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MEETING REVIEW

Once upon a dish: the next frontier in engineering multicellular systems

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ABSTRACT

In June 2022, the second meeting on 'Engineering Multicellular Systems', organized by the European Molecular Biology Laboratory and the Institute of Bioengineering of Catalonia, took place in Barcelona. Stem cell and systems biologists, physicists and engineers from all over the world gathered to discuss how recent breakthroughs in organoid technologies, engineering and mechanobiology are boosting our understanding of early morphogenesis, organogenesis and organ function with applications in tissue engineering, disease modeling and drug screening. The meeting was organized with sustainability in mind, and included an ethics session and an outreach public activity.

Introduction

The second EMBL-IBEC conference, held in Barcelona in June 2022, involved stimulating discussions on a very diverse range of topics with a central focus on 'Engineering Multicellular Systems'. The second meeting was organized and opened by Xavier Trepat [Institute of Bioengineering of Catalonia (IBEC), Barcelona, Spain] and James Sharpe [European Molecular Biology Laboratory (EMBL), Barcelona, Spain]. Vikas Trivedi from EMBL had a key role in organizing the meeting with co-organizers Miki Ebisuya and Kristina Haase (both from EMBL, Barcelona, Spain), as well as Nuria Montserrat and Josep Samitier (both from IBEC, Barcelona, Spain). The conference took place at the Barcelona Biomedical Research Park (BBRP), which is a unique creative hub of different biomedical sciences in Barcelona. The idyllic location of the BBRP building, with fantastic views of the Mediterranean Sea, inspired many lively discussions during the coffee breaks where people met in person after more than 2 years of virtual meetings. As this was the second meeting, with the previous one having taken place in February 2020 (Haase and Freedman, 2020) – just before the start of the pandemic – James Sharpe noted in his opening speech how innocent we all were back then, not thinking that, as in a Hollywood movie, the world would change from one day to the next due to a new virus.

From the scientific perspective, the meeting covered a broad range of topics, all of them around the central question of how functional organs and complex organisms emerge from the interaction of cells and lower-order structures. This question is not new; it has been a central question in developmental biology for centuries. Our avid hunt to understand how we are formed from a tiny embryo goes back to Hippocrates (460-370 BC) and Aristotle (384-322 BC). Aristotle studied embryos by opening up bird eggs and dissecting them to observe their different developmental stages. Back then, he could only inspect to try to understand how life emerges. Today, we know that embryos are composed of cells that have an intrinsic ability to self-organize into complex tissues and organs, with intricate architectures and specific functions. For almost a century, several model organisms have been used to understand these processes mainly using genetic approaches. However, in the past few decades, our understanding of organ development and maintenance, combined with our ability to grow pluripotent and adult stem cells, have revolutionized our potential to build tissues and organ-like structures *ex vivo* using bottom-up and top-down approaches. The advantage of the *in vitro* models is that they allow experimentation under a well-defined controlled environment where cells can be captured with spatiotemporal resolution. In the case of human tissues, this is crucial due to technological challenges and ethical concerns. Yet how functional complex tissues, organs and organisms emerge from the interaction of cells and molecules remains a fundamental question in biology. Spanning from stem cell and developmental biology to biophysics, engineering and robotics, the meeting shed some light on this fundamental question. Here, we highlight some of the results that were discussed in each discipline to provide an overview of the breadth of this meeting and especially its implications for the developmental biology community (Fig. 1).

Symmetry breaking, shape and fate in early embryos: deconstructing complexity

Symmetry breaking and self-organization are two essential processes for the development of multicellular systems. Prisca Liberali from the Friedrich Miescher Institute for Biomedical Research (Basel, Switzerland), opened up the meeting by discussing work on self-organization, heterogeneity and symmetry breaking in mouse gastruloids, aggregates of embryonic stem cells that recapitulate key aspects of gastrula-stage embryos (van den Brink and van Oudenaarden, 2021). Liberali uses mouse gastruloids to understand symmetry breaking in the mammalian embryo. She presented unpublished data using high-throughput imaging approaches to study the symmetry breaking in gastruloids with spatiotemporal resolution. Reconstruction of the developmental trajectory in pseudotime revealed that gastruloids break symmetry at ∼72 hours (h), the time at which the structure exhibits heterogeneity in Sox2 expression. In addition, she found that a population of cells that expresses high levels of Sox2 appeared in the core of the organoid after 108 h and re-expressed pluripotency genes and some genes of the primordial germ cell (PGC)-like signature. However, direct comparison with bona fide PGC cells revealed that they are not true PGC cells. According to these large-scale phenotypic characterizations, gastruloids are more variable in shape at around 96 h and become very homogeneous at 120 h. Liberali's

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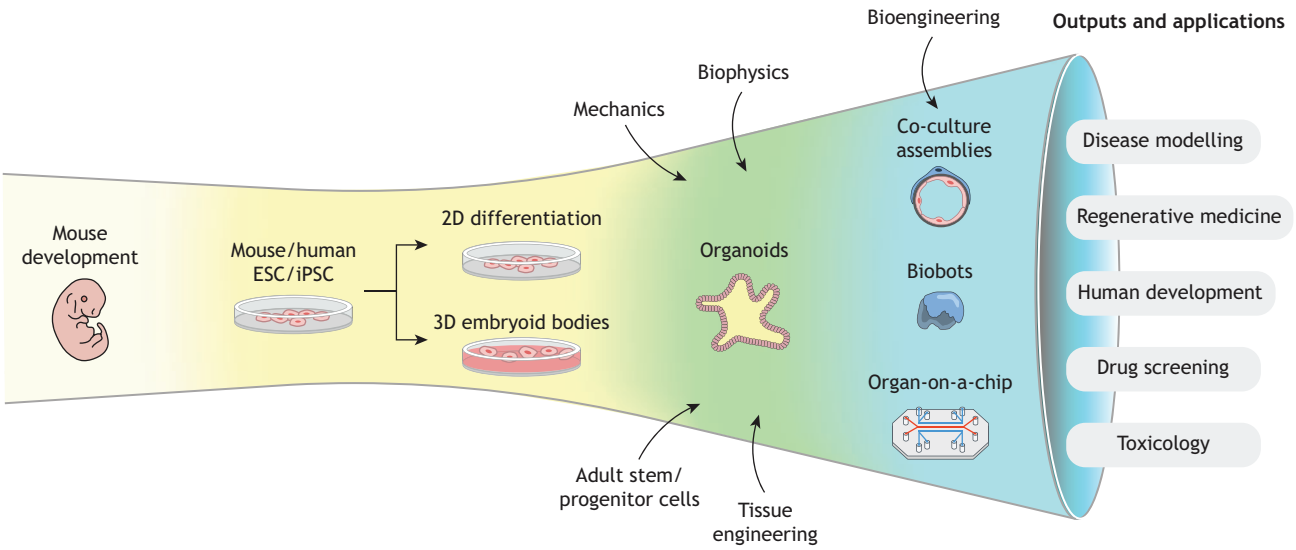

Fig. 1. An interdisciplinary roadmap for building multicellular systems: from stem cells to organoids, organs-on-chips and biobots. The use of mouse and human stem cell models (including embryonic stem cells, adult stem cells and induced pluripotent stem cells), in combination with advanced extracellular matrices, mechanical cues, biophysical methods and tissue engineering approaches, have greatly advanced our ability to build multicellular systems *in vitro*. These interdisciplinary efforts resulted in the generation of novel human organoid models, 2D and 3D co-culture systems, organs-on-chips and biobots. Such human models generate a springboard of opportunity not only to elucidate the principles of human development, disease, homeostasis and regeneration but also for establishing drug screening and toxicology platforms. ESC, embryonic stem cell; iPSC, induced pluripotent stem cell.

data support the growing idea in the organoid field that variability should be seen as an opportunity, rather than a disadvantage, to better understand developing systems because this increased heterogeneity could explain how cells find their ways to differentiate during the early embryonic stages. Although whether this concept holds true in the real embryo has yet to be proven.

Following up on the same question but from a biophysics approach, Vikas Trivedi from the EMBL (Barcelona, Spain) examined how forces contribute to symmetry breaking in gastruloids. Trivedi′s group aims to understand how networks of genes integrate with the forces and mechanical properties of the cells to form self-organizing structures at early developmental stages. It is well described that in gastruloids there is a polarized expression of the mesodermal transcription factor brachyury (Bra/T) (Anlaş et al., 2021 preprint). Using a combination of mechanical measurements, single-cell sequencing and mathematical modeling, Trivedi presented evidence that coordinated changes in tissue rheology and cell signaling guide the timescale and the size of the Bra/T pole in the gastruloid model (Oriola et al., 2022). Trivedi also emphasized that the initial culture conditions of pluripotent stem cells affect the dynamics of the symmetry-breaking event in gastruloids and that there might be more than one way of achieving symmetry breaking in this model. Trivedi concluded that brachyury^{+} cells control tissue rheology and are drivers of the symmetry-breaking process in gastruloids.

Continuing symmetry breaking and cell fate decisions to address how forces control global tissue pattern and shape, Pierre François Lenne from the Institut de Biologie du Développement de Marseille (France), put forward the idea that many multicellular systems self-organize without prior assignment of cell properties. Using elegant live imaging, he showed that collective cell movement of T/Bra cells and cell state transitions cooperated to pattern and shape gastruloids. Lenne observed that, in gastruloids, the collective movement of cells towards the symmetry breaking point creates an axial flow, and that tissue flows contribute to the polarization process (Hashmi et al., 2022). But how tissue flows are related to brachyury-expression fields is an open question and he emphasized that it will be challenging to capture the dynamics of these processes even in gastruloids.

Following on from deconstructing the early embryo, Marta Shahbazi from the MRC Laboratory of Molecular Biology (Cambridge, UK) presented work on the coordination between cell fate decisions and tissue shape changes. Shahbazi established a new 3D model to maintain mouse pluripotent cells as self-renewing organized epithelial structures. Analysis of the transcriptional profile revealed that the epithelial structures have a similar profile to the embryonic epiblast tissue of early post-implantation mouse embryos. She expects to use this system to deconstruct the complexity of early mammalian development in the dish and address the intriguing questions of how morphology and shape influence cell fate, and vice versa.

In summary, this set of talks highlighted that symmetry breaking, tissue shape and cell fate are processes regulated by both gene expression patterns as well as mechanics, and that self-organization is a coupled mechanochemical process. Although these presentations highlighted gastruloids as an interesting system for studying early events of symmetry breaking in development, they also raised the issue of whether more data are needed to benchmark the system and whether similar processes arise inside the real embryo.

Explaining morphogenesis with the lens of biophysics

Evident in the meeting, and becoming increasingly recognized, is that to understand self-organization during morphogenesis requires combining several disciplines. It is no longer a classical developmental biology question; instead, it is a systems-level question that requires a systems-level understanding. During development, tissues acquire function and shape via differentiation and morphogenesis, which are driven by coordinating cellular forces and shapes at the tissue scale. The meeting integrated developmental biology talks with biophysics talks applied to biological questions that spanned from topological defects to flow as a source of information. Aurélien

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Roux from the University of Geneva (Switzerland) examined the role of topological defects in self-organization using myoblasts as a model system. He showed that myoblasts self-organized around integer topological defects resembling spiral and aster-like structures to establish complex multicellular architectures. When differentiation of cells was inhibited, these arrangements could drive the growth of impressive swirling protrusions that look like tornadoes. On the contrary, when cells differentiated, the arrangements triggered the generation of asters. Roux further showed that topological defects can generate force gradients that concentrate compressive stresses. From these studies, Roux proposed that integer topological defects act as mechanical organizing centers that control differentiation and morphogenesis (Guillamat et al., 2022).

Following on the role of mechanics and topological defects, Kinneret Keren of the Israel Institute of Technology (Haifa, Israel) shifted from mammalian models to the interesting Hydra, which has been very attractive for developmental biologists due to its ability to regenerate its simple body. Keren discussed how topological defects drive regeneration in Hydra and how mechanical forces contribute to the formation and stabilization of the body plan during morphogenesis. She showed that defects in the nematic organization of the actin fibers provide information on the morphogenesis process and that the topological defects in the nematic order that emerge during the early regeneration phase can be used to trace the morphogenetic process (Maroudas-Sacks et al., 2021). She concluded that the nematic actin fiber orientation field acts as a mechanical morphogen that interacts with other mechanical and biochemical morphogens, toward the robust formation of functional tissues in regenerating Hydra.

Moving towards morphogen signals and information flow, Karen Alim from the Technical University of Munich (Germany), presented some exciting work on how flow could encode information that can influence self-organization. Her lab is using *Physarum polycephalum*, a simple unicellular slime mold, to study how its network-like body adapts to its environment. The beautiful networks that the slime mold is forming are dynamic and continuously self-organize its structure to enable it to solve a task as complex as the shortest path through a maze. She showed that flow shear stress drives the adaptation process of the complex networks that the mold generates. Alim presented further evidence that an external stimulus, such as a nutrient source, was sufficient to drive adaptation, in addition to a coordinated and stimulus-specific network adaptation. She concluded that the memory of the stimulus location is retained as an imprint in the network architecture (Kramar and Alim, 2021) and this memory impacts the overall network, its size and its function (Fleig et al., 2022). Whether similar information is also encoded in other flow networks in higher order organisms; for example, vascular networks in mammalian organs, such as the lung, is an intriguing question that awaits an answer.

Exploring pressure as another crucial physical parameter for developing cellular systems, Morgan Delarue, from the Laboratoire d'Analyse et d′Architecture des Systèmes (Toulouse, France), explained how the study of the cytosolic rheological properties of the unicellular growing budding yeast, *Saccharomyces cerevisiae*, can reveal universal principles of how pressure regulates cellular and organismal growth. He showed that compression can affect the motion of macromolecules inside the cell, causing protein synthesis to decrease and therefore reducing the amount of biomass available for the organism to grow. Whether this translates to multicellular systems, such as mammalian tissues, is still to be elucidated but provides further evidence of how important physical parameters are in living systems.

Applying robotics to cellular systems to engineer tissues in a dish

Modular tissue-engineering approaches provide a promising strategy for building complex living structures through the co-assembly of microscale tissue units. Using bio-fabrication tools, multiple cell types of muscle, neural and vascular cells can be combined to generate structurally different compartments of human organs that resemble some of the tissue functions. In the meeting, the crucial relationship between new multicellular systems and novel robotics and engineering approaches was evident in many talks. Samuel Sánchez from the Institute for Bioengineering of Catalonia (Barcelona, Spain) presented his exciting work on 3D bioengineering of biohybrid robots (3D biobots) and 3D actuators that are established in his lab. Sánchez used a combination of hydrogels, nanoparticles and skeletal muscle cells to print tissue-like structures, which developed 3D actuators and swimmers. Using electrical stimulation, they could train the muscle to contract and study the effect on maturation. The key to biobot performance though was the addition of a spring resembling the presence of a bone-like structure, which enhanced muscle performance. The generation of such models that allow control over muscle contraction and permit the measurement of actual forces creates opportunities for novel drug evaluation and screening approaches (Guix et al., 2021). Along similar lines, Selman Sakar from the École Polytechnique Fédérale de Lausanne (Lausanne, Switzerland) presented work on engineered tissues with a focus on connective tissue and muscle. Sakar highlighted the importance of using synthetic support structures made from hydrogels or elastomers to constrain contractile tissues, such as the muscle, which do not have a stable equilibrium morphology. Sakar further discussed how mesoscale physical principles of morphogenesis can be harnessed for the controlled self-assembly of tissues with complex equilibrium shapes without support structures (Mailand et al., 2022). To identify these principles, his lab is using advanced microscopy, robotic microsurgery, microtechnology, wireless actuation and finite element modelling. Combined with efforts in the development of tunable matrices and optogenetic stimulation, Sakar envisions the conception of reconfigurable and self-healing robots that are autonomously assembled from living matter.

Roger Kamm from the Massachusetts Institute of Technology (Cambridge, MA, USA), presented two co-culture models that have been recently developed in his lab and their applications. The first model involved the co-culture of separately generated spinal cord neurons and skeletal muscle cells that formed neuromuscular junctions in a 3D environment (Osaki et al., 2020). They successfully used the model to study the role of TDP-43 and showed that it causes muscle deterioration that affects muscle performance. Kamm also presented a neurovascular model of the blood-brain barrier and applied it to study Alzheimer′s disease (Hajal et al., 2022). The analysis revealed the distribution of amyloid-β protein in the brain extracellular matrix and its progressive accumulation at the vascular wall. This caused increased vascular permeability, which is a characteristic of cerebral amyloid angiopathy. Kamm highlighted the importance of using complex models to probe disease mechanisms and also for the development of new drugs.

Anna Herland from the KTH Royal Institute of Technology (Stockholm, Sweden), also focused on the neurovascular unit (NVU). Her lab is using microengineering approaches to generate

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3D microfluidic NVU models that allow direct interaction between the human endothelium and perivascular cells. This configuration resulted in higher barrier function and response to acute inflammation that more greatly resembles an *in vivo* response compared with traditional culture. Herland has further developed the NVU model and adapted it to a compartmentalized chip system. Additionally, they have developed protocols to derive all NVU-relevant cells from pluripotent stem cells. Herland emphasized the importance of generating such complex *in vitro* systems to establish patient-specific drug screening approaches in the future.

As the size of engineered tissues becomes larger, the cells in the middle do not have access to the nutrients because there are no blood vessels to facilitate the transfer. Vascularization is important for the growth, long-term maintenance, maturation and functionality of tissues. Thus, finding ways to vascularize the *in vitro* generated models is of tremendous importance to develop *in vitro* tissues and organs that resemble the adult tissues. Cristina Barrias from the Instituto de Investigação e Inovação em Saúde (Porto, Portugal), discussed different modular tissue engineering approaches for building vascularized microtissues from the bottom up. She emphasized that microtissue units present a high surface area, which facilitates the diffusion of oxygen and molecules, through interstitial gaps, affording a useful tool for generating densely cellularized 3D constructs. Barrias outlined different approaches to engineering vascularized microtissues and described some of their applications in the fields of regenerative medicine and *in vitro* tissue modelling.

Advanced human self-organizing functional organoid models to study tissue homeostasis, regeneration and disease

Organoids from different tissues have been generated in recent years from human pluripotent stem cells and tissue-resident stem/progenitor or differentiated cells. These organoids resemble some aspects of the tissue function and are revolutionizing the field of translational medicine due to their ability to model human diseases. Several talks were oriented at showcasing the new developments and applications of the most advanced organoid models at present. Sasha Mendjan from the Institute of Molecular Biotechnology (Vienna, Austria) presented impressive contractile cardiac organoids. The Mendjan lab used human pluripotent stem cells and the mouse embryonic developmental principles as a guide to generate self-organizing cardioids that formed a chamber-like structure containing a cavity. Mendjan showed that cavity morphogenesis is governed by a mesodermal WNT-BMP signaling axis and requires HAND1, a transcription factor associated with developmental defects in the heart chamber (Hofbauer et al., 2021). After the establishment of the model, Mendjan challenged its regeneration potential using cryoinjury. Cryoinjury initiated a cell type-dependent accumulation of extracellular matrix, an early hallmark of both regeneration and heart disease. Current work in Mendjan's lab focuses on advancing the complexity of the cardioid model and establishing it as a powerful platform with which to study the mechanisms of congenital heart defects.

From contractile cardiac muscle, Mina Gouti from the Max Delbruck Center of Molecular Medicine (Berlin, Germany) presented contractile human neuromuscular organoids (NMOs). Gouti used human pluripotent stem cell-derived neuromesodermal progenitors (NMPs), the building blocks of the posterior body, to simultaneously generate spinal cord neurons and skeletal muscle cells that self-organize in 3D to generate functional NMOs. NMOs contain functional neuromuscular junctions; they contract and develop central pattern generator-like neuronal circuits (Faustino Martins et al., 2020). She presented unpublished data showing that NMOs can be used to model spinal muscular atrophy pathology. Focusing on the development of advanced human neuromuscular models, Gouti also presented an unpublished 2D self-organizing functional neuromuscular junction model. She highlighted the importance of being open and choosing the right *in vitro* model depending on the question, as both 2D and 3D human models have their own advantages and limitations. In the future, both models can be used as preclinical models for neuromuscular diseases and personalized therapies.

With a focus on organoids generated directly from tissue, Meritxell Huch from the Max Planck Institute of Molecular Cell Biology and Genetics (Dresden, Germany) has pioneered the generation of different organoid models from resident tissue stem cells, including stomach, liver and pancreas. Huch focused on liver organoids and their ability to regenerate in a dish. She showed that liver regeneration is driven by a cellular plasticity mechanism that enables the activation of adult differentiated liver cells into proliferating progenitors. Huch identified that genome-wide remodeling of the transcriptome and epigenome of the cell (specifically, the DNA methylome) are the major drivers of progenitor activation both during organoid initiation and after tissue damage *in vivo* (Aloia et al., 2019). She also highlighted the importance of preserving tissue architecture in organoids, and not just adding cells together without recapitulating the native tissue. Her latest discoveries show that the same niche cell can exert a pro-regenerative or pro-quiescent signal depending on whether the niche cell did or did not establish cell contact with the epithelium (Cordero-Espinoza et al., 2021). The Huch lab is currently applying these organoid models to study both tissue regeneration and cancer at different biological levels.

Following up on the generation of cancer organoid models, Talya Dayton from the lab of Hans Clevers at the Hubrecht Institute (Utrecht, The Netherlands) developed the first patient-derived tumor organoids (PDTOs) from low-grade pulmonary neuroendocrine tumors and an understudied subtype of high-grade neuroendocrine cancer. Neuroendocrine cells have been very difficult to identify and culture. Dayton performed targeted screening of growth factor components in chemically defined culture media, which uncovered that a subset of pulmonary neuroendocrine tumor cells that expressed high levels of the epidermal growth factor receptor and whose growth in culture depended on epidermal growth factor. Using a multi-omics analysis approach, Dayton demonstrated that PDTO lines retain intra-tumoral heterogeneity and the major gene expression patterns observed in their parental tumors. Dayton is currently establishing her own lab at the EMBL (Barcelona, Spain), where she will continue using tractable organoid systems to engineer organoid models of neuroendocrine cancer and other neuroendocrine-related diseases to establish novel therapeutic approaches.

Although human organoids offer fascinating capabilities to study the human-specific aspects of development and disease, we cannot ignore the fact that they are very time-consuming and expensive models. Jochen Wittbrodt from the Centre for Organismal Studies (Heidelberg, Germany) presented organoids that rapidly developed from teleost species, such as medaka and zebrafish. He emphasized that these organoids offer an easier and quicker way to address multiple aspects of development and disease, and to probe the interactions of organoids with variable physical environments. As an example, he presented work on the generation of retina organoids and how physical constraints are important for the generation of retinal stem cell populations (Zilova et al., 2021).

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Short talks, posters and ethics sessions – a springboard for discussion

As alluded to earlier, in addition to the main scientific talks, the meeting also held short talks and poster sessions, as well as business and ethics sessions, which enriched and complemented the wide range of scientific discussions. The broad range of short talks, selected from the best abstracts, was a clear reflection of the quality of the meeting. Mostly presented by young investigators or trainees; these ranged from building stem cells from different animal species to developing pancreatic cancer organoid models for drug screening, from modeling muscular dystrophy in organ-on-chip models to investigating novel modes of cell migration using engineered microchannels or using bioelectricity to control cell directionality. These sessions were complemented by two brilliant talks on ethics in engineering multicellular systems. Amy Hinterberger (King's College London, UK) discussed the social and ethical implications of new biotechnologies. She presented a case study in which she interviewed 25 researchers and discussed their responses to a series of questions to review how human attributes are modeled and understood in multicellular living systems. Hinterberger cited the advantages of using organoid models for '3R (replace, reduce and refine)' purposes. However, she also noted that due to organoids, for example, new uses of the animals in the lab may arise and emphasized the importance of bringing human and animal ethics committees into a conversation. She closed by affirming that organoids are conduits through which humans realize visions for the future and this attribute makes them political and social.

Matthew Sample, from Leibniz University Hannover (Germany) emphasized that while ethical questions require special attention due to the emergent potential of multi-cellular living systems, the answers need to come from a collective action that involves the scientists and public – not from bioethics experts alone. Drawing on insights from previous STEM-humanities collaborative workshops (Sample et al., 2019), Sample discussed the importance of asking the right questions regarding the use of multicellular systems and encouraged us all to decide what organoids mean and how we can best use them for the benefit of society. In the end, he stressed the importance of including public engagement sessions in conferences to enhance the communication between scientists and the public.

From embryos to organoids and back: deconstructing to reconstruct

Different organisms from slime molds to Hydra, medaka and zebrafish to mice and humans were discussed in the meeting, mingled in talks that focused on a broad range of topics from mechanobiology and how forces influence self-organization and tissue morphogenesis, to symmetry breaking and regeneration, to building and re-building functionality. Assembling the different themes touched upon in this year's talks brings us the realization that, despite the broad range of model organisms and disciplines, we are all united by the quest to find answers to the same ancient question: 'How are we built?'.

The broad breadth of the meeting gave us the opportunity to appreciate once more the importance of taking an interdisciplinary approach to address these key questions and overcome challenges in developmental biology. For many years, developmental biologists have used embryos to learn about cells, tissues and organs. However, studies in tissue mechanics and engineering approaches were difficult to implement in the embryo and thus our studies concentrated on gene function. The generation of organoids from pluripotent stem cells, which resemble early developmental stages, tissues or even mini-organ-like structures, now gives us the unique opportunity to better understand what is happening in the embryo (reviewed by Terhune et al., 2022).

Understanding how cells and organisms respond to changing environments will be of fundamental importance to answering these questions. One bonus is that these approaches will provide us with the means to achieve reproducible *ex vivo* models that, in return, will facilitate answers to the very same questions around 'How are we built?'. Following the rationale of the physicist Richard P. Feynman, who said, "what I cannot create, I do not understand" if we understand, we can build, and if we build, we can also gain further understanding. In that regard, the future is bright. There are plenty of multicellular models that need building from scratch or require great refinements to recapitulate the function, architecture and shape of the native tissue 'in a dish'. In the meeting, Vikas Trivedi noted in his closing remarks the broad range of organoid models discussed, including gastruloids, brain organoids, cardioids, neuromuscular organoids, retina, liver, pancreatic cancer and PDTOs. Apart from organoids, organs-on-chips and biobots give the opportunity to engineer complex systems and study the interactions of different organs with the environment. Moreover, as we used embryos to learn about cells, tissues and organs for so many years, we now have the unique opportunity to use what we learned from the different organoid models to better understand the embryo. We can even use the knowledge gained from the culture and engineering conditions to maintain organoids to improve our ability to culture embryos. In this respect, Jacob Hanna from the Weizmann Institute of Science (Rehovot, Israel) gave a fascinating talk on the *ex vivo* culture of mouse embryos. His lab has established custom platforms for growing mammalian embryos *ex utero* from early (E5.5) to advanced (E11.5) stages of development (Aguilera-Castrejon and Hanna, 2021). The ability to grow the embryos *ex vivo* for such a long time, in combination with advanced imaging technologies, will allow us to study stem cell transitions during embryogenesis and organogenesis, and to directly compare the processes with *in vitro* models in a way that was unthinkable only half a decade ago. Thus, a two-way approach between the *in vivo* and *in vitro* models will be instrumental to understanding how life is built. Advanced technologies, such as human pluripotent stem cells and organoids, as well as single-cell sequencing in combination with mathematical models and biophysics approaches, have revolutionized our understanding of how multicellular systems are built. This acquired knowledge will certainly help us build these multicellular systems better, allowing closer recapitulation of the organs and embryo in a dish. On the other hand, these advances have recently raised certain ethical concerns that demand our attention to find the best ways in which to use our powerful models for the benefit of society.

In conclusion, as highlighted in this year's talks, the field has experienced great advances in the barely 2 years that separated the very first meeting from this year's second edition. This leaves us with great expectations for the future, eagerly looking to the uncharted new territories that the field will be exploring in the coming years that we hope will be communicated in the next edition of this meeting in 2024.

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