

Stephen Ferguson: I think successful systems will need to effectively model an important subset of "normal" biology to warrant adoption. If you are modeling some bit of normal biology that is relevant, then I think that will have a stronger opportunity for adoption with these emerging *in vitro* models.

Todd Shelper: I think if you could find a hit compound or a lead compound that was identified in 3D but not found in a 2D system and then made it all the way through the drug discovery pipeline, that might provide strong evidence of its value.

Sitta Sittampalam: I agree. That is the same kind of challenge HTS had in the early days. So it is very similar.

I would like to thank you all very much for participating in this discussion. In summary, I think we agree that this field is still exploratory, but there is a rapid awareness in the academic, pharmaceutical, and biotech worlds of 3D technology. Cost and accessibility are things that people have to think about. Another important point that was brought out is that 3D cultures are currently much more prevalent in the oncology world and other areas are maybe a little bit behind because of the technical challenges in producing other 3D tissues.

The validity of the system has to be still proven in multiple ways—2D versus 3D versus in vivo—and the proof of validity is physiological relevance. How good is the model physiologically, how well can it mimic what is happening in vivo, whether it is a 3D coculture or a 3D organoid, and how well can we use that data?

Then there are obviously analytical challenges in measuring some of these activities in 3D constructs. We also discussed the use of bioprinting, the use of stem cells to enhance the formation of 3D cultures, and about cocultures versus organoids. Another important point was whether a different result seen in 3D versus 2D is a better result, or just a different result. I think the jury is still out on that one. Finally, many of you pointed out that the regulatory bodies need to adapt this, and there are some efforts going on through the Center for Responsible Science and some discussions with the FDA, specifically in the cardiac preclinical arena.

In terms of costs, eventually the costs of these systems will probably come down. Primary cells are still quite expensive and are much more difficult to use.

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