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MEETING REVIEW

Once upon a dish: engineering multicellular systems

Kristina Haase[1,*] and Benjamin S. Freedman[2,*]

ABSTRACT

In February 2020, the European Molecular Biology Laboratory (EMBL) and the Institute for Bioengineering of Catalonia (IBEC) joined forces to unite researchers from all over the globe to discuss emerging topics in 'Engineering Multicellular Systems'. As we review here, key themes that arose throughout the meeting included the ethics of organoids in developmental biology, bottom-up versus top-down models, tissue organizing principles, and the future of improving these systems to better mimic the natural world.

KEY WORDS: Embryoids, Morphogenesis, Organ-on-chip, Patterning, Signaling, Synthetic embryos, Vascularization

Introduction

The first EMBL-IBEC Winter Conference, held in Barcelona in February 2020, involved lively discussion of many topics surrounding 'Engineering Multicellular Systems'. This inaugural meeting was organized and chaired by James Sharpe (EMBL Barcelona, Spain) and Xavier Trepat (IBEC, Barcelona, Spain). Co-organizers included Nuria Montserrat and Josep Samitier (both from IBEC, Barcelona), as well as Miki Ebisuya and Vikas Trivedi (both from EMBL Barcelona). The Conference took place at Gaudi's La Pedrera – an architectural landmark known for its non-conventional modernist themes, and one which was well-suited to the discussion of topics that spanned developmental biology and tissue engineering.

The focus of this meeting was on emerging tools such as stem cell technologies, synthetic biology, organ-on-chip systems and regulated mechanobiology. Both bottom-up and top-down approaches were seen in the talks, and several key themes arose throughout the meeting. As we highlight here, a recurrent and important theme that emerged was that of developing robust model systems to understand tissue and organ formation and function.

Synthetic embryos, embryoids and gastruloids: from ethics to biology

The Mayor of Barcelona, Ada Colau, kicked off the meeting by emphasizing the city council's long-standing commitment to scientific research. She explained that, in an age of big data and transhumanism, the government can play an important role in facilitating public discussion and developing science policies. This preceded discussions at the meeting regarding gastruloids and other multicellular structures resembling human embryos that offer rare insights into biology, but that also raise ethical questions. For instance, if synthetic embryos could turn into a pregnancy, would they be legally equivalent to embryos generated by *in vitro* fertilization? Insoo Hyun (Harvard Medical School, Boston, USA) likened his role to a 'secular priest', to whom scientists go for ethical guidance. In addition to classical mechanisms such as informed consent and review boards, he proposed a 'new engineering' approach for bioethics: recognizing that experimental design choices are not value neutral, the decision for how to conduct an experiment can be arrived at through collaboration between researchers and ethicists, using sensible compromises and guidelines (Hyun et al., 2020). For example, 'unnecessary' biological completeness can be purposely avoided by engineering less controversial systems, where these can achieve similar insights.

What is the rationale to construct synthetic embryos? Magdalena Zernicka-Goetz (University of Cambridge, UK, and California Institute of Technology, Pasadena, USA) stressed the importance of these systems for understanding miscarriages, 30% of which occur at the implantation stage. She highlighted how synthetic structures provide a much-needed surrogate for actual human embryos, which can be difficult to obtain and are often of sub-optimal quality. As an example, she showcased how synthetic embryos built from three component stem cells, incorporating trophectoderm and embryonic stem cells encased in a softer 'shell' of primitive endoderm, have revealed an FGF4-dependent feedback loop mediated by β-integrin signaling between epiblast and primitive endoderm – a discovery that would have been difficult to make any other way (Sozen et al., 2018).

How far can synthetics actually develop? Alfonso Martinez Arias (University of Cambridge, UK) focused on anterior-to-posterior axis formation in elongated gastruloids, the localized gene expression patterns of which resemble that of day 9 post-conception mouse embryos to an uncanny degree. Despite this advanced level of spatial organization at the gene expression level, gastruloids exhibit limited morphogenesis compared with the embryo. One missing piece of the puzzle may be the extracellular matrix, which, when supplemented to gastruloids, enables them to produce striped segmentation patterns reminiscent of those that emerge during somitogenesis (van den Brink et al., 2020).

Eric Siggia (The Rockefeller University, New York, USA) discussed the importance of geometry in 2D colonies of embryonic stem cells, which exhibit primitive streak-like patterning when physically confined. This reflects a role for BMP signaling; Siggia noted that the expression of BMP receptors is restricted to the basolateral surface in these cells, so when these structures are grown in 3D, these receptors become more exposed, enabling low levels of BMP and Wnt to induce symmetry breaking events resembling those observed around the embryonic node. By generating tripartite gastruloids, it may be possible to mimic the hypoblast layer underlying the epiblast in order to better recapitulate the geometry of signaling and patterning. These sessions provided an exciting sneak preview of the next phase of human developmental biology research, coupled with a thoughtful commentary on the ethical considerations required as synthetic embryos grow ever-closer to the real thing.

The balance between emergence and control in engineered living systems

Both bottom-up and top-down approaches to engineering living systems have inherent advantages and disadvantages. For example,

[1]EMBL Barcelona, Carrer/Dr. Aiguader 88, Barcelona 08003, Spain. [2]Division of Nephrology, Kidney Research Institute, and Institute for Stem Cell and Regenerative Medicine, Department of Pathology (Adjunct), and Department of Medicine, University of Washington School of Medicine, Seattle, WA 98109, USA.

*Authors for correspondence (kristina.haase@embl.es; benof@uw.edu)

K.H., 0000-0003-3321-4286; B.S.F., 0000-0003-2228-7383

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building bio-systems bottom-up from single components can lead to oversimplification, and conversely complexities stemming from a top-down approach can challenge our understanding of intertwined biological relationships and mechanisms. This meeting was host to a variety of researchers using both strategies – either to fully control (as in synthetic biology) or direct (using tools such as microfluidics) the emergent behavior of multicellular systems using *in vitro* tools. Although the reductionist point of view is to break everything down into simple components, a functioning cell and, furthermore, a functional tissue is not simply the sum of its parts. Regardless of the shortcomings, breaking these complex tissues and cells down to simpler components, by trying to direct patterning or behaviors through regulation of genetic programming, biochemical and mechanical means, allows us to gain a deeper understanding of the complexities of the collective and emergent behaviors necessary for life.

With the aim of building tools to understand developmental biology, several researchers are using a bottom-up approach to understand the fundamentals of development. For example, tissue patterning is a tightly regulated process dependent on a number of temporally and spatially dependent cues. Morphogen gradients are one of these cues; however, details behind the mechanisms of their development remain unknown. Using cell culture, genetic engineering, time-lapse imaging and mathematical modeling, Pulin Li (Whitehead Institute, Cambridge, USA) demonstrated how Sonic Hedgehog (SHH) signals can be reconstituted in a petri dish as spatial morphogen gradients. By engineering knockouts and tunable closed-loop circuits into the SHH pathway, she was able to show how an evolutionarily conserved negative feedback loop helps buffer changes in morphogen production rates to ensure robust amplitude and length scales of these gradients. Also focusing on developmental signaling, Wendell Lim (University of California, San Francisco, USA) showed us one of his lab's latest novel synthetic tools that allows them to direct self-organization between cellular aggregates in 3D. Recently, they created synthetic Notch ligand-receptor pairs of adhesion proteins, which can be controlled (by co-culturing ligand and receptor cell lines) and are capable of reporting pathway activity (i.e. via fluorescence indicators) in response to differentiation. By transducing these synthetic circuits into non-organizing wild-type fibroblasts, they were able to direct cells into self-organizing patterns similar to layered structures seen during development. More recently, they have developed synthetic diffusible morphogens, the outputs of which can be programmed to generate diverse gradient interpretation patterns similar to the segmentation patterns observed in natural development.

In contrast to synthetic circuitry and genetic manipulation, others have focused on controlling absolute cell position in an effort to promote differentiation and organization. Wei Sun (Tsinghua University, Beijing, China, and Drexel University, Philadelphia, USA) shared his lab's development of a number of 3D cell printing strategies, which allow them to print multiple cell types simultaneously and in a number of varied bio-gels. Importantly, they recently demonstrated the ability to print human induced pluripotent stem cells (iPSCs) with high viability and showed that these maintain pluripotency in uniform aggregates. Bioprinting is a particularly useful strategy for teasing apart how spatial patterning and densities play a role in tissue formation at the single cell level, complementing microfluidic techniques that can influence single cells (e.g. encapsulation techniques) or multicellular aggregates (e.g. organ-on-chip designs). For example, Danijela Vignjevic (Institut Curie, Paris, France) demonstrated a variety of engineering strategies they use in the lab to control cellular patterning and to elucidate the role of fibroblasts in epithelial migration, proliferation and invasion. Fibroblasts in particular are becoming well-known for their role in both gut homeostasis and cancer metastasis – two focal points of research in her lab.

Mechanical cues are also now well-known to play a role in driving cell and tissue formation and function. Moreover, tissues must be able to respond to these cues in an acute and spatially accurate manner to maintain homeostasis. For example, injury to arteries and veins initiates a cascade of events that result in local clot formation. Hongxia Fu (University of Washington, Seattle, USA) highlighted the importance of local activation of Von Willebrand Factor (VWF) – an endothelial-regulated protein that plays a role in binding platelets. Considering that VWF has been touted as a mechanical flow sensor, she constrained VWF proteins in a microfluidic chip in order to examine their interactions with platelet membrane receptors under controlled flow regimes. Sufficient tension (induced by shear flow) was required for VWF to bind platelet membrane receptors and, interestingly, binding was deactivated shortly after the tension was released, as would be required in normal blood circulation. Control over mechanical cues, such as on-chip flow rates, was also a focus of the talk by Ryuji Morizane (Harvard Stem Cell Institute, Cambridge, USA), who demonstrated the increased propensity for endothelial vascularization of kidney organoids on-chip. By culturing organoids under a flow regime, he and his colleagues noted enhanced endothelial gene expression and vessel formation, which also contributed to increased morphological changes (arrangement of tubular epithelial cells and podocytes) and expression of podocyte transcription factors that are markers of adult maturity.

Although we are not able to cover all talks here, we would like to note that most, if not all, of them fit within the context of employing some level of control on cellular systems. Whether full regulation or partial control is achieved, these strategies are instrumental for understanding the principles that influence self-organization. Much like the abstract paintings of Picasso, which incorporate familiar elements in new and surprising patterns, natural tools are being redeveloped and repurposed to effect changes within cells themselves (e.g. via mutants and synthetic biological components), or to alter the surrounding microenvironments (e.g. via non-natural on-chip environments). This can provide many insights into the developmental and regenerative capacity of complex cellular systems.

Organizing principles at the tissue scale

How do tissues produce, maintain and modify their complex architectures? Several talks grappled with these questions, which are central to organogenesis, homeostasis and disease. Arthur Lander (University of California, Irvine, USA) noted that embryos are highly robust and that sizes and patterns are specified with remarkable precision. In discussing how regulation of stem cell behavior achieves size control, he explained why integral negative feedback, an engineering design principle capable of establishing a 'set point' towards which systems repeatedly converge, is essential. For pattern control, he talked about the *Drosophila* wing imaginal disc, and the ability of gradients of the morphogen Decapentaplegic (Dpp) to scale automatically, so that pattern becomes coupled to disc size. He argued that simple models that attribute scaling to the effects of an expander molecule such as the secreted protein Pentagone (also known as Magu) cannot explain the behavior of the wing disc; instead he implicated feedback regulation of receptor and co-receptor function as playing a key role in the scaling process (Zhu et al., 2019 preprint).

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Xavier Trepat (IBEC, Barcelona, Spain) used organoids to map the mechanical forces that shape intestinal villi and crypts. Combining enteroids with 2D culture and traction-force microscopy, he discovered a crucial role for tension in migrating transit amplifying cells in organizing and compartmentalizing the stem cell niche. Pharmacological inhibition of myosin II rapidly and reversibly ablated these forces, suggesting a 3D vertex model for surface tension within the epithelium. Using human kidney organoids as a model, Nuria Montserrat (IBEC, Barcelona, Spain) elucidated the importance of microenvironment stiffness. Transplanting organoids into the chorioallantoic membrane of the chick egg (which exhibits ∼1 kPa stiffness) encouraged growth and maturation of the organoids, and also provided a source of much-needed vasculature. The chick embryo could be partially substituted by a bioengineered hydrogel, the stiffness of which could be tuned to accelerate organoid formation, providing a longer-term system with greater flexibility and design options.

Disease can be thought of as a situation in which principles of tissue organization have gone awry. Benjamin Freedman (University of Washington, Seattle, USA) discussed a human organoid model of polycystic kidney disease, a Mendelian disorder in which tiny tubules expand to form fluid-filled sacs. Culture of organoids carrying disease-associated mutations revealed that the phenotype is highly sensitive to its microenvironment. In addition, the exposure of organoids to physiological levels of fluid shear stress induces cyst expansion, underlining the importance of mimicking tissue fluidics in modeling disease. Focusing on breast cancer, Zev Gartner (University of California, San Francisco, USA) described a pivotal relationship between two neighboring types of cells: myoepithelial cells and luminal cells. In the bi-layered mammary epithelium, myoepithelial cells act as shepherds, restricting luminal cells from escaping the epithelium. Self-organization of these two cell types progresses toward a structure that minimizes overall tissue surface energy, and can be predicted using lattice-based models. Mutations in breast cancer driver genes decrease interactions between luminal epithelial cells and the basement membrane, perturbing the delicate balance and enabling escape. Knockdown of talin 1 in mutant cells, however, can correct self-organization. Collectively, these talks emphasized the role of the microenvironment, epithelial adhesion and feedback loops in controlling the set points of tissues in both development and disease.

The future of engineering multicellular systems

Multicellular systems (cell spheroids, organoids, organ-on-chip, etc.) have emerged as powerful tools used to mimic organ-like features, and many of them demonstrate heterogeneous complexities similar to those seen *in vivo*. Several speakers highlighted the importance of including vasculature in these models – an important feature of any functional tissue. Given recent advances in stem cell technologies, it is no surprise that we are collectively approaching fully vascularized engineered tissues and organoids. Josef Penninger (University of British Columbia, Vancouver, Canada) has been generating mutant embryonic stem cells (ESCs) for a number of years, culminating in a mouse ESC mutant Haplobank, which he described by drawing parallels to the meiotic and mitotic cells that decorate gowns and tapestries in Gustav Klimt's paintings, which symbolize the attractive combinations of and variations in life. He also presented beautiful vascular organoids that can be generated by directing the fates of multiple types of human PSCs *in vitro*. Upon exposure to hyperglycemia and inflammatory cytokines, these human PSC-derived vascular organoids recapitulate key features found in diabetic vasculopathies, and they anastomose with mouse vasculature following implantation (Wimmer et al., 2019). The ability to reproducibly direct stem cell fates into coordinated and collective behaviors is quite appealing; however, this totally emergent behavior cannot be fully controlled, and thus others have tried to constrain their collective behaviors.

A leader in the field of engineering multicellular systems, Roger Kamm (Massachusetts Institute of Technology, Cambridge, USA), highlighted the benefits of controlling innate cellular self-assembly processes *in vitro*. He also described the potential to develop functional biological tissue alternatives, as detailed in a recent white paper (Kamm et al., 2018). Neurovascular models of a vascularized blood brain barrier and a neuromuscular junction were shown as examples of how engineering, when combined with emergent cell behaviors, can lead to the design of predictive disease models, including models of Alzheimer's disease and amyotrophic lateral sclerosis (ALS). Building model systems in this manner was also a focus of a former Kamm lab member Kristina Haase (EMBL Barcelona, Spain). Kristina showed how macroscale chips can be designed and used to model specific tissue-like vessels. By optimally integrating the minimum and essential components of endothelial and stromal cells on-chip, she showed how vessels self-assemble into perfusable networks – making them uniquely accessible for testing of small molecules, antibodies and/or immune cells, as shown by one of her recently published models of placental-like vasculature (Haase et al., 2019). As an alternative approach to semi-controlled emergent systems, others have been using highly-controlled approaches to generate patterned vessels. Maria Bernabeu (EMBL Barcelona, Spain) showcased her fantastic endothelial-lined vessels, generated by either pre-patterning or laser-ablation techniques in collagen gels. Precise control over vessel geometry (down to a single endothelial cell wrapping in a capillary) allowed for examination of the binding capacity of *Plasmodium falciparum*-infected erythrocytes to the endothelium under quantifiable flow regimes (Bernabeu et al., 2019). Cutting-edge vascularization strategies such as those mentioned here are necessary to develop fully functional organoids or other large-scale tissue structures; without them, organoids remain more primitive.

Somewhere between full control and emergent behavior lies the work of Matthias Lütolf (École polytechnique fédérale de Lausanne, Switzerland), who has been steering emergent behaviors through design to generate deterministic organoids (Brassard and Lutolf, 2019). As an example, Matthias showed how designer matrices can be used to control the fate of primary intestinal stem cells within patterned pockets, resulting in some cases in tubular organoid-like guts with fully accessible crypts and villus-like domains on-chip. These hybrid organoids-on-a-chip allow for implementation of physiologic-like cues, and even the integration of endothelial lined vessels – again stressing the need to incorporate vessels on-chip. With the use of human PSCs, and with an increasing pool of knowledge developing around cell self-assembly, designer chips can be used to direct cell fate and co-ordinated behaviors. We are now at the forefront of this field, witnessing evolution towards fully functional and vascularized lab-grown organoids and tissues.

Conclusions

Set in an inspiring venue, the meeting was imbued with references to science in art and engineering, from spiral nautili in Gaudi's stonework to meiotic ova in the murals of Gustav Klimt. Three well-attended poster sessions allowed for attendees to meet each other and engage, and an exciting series of shorter talks was selected from amongst the abstracts. As James Sharpe noted in the final wrap-up,

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the beauty of the natural world is no accident – it is the product of millions of life lines of evolution, guided by clear design common principles, as illustrated by the wings of bats and birds. Many of the machines we use today, such as the automobile, have evolved through similar phylogenies. Thus we should not fear imperfection at this stage, but rather jump in with both feet and start trying to engineer multicellular systems. Through this process, science provides a lens through which we can understand nature's beautiful forms and gain a deeper appreciation of how they all come together.

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Competing interests

Dr Freedman is an inventor on patent applications related to kidney organoids and is an advisor for Chinook Therapeutics. The authors declare no other competing or financial interests.

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