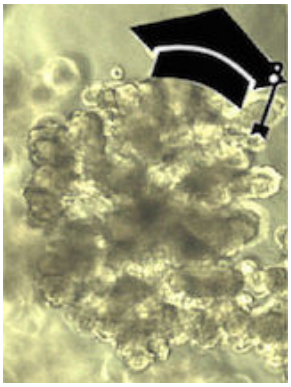
Improving *in vitro* models: The coming of age of organoids

Commentary by Amélie Rezza, PhD 27/03/2018

When you know even just a little about experimental research, in biology or any other science, how it's done and how it works, it quickly becomes obvious that it's all about the model. In biomedical research, for example, the overall aim is to better comprehend the human body and diseases that affect it, to understand their emergence and their development in order to prevent and cure them. So, very rapidly, the question of the model's relevancy becomes central: What organism to use for *in vivo* studies? What cellular system for *in vitro* experiments? Hence, we as a community are always looking for the best way to recapitulate what happens in patients, looking for the «perfect» model.



In vitro models have always been a central feature of biomedical research, and it's safe to say their use is only going to increase in the future. There is an endless list of *in vitro* models depending on the number of cell types involved, the material needed, the complexity of the system, but also what it looks like. Historically, *in vitro* studies started using «cells in a dish» as a layer or in suspension, now called 2D systems, but there are now many other ways to culture cells in complex structures by adding one dimension, called 3D systems. It's been known for the past 50 years or so that if you take cells from an organ, dissociate them, and put them back together in a dish (in appropriate conditions—that's the trick!), they can reorganize and reform the organ of origin, albeit a smaller/simpler version of it. These mini-organs, or organoids, with self-organizing properties, have been first observed in now «classic» studies in embryonic chick tissues,[1] but have been shown to exist in virtually all developing vertebrate organs.

Organoids are defined today as 3D structures that develop from stem cells and self-organize through cell-sorting and spatially restricted lineage commitment. In other words, these structures form through cell–cell «recognition» and organize in 3D thanks to spatial constraints, while stem cells give rise to different committed cell types. In a groundbreaking paper in 2009, Hans Clevers and collaborators showed that they could recreate mini-guts *in vitro*, with crypts and flat villi, from just intestinal epithelial stem cells.[2] In these intestinal organoids, multipotent cells can give rise to all epithelial cells of the intestine, absorbent and secretory cell types, just like it happens *in vivo*, and they can be kept in culture for months, if not years. Organoids can be formed from pluripotent stem cells (PSCs, embryonic or induced), or organ-specific adult stem cells (aSCs), and as of today, they can be used as a model for more than 10 organs, from the gut to the brain, PSCs and/or aSCs, depending on the organ. Interestingly, they can also be formed from tumors.

Organoids might have been mostly used for developmental biology studies at their inception, with a particular interest for organogenesis, but in the past nearly 10 years now, they have regained popularity in many other areas of biology and are being recognized as powerful models for various applications. (They have just been named «Method of the Year 2017» by Nature Methods.)[3] In cancer research, for example, organoids or, rather, tumoroids, represent an unexpected method to preserve clinical tumor samples, as it allows maintenance of premalignant cancer cells in culture. Indeed, the Human Cancer Model Initiative

announced in July 2016 that it would include tumoroids in its «bank» of cancer models.[4] Institutions from different countries are involved in this effort to better capture the diversity of cancers subtypes, and these models are to be shared with the entire scientific community. Importantly, maintaining cancer cells in culture also means they are proliferating, significantly increasing the amount of material available for deeper analyses. Recently, researchers have used organoids to expand cells from three primary liver cancers and perform gene expression analysis, opening the door to biomarker discovery for rare cancer subtypes.[5] Organoids have also been used in drug discovery with very conclusive results. In May 2017, the FDA expanded its indication of Kalydeco, a cystic fibrosis drug, from 10 to 33 mutations in the *CFTR* gene.[6] This decision was solely based on *in vitro* tests on patient-derived intestinal organoids, clearly demonstrating their promising preclinical potential.

The list of possible applications goes on, including personalized medicine, disease modeling, and host-microbe interaction, but organoids still have some flaws. In some cases, the organoids don't include all cell types, their structure is not relevant to the *in vivo* organ, or they show an immature function. For some applications, some studies requiring more complex settings, these models might soon be outperformed by more elaborate 3D systems such as organs-on-chip and 3D bioprinting. It is still too early to tell which particular model will win the award for best, most relevant model, but it is interesting to note that besides being a technology, a method (2D, organoids, organs-on-chip), what will be deemed the best model will also depend on the choice of cells used on the platform. The holy grail is, of course, the use of primary human cells, but they are not always available (neurons are a major problem, obviously). So, as alternatives, cells derived from human induced PS (hiPS) cells, or primary cells derived from rodent models are particularly interesting, even if hiPS-derived cells show some immaturity and primary rodent cells are, well, not from humans.

So, yes, there really is no «best, most relevant model» for *in vitro* studies today, and there might never be just one, as it all depends on the application.

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References:

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