Ask the experts: current obstacles and future developments in cell culture

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In this 'Ask the experts' feature, a panel of international experts share their perspective on current obstacles and future developments in cell culture.

Meet the experts

Oren Ben-Yosef

Oren Ben-Yosef joined Biological Industries (Israel) in 2016 as a Technical Support Specialist. Ben-Yosef has a BSc in Biotechnology Engineering and a PhD in Medical Sciences from the Technion Israel Institute of Technology (Israel). His previous research focused on embryonic stem cell (ESC), and induced pluripotent stem cell (iPSC) culturing methods and differentiation.

Jorge S Burns

Jorge S Burns is Project Coordinator for the GRABTOP Project, which aims to develop a novel graphene biosensor testing osteogenic potency for capturing best stem cell performance for regenerative medicine. He is part of the Faculty of Medical Engineering at University Politehnica of Bucharest (Romania).

Justin Colacino

Justin Colacino is the John G. Searle Assistant Professor of Environmental Health Sciences in the School of Public Health, University of Michigan (MI, USA). His research focuses on understanding environmental and dietary factors in carcinogenesis and cancer prevention. Specifically, the goal of his research is to characterize the environmental susceptibility of normal human stem cell populations, elucidating the etiology of sporadic cancers. His research group combines wet lab bench work, and bioinformatic and statistical analysis of large scale genomic and epidemiologic datasets to translate findings from *in vitro* models to the population level.

Daniel J Kota

Daniel J Kota is an Associate Scientist and Director of Emory Personalized Immunotherapy Core (EPIC; Emory University, GA, USA). Native of Brazil, Kota received his PhD in medical sciences, completed a postdoctoral training in neurotrauma and ran a research program as faculty, unlocking the therapeutic properties of adult stem cells across the USA at the Burnham Institute (CA, USA), Tulane University (LA, USA), Texas A&M University, the University of Texas (both TX, USA) and Sanford Health (SD, USA). Kota has a passion for translational science, oversees the day-to-day operations of EPIC and is actively involved in the scientific aspects of every project from concept to execution.

Bruno Péault (University of California at Los Angeles, CA, USA)

Bruno Péault is Professor and Chair at the University of Edinburgh (UK), Center for Vascular Science and MRC Center for Regenerative Medicine (UK), and Professor of Orthopedic Surgery at the University of California at Los Angeles (UCLA; CA, USA). Péault's research includes developmental hematopoiesis as well the identification, purification and characterization of diverse classes of stem cells present in adult organs. Prior to joining UCLA and the University of Edinburgh, Péault served as a Professor of Pediatrics and co-director of the Stem Cell Research Center at the Children's Hospital of Pittsburgh (PA, USA), as research director at the Centre National de la Recherche Scientifique (France) and department head at the Institut National de la Santé et de la Recherche Médicale (France).

How have cell culture needs changed since you began working in this field?

Oren Ben-Yosef

I think it depends who you ask. Researchers and labs will tell you that the needs are the same – good looking cells, or colonies that expand indefinitely and differentiate to whatever somatic cell or cells they are researching. The industry, the pharma companies and everybody who is doing clinical applications will tell you that the needs have changed drastically; more regulatory requirements call for more defined culture conditions and the cells must be expanded better. Time is money and a shorter culture time to get the same amount of cells, or more cells, saves money.

Jorge Burns

When I began working in the field, it was a genuinely *in vitro* world. To address needs of convenience and safety, this was replaced by disposable plastic cell culture. Over the last three decades, increased sophistication has greatly improved primary human cell culture. Lessons learned from immortal human cancer cells are now being tailored for a much wider range of cell and tissue types. This in turn has revolutionized our understanding of pathological processes in many previously untreatable diseases. In particular, appreciation that tissue specific stem cells may serve as therapeutic agents has driven huge expansion and progress across academic, industrial and clinical sectors. Multidisciplinary collaboration is now fundamental.

Justin Colacino

The field has changed dramatically since I first started performing cell culture back in 2005. There has been an explosion of methods to grow patient-derived tissues in three dimensions. We can now generate iPSCs from anyone, and then use directed differentiation in a dish to push these cells into an expanding array of cell types. Advances in microfabrication now allow us to better mimic the physical environment within a tissue and to even model how multiple tissues communicate. On top of this, agencies such as the US Environmental Protection Agency (USA) and the National Institutes of Health (UK) are making concerted efforts to replace animal experiments with well-designed and validated *in vitro* experiments. In short, it is an exciting time to be doing cell culture.

Daniel Kota

There has been a significant increase in demand for consumables, a proliferation of good manufacturing practice facilities and multiple attempts to solve large-scale manufacturing. This has all been triggered by the 'discovery' of stem cells, such as mesenchymal stem/stromal cells (MSCs), ESCs, iPSCs, along with tissue-specific stem cells including neural or adipose cells, combined with the most recent excitement caused by CAR T, NK cells and dendritic cells for immunotherapy applications. These cells are being used in both basic and clinical research, at small and large manufacturing scale worldwide. Hence, it is not unusual to experience backorders of nutrient media and plasticware. In addition, the price of serum has seen an increase in 1000% for the past 10 years. Human platelet lysates and chemically defined media, although increasing in availability, offer the same high prices.

There is also a need for a hybrid workforce that combines cell culture, regulatory and clinical knowledge that neither laboratory technicians nor medical technologists alone offer, and there is still no consensus on the optimal bioreactor or large-scale technology for a lot of cell types, including MSCs.

Bruno Péault

I cannot say that our actual needs have much changed. Generally speaking, we culture cells either to ‘expand’ them or to study their biology in a relatively simple setting. Even though the cells we are studying have diversified dramatically, what we expect from cell culture remains essentially the same.

There are however some evolutions such as cell culture under different oxygen concentrations, which has exploded in recent years. It has been discovered that culturing cells in lower oxygen concentrations, such as 5% and even 2%, dramatically changes their behavior and properties. The reason is that genes encoding proteins named hypoxia inducible factors are activated under lower oxygen concentration. Changing cell properties under hypoxia is not a mere culture artefact though, but reflects the fact that, depending on the extent of vascularization, some tissues in the body are naturally hypoxic. A whole area of pathobiologic research tries to understand how oxygen concentration regulates cell function, which is of high relevance in organ development and regeneration, as well as cancer.

In another perspective, cell culture is increasingly combined with biomaterial science to determine optimal cell survival and growth conditions, in two and three dimensions. To this aim, cells are cultivated in the presence of adhesion molecules, synthetic matrices and scaffolds. Beyond basic biology, this research aims to determine optimal ‘packaging’ conditions for therapeutic cells to be transplanted into patients.

With the important role that cell culture plays in the approval process of cell therapy, how can developers simplify this process?

Oren Ben-Yosef

It is important to work from the ground up, have a good regulatory team in place, including quality assurance and regulatory affairs, and obviously work with the highest order suppliers, so all the raw materials are defined, known and approved. For instance, Biological Industries has media that have a Drug Master File (DMF), submitted to the US FDA and Health Canada, that contains detailed information about the ingredients, manufacturing facility and procedures involved in the production of a specific product. The process of registering a new cell therapy product with the FDA is significantly shortened when utilizing a product with a DMF. It also demonstrates to the consumer that a company that manufactures such a product conducts its manufacturing operations in a transparent and correct manner and complies with quality and safety requirements.

Jorge Burns

Simplification is key to the broad adoption of cell culture conditions and practical transferability from academic to clinical scenarios in a cost-effective manner. At the same time, simplification must not undermine appropriate mimicry of *in vivo* conditions. Compromises are perhaps inevitable, but advances in microfluidics, new material nanotechnology and improved biomarker discoveries are conspiring to increase the choices available for changing and controlling cell micro-environments. Despite many challenges, those novel options allow an optimistic outlook.

Justin Colacino

An area where cell culture product developers can have a significant impact in the rigor and reproducibility of the field is in developing media and other culture components which are fully-defined. Many of the products we use in our work are biologically-derived, and thus susceptible to significant lot-to-lot, and thus experiment-to-experiment, variation. By developing fully-defined cell culture reagents, product developers can help reduce this variation and improve reproducibility across multiple laboratories.

Daniel Kota

Developers have already begun and must continue to work with manufacturing sites on personalized media that come ready-to-use. The reduction of reagent amalgamation and media changes that cell processing facilities have to perform not only decreases contamination risks and human error, but also alleviates the regulatory burden. It should be noted that the latter should always be in mind when developing any cell culture reagent.

Bruno Péault

Culture complicates approval by regulatory agencies. The culture process is however generally rather simple and highly standardized. Cell cultivators just have to bear with regulations and I don’t really see many ways around this. It is however sometimes possible and more original to simply bypass the culture step. This is one of our strategies,

using selected native ‘unexpanded’ stem cells. Contrary to conventional thinking, you do not necessarily need to ‘expand’ stem cells in culture prior to therapeutic use. If you have the right stem cells you do not need lots of them, although this does not apply in all instances. You remain, of course, dependent on culture if you are using pluripotent cells, such as ESCs, iPSCs or conventional MSCs, which are, in essence, selected by the culture process.

What is the biggest obstacle remaining in the field?

Oren Ben-Yosef

The biggest obstacle, albeit necessary, is the regulations in place for cellular research, and clinical research in particular. Regulations are placed for a reason, and a good one at that. They might hinder some projects, but if your research is robust enough, and if you have a good support from your suppliers, it becomes less of an obstacle and more a hurdle that shows that you are well on the way to achieve what you want – good, consistent research that can benefit mankind.

For example, as a manufacturer for the life science industry, Biological Industries is fully committed to the Current Good Manufacturing Practices comprising applicable local law and international guidelines adopted by the FDA, as well as the European Medicines Agency. Our quality systems also comply with the international standards for Quality Management Systems as defined by the ISO 13485:2016 and are routinely audited by an accredited external body. Our manufacturing facility is an ISO 9001:2015 and ISO 13485:2016 certified facility and our controlled environment clean rooms are graded from ISO 8 up to ISO 5 (classified from class 100,000 to 100).

Jorge Burns

An understandable focus on isolation and maintenance of pure cell cultures, often expanded as monolayers, is being superseded by a growing need to understand cell–cell interactions between different cell types, also within organoid tissue-like contexts. Vascular development, cell homing, extracellular vesicle mediated intercellular communication and appreciation of mitochondrial homeostasis are areas of research that will require complex analysis. Fortunately, progress in instrumentation and technological know-how including label-free and single-cell analysis will facilitate steady progress. Ultimately, accurate measurements and identification of key active components will lead to approved potency assays that are critical for designating the quality of a cell product for therapeutic use.

Justin Colacino

I think the biggest obstacle that we currently are facing is how to accurately model the full diversity of cell types within a tissue and their interactions. In our lab, our cultures typically have, at most, two to three different major cell types present. We know, especially in cancer, that the stromal fraction of the microenvironment plays a huge role. Capturing these interactions in a physiologically relevant way remains a major challenge for us.

Daniel Kota

The biggest challenge remains the translation from bench to bedside. It is not possible for a research lab to undertake the costs of large-scale manufacturing. Therefore, most of it is done offsite or by private companies and ended up ‘lost in translation’ simply because the nuances and conditions seen in the laboratory cannot be fully replicated. Many basic studies are performed with fetal bovine serum, which is not used in patients. The use of the patient’s plasma, platelet lysate or chemically defined media may never replicate the specific characteristics observed on the bench or animal models. Likewise, the use of bioreactors may alter cells that were originally cultured in flasks. We know that shear stress can change the properties of MSCs when compared to static cultures [1], along with doubling time and confluence. Again, it comes down to a lack of defined mechanisms, but also possible complications and side effects that may originate from large-scale manufacturing that are often overlooked.

Bruno Péault

I would not call it an obstacle but more a complication or bias which is too often overlooked. This is the fact is that culture dramatically changes gene expression in cells. Some cells, like MSCs, have long been characterized exclusively as cultured cells. Now that we know what innate MSCs are within tissues, we can make a direct comparison and we have realized that MSCs are dramatically modified by culture. MSCs are the most commonly used human stem cells in the clinic, with more than 800 trials to date. Many users assume that cultured MSCs are exact replicas of native, tissue resident MSCs, which is wrong. Quantitatively, as well as qualitatively, MSCs are largely a product

of *in vitro* culture. This is of course likely applicable to other cultured cells. This is why culture assays often cannot replace *in vivo* experiments.

What is the best/most effective technology innovation you've seen in the last 5 years?

Oren Ben-Yosef

It is not a 'technology' *per se*, but I think that, finally, researchers have started looking more at the 'macro' rather than the 'micro'. Organoids, a popular research method in recent years, are 3D *in vitro* grown structures derived from pluripotent stem cells (PSCs) or adult stem cells that self-organize into near-native microanatomy with organ-specific differentiated cell types and tissue compartmentalization. The reason I think this is the most effective technology is that you can't take a cell population out of context, for example it is native tissue or organ, and expect it to behave as it does *in vivo* or *in situ*. Therefore, creating the microenvironment of the organoid is far more indicative to PSC research than any changes in incubators.

Jorge Burns

Many technological innovations emerging in the last five years have a long history in the making. What is most significant is that cells have been approved as therapeutic agents and supporting technologies have become more refined. Significant advances in imaging technology have improved diagnosis and our ability to track therapeutically applied cells. Use of theranostics when monitoring new therapeutic interventions will help accelerate clinical trials. If pressed to describe the best/most effective technological innovation in the last 5 years, then advances in immunology are clearly outstanding.

Justin Colacino

While it is not an innovation that is specific to cell culture, the ability to comprehensively profile large population of single cells using unbiased analyses is, I believe, pushing our field forward more than any other. In our own work, we are using single cell RNA-sequencing to map our primary human cultures and have uncovered new and exciting biology to pursue. These studies are informing our *in vitro* work by giving us a comprehensive catalog of the cell types and phenotypes in our cultures. We can now understand, in exquisite detail, the effects of altering media composition or exposing the cells to an environmental stressor, on a cell type specific basis.

Daniel Kota

Any technology that automates cell isolation, such as Sepax Technologies, Inc. (DE, USA), processing, assessment, for example cell counters or benchtop flow cytometry, or storage, such as CellSeal from Cook Regentec (IN, USA), are improved technologies.

Bruno Péault

I may have missed something but in our areas of investigation I am not aware of any major recent innovation as regards cell culture. At least with regard to basic research, cell culture has changed amazingly little. If we go back 25 years, for instance, we used the same incubators, the same plasticware, the same media and the same antibiotics, and we were equally dependent on the use of animal serum. If you compare with the evolution of molecular biology techniques, for example, the difference is absolutely striking.

How do you think cell culture processes, protocols & products will evolve in the next 10 years?

Oren Ben-Yosef

In one word: suspension. Clinical applications require more and more cells to be effective. While differentiation protocols are regularly refined, generally, the more cells you have, the more end product you get, be it other cell types, secreted proteins, vaccines etc. There is a lot of work being done to adapt PSC and MSC culture to 3D culture conditions, which include better suited media, microcarriers and/or hydrogels, organoid and aggregates formation, better suited bioreactors and many other advances happening right now, all with the end goal of getting more cells each passage while maintaining their characteristics and markers.

Jorge Burns

Cell culture processes, protocols and products are going to be increasingly influenced by information and communication technology and the elephant in the room is how data handling will need to be controlled and regulated

within increasingly knowledge-based economies. Regulations regarding privacy of information will impinge upon laboratory activities as operating procedures become increasingly standardized.

Accordingly, there will be a period requiring increased investment in robust biomarker discovery and biosensor design to improve monitoring and quality control. However, key to implementation will be focus on efficiency, sustainability and cost reductions for broad use. Over the coming years, bioreactors and perhaps implementation of robotics guided by artificial intelligence may become key elements of industrial scale processes that bring cell-based therapeutics to a demographically growing market. Novel products that specifically activate and regulate innate stem cells for improved healing are also likely to emerge.

Justin Colacino

I expect that the next 10 years will see an explosion in the number of available patient-derived organoids in biobanks. I hope that these tissues will be collected from epidemiologically characterized individuals who, in aggregate, better represent human genetic diversity. I expect that these organoids will allow us to make significant progress in truly understanding the biology of gene–environment or gene–pharmaceutical interactions. I also expect that we'll see significant improvement in 3D models of the tissue microenvironment through improved culture protocols, medias and matrix development.

Finally, I suspect that we'll see dramatic democratization of the 'organ-on-a-chip' methods through increased access to microfabrication facilities and 3D printers. Compared to the organoid models our lab typically uses, the culture conditions for 'organs-on-a-chip' are complex, as they require culture conditions that are simultaneously suitable for multiple cell/tissue types. These conditions can range from differences in growth matrix, to media formulation, to oxygen concentration. As we overcome these challenges, however, we will be closer than ever to accurately modeling human physiology in a tissue culture dish.

Daniel Kota

In the next 10 years, it is safe to say every major hospital will have its own cell manufacturing facility since cell therapy will be extremely personalized and the technology to manufacture cells will be available to all. We will see culture media that are personalized for each cell application, with ready-to-use, off-the-shelf products that will minimize preparation and media change. Finally, automated, closed systems will become more available and the current need for trained workforce will be alleviated.

Bruno Péault

It is hoped that evolution will be seen in 3D culture systems to mimic more closely tissue structure. On the other hand, a major challenge has been, for decades, to maintain 'natural' stem cells such as hematopoietic stem cells in an undifferentiated state *in vitro*. Progress in this direction would represent a major breakthrough.

Disclosures

The opinions expressed in this interview are those of interviewees and do not necessarily reflect the views of Future Medicine Ltd.

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References

1. Diaz MF, Vaidya AB, Evans SM *et al.* Biomechanical forces promote immune regulatory function of bone marrow mesenchymal stromal cells. *Stem Cells*. 35(5), 1259–1272 (2017).