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WHITE PAPER

COVID-19 AND ORGANOIDS: MINI-ORGANS WITH MASSIVE POTENTIAL

From modeling pathogenesis[1] and fast-tracking drug discovery[2] to predicting the efficacy of cancer treatments[3] and even improving pregnancy outcomes[4], organoids are both revolutionizing therapeutic development and advancing our understanding of human biology. With a pandemic in full swing, researchers have been turning their attention to how organoids can help curb the dramatic consequences of the COVID-19 crisis. Here we take a look at the enormous potential of organoids in COVID-19 research and beyond by exploring past, current and emerging research.

A PATIENT IN A PETRI DISH

By harnessing the unique power of stem cells along with the right conditions, a few starting cells can be encouraged to spontaneously assemble into complex, self-organized 3D cultures – known as organoids. As these cultures resemble miniature organs - emulating everything from mini-guts, lungs and brains to mini-hearts - organoids are proving to be a powerful research tool across the life sciences.

When the novel coronavirus emerged in China and rapidly spread across the world, it didn't take long for researchers to begin to utilize organoids to better understand, treat and prevent disease. In fact, a wealth of research papers investigating SARS-CoV-2 in a variety of organoids were published the very same year that COVID-19 turned pandemic.

Although it's difficult to argue that the COVID-19 pandemic has had many positive effects, organoid research is an area that has seen rapid, unparalleled growth. If, or perhaps when, another pandemic emerges, this growth could elevate organoids to one of the most powerful tools at our disposal.

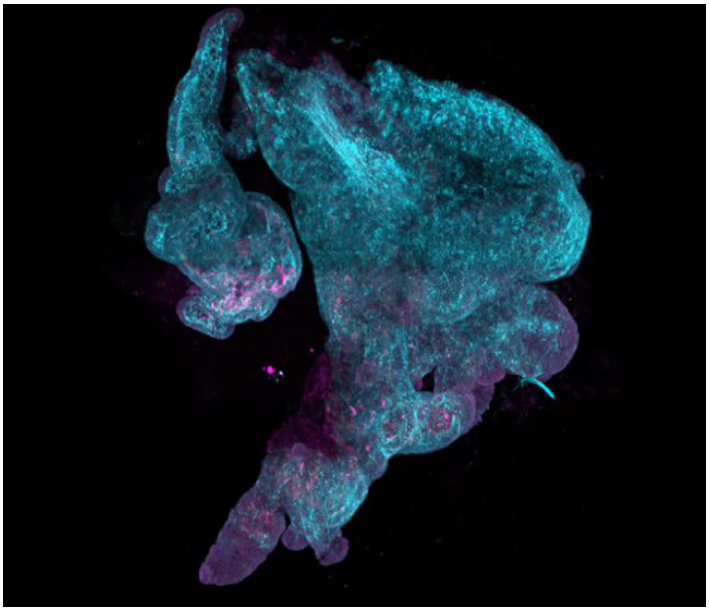

FIGURE 1. An Organoid model of SARS-CoV-2 Infection. Human intestinal organoids expressing the ACE-2 Receptor, cultured using Cultrex UltiMatrix BME.

GOING VIRAL: ORGANOIDS AND COVID-19

Modeling viral infections and their pathogenesis not only enables us to better understand the problem but also, crucially, to identify suitable drug targets. Being able to do this rapidly and effectively is perhaps never more important than when the world is gripped in the midst of a global pandemic.

Of course, organoids already have a track record in the realm of viral research – Zika is a good example. Researchers grew brain organoids infected with the Zika virus and observed a dramatic difference in size compared to non-infected cultures[10] - which ultimately provided the definitive link between babies born with microcephaly and Zika virus infection. Brain organoids have since been used to disentangle the intricate complexities of brain microcephaly[11], and even employed to identify novel and promising treatments[12]. Similarly, organoids have been successfully used to model many infectious viral diseases – including rotaviruses[13] and respiratory syncytial virus (RSV)[14].

MODEL BEHAVIOR: MODELING COVID-19 INFECTION IN ORGANOIDS

With this precedent, research has looked to organoids to study COVID-19 (Figure 1). To find out what cells or tissues SARS-CoV-2 infects – known as its viral tropism – researchers directly infected various types of organoids. Lung, brain, kidney, heart, and intestinal organoids[15] can all support SARS-CoV-2 and these studies have helped explain how the virus spreads throughout the body and causes some of the more unusual effects of COVID-19 infection – such as gastrointestinal and neurological complications.

At the beginning of the pandemic, a group of researchers noticed that gastrointestinal symptoms and viral RNA in stool samples were very common in patients infected with COVID-19. Having already developed a protocol for producing intestinal organoids from adult stem cells, the team harnessed these capabilities to model COVID-19 infection[16]. They surmised that since the intestinal villi expressed high amounts of ACE2 – the human protein SARS-CoV-2 uses to gain access to our cells – intestines could facilitate infection.

Indeed, the team were one of the first to show that SARS-CoV-2 productively infects human gut enterocytes in both proliferating

and differentiated organoids[16]. As well as identifying a huge upregulation in interferon-related genes – a classic sign of viral infection – the authors also noted more unusual cellular responses that could represent a target for curbing viral productivity. Find out more by listening to this recent COVID-19 Symposium.

Modeling of COVID-19 in airway organoids has shown that the virus induces the production of proteins known as chemokines and cytokines[17]. In some people with severe COVID-19, this can trigger a massive immune response known as a cytokine storm – that can be deadly. Excessive release of pro-inflammatory cytokines such as IL-6, IFNγ, IL-10, GM-CSF and CRP, can be closely monitored in organoids, using research solutions like the Luminex® kits for multiplex cytokine assays, to understand severe COVID-19 disease progression.

As discussed, modeling viral infection is important for several reasons and identifying potential targets for treatment is a big one. Since SARS-CoV-2 attaches and infects cells via ACE2, interrupting this mechanism has been a focal point for drug design. In one study, Penninger and colleagues infected vascular and kidney organoids to show that soluble ACE2 inhibited SARS-CoV-2 infection[18].

While this is an interesting target, many other host factors play a role in the infection process. By employing CRISPR technology, scientists have been able to create knockouts in organoids to screen for these. By individually knocking out genes in organoids and observing the effects on infection, researchers were able to identify a protein known as TMPRSS4 as an essential host factor[19] – and a possible drug target. Elsewhere, scientists have deployed CRISPR to knock out every single gene in the human genome in lung cells to find genes and pathways required for infection[20]. Coupling this type of genome-wide CRISPR screening with organoid technology has the potential to rapidly identify host factors for infection while simultaneously providing insights on pathogenesis.

ORGANOIDS CAN SPEED-UP DRUG DISCOVERY

Typically, it takes approximately ten years to get a drug from the lab to the patient - and it comes with the hefty price tag of over $2.5 billion[21]. Why is it so expensive? Partly because the success rate is low - with only 5% of drugs that proceed to the clinical trial stage making it to patient[22]. Clearly, this is not ideal, pandemic or otherwise.

Organoids offer exciting new models to streamline the testing of candidate therapies and speed up the drug screening process. Since they closely mimic human tissues, they offer the promise of 'real' human data on drug pharmacodynamics. And, organoids can be used in a high-throughput capacity – enabling the screening of lots of candidates, fast. This is set to get *even faster* with technologies such as 3D bioprinting on the horizon. In fact, cutting-edge 3D bioprinters capable of printing lifelike structures with intricate, microscopic features, in 96 well plates and in under 30 minutes have been announced[23].

In the year and a half since COVID-19 took center stage, large scale drug screens on organoids are well underway and numerous candidate leads have been established. Screening over 1,000 FDA-approved drugs on lung organoids infected with SARS-CoV-2 pseudoviruses, Chen's team at Weill Cornell Medicine in New York City were able to identify several drug candidates that could be repurposed to treat COVID-19 patients[24]. The feasibility of the candidates was confirmed by observing organoids infected with live SARS-CoV-2 as well as in an *in vivo* mouse model.

Using a similar workflow, more than 1,000 drugs were tested on iPSC-derived colorectal organoids[25]. Several promising candidates overlapped with Chen's study – including mycophenolic acid (MPA), quinacrine dihydrochloride (QNHC) - indicating multi-tissue effectiveness.

In addition to drug screening, organoids offer an appealing platform to test the response of potential vaccines. The ability to rapidly and accurately assess candidate vaccine response could translate to savings in both time and resources during the vaccine development process. Although no vaccine has emerged as a result of organoid research as yet, studies have shown a measurable immune reaction in tonsil organoids treated with a COVID-19 candidate. Moreover, as new variants continue to emerge across the world, organoid platforms could be employed to keep an eye on the effectiveness of vaccines.

COMBATTING COVID-19 VARIANTS

Emerging SARS-Cov-2 variants are keeping researchers – and the rest of the world – on their toes. Making sense of how mutations influence transmission, pathogenesis, disease severity and the effectiveness of therapeutics is of critical importance to the progression of the pandemic.

In a recent preprint, Clevers and colleagues used human airway, alveolar and intestinal organoids to provide new insight into the behavior of the B.1.1.7 variant[26]. They discovered that the variant – first identified in the UK – sheds more viruses and for longer and could outcompete its ancestral strain in terms of replicative fitness. These results help explain its observed increased disease severity and transmissibility.

By offering a rapid strategy to assess the tropism and fitness of new variants, organoids can help us quickly respond with preventative measures. For instance, we could monitor the effectiveness of vaccines or detection tests as new variants emerge and adjust formulations accordingly.

AN EXCITING FUTURE

The growth of organoid technology is certainly paving the way for novel and promising clinical applications for treating COVID-19 – and other diseases. Not only have organoids provided us with a far more accurate image of what host factors are essential for a COVID-19 infection as well as better visualization of viral spread, but they offer us a rapid and viable platform to identify drug targets and drug candidates.

And with 500-1000 animals needed to optimize a new antiviral compound for clinical trials[22], organoids are increasingly being seen as an ethical choice. While it is recognized that organoids may not completely eliminate the need for animal testing, the

progression of technology promises to reduce its necessity and improve the success rate of drug development.

As research continues to broaden our knowledge on organoids and progress is made with cutting edge technologies - such as organ-on-a-chip and personalized organoids – applications become ever more limitless. Bio-Techne is striving to help make these innovations possible with a full scope of high-quality products related to organoid research.

Growing organoids that recapitulate the micro-anatomy of a real tissue or organ calls for an environment that can be difficult to reproduce in culture. Bio-Techne's new Cultrex™ UltiMatrix™ Reduced Growth Factor Basement Membrane Extract is an advanced, well characterized matrix with proven efficacy in 2D, stem cell, organoid and other 3D cultures and applications. Culturing organoids typically requires a range of highly consistent growth factors, cytokines and small molecules. For example, vascularization of brain organoids using a patient's own pluripotent stem cells[9] was supported by applying a set of GMP-grade growth factors and cytokines to induce signaling pathways and differentiation – including BMP4, VEGF and FGF2. Alongside a full selection of these molecules, Bio-Techne's bioactive cytokines, growth and differentiation factors help optimize cell growth, maturation, and viability in a reproducible manner.

Bio-Techne responded at the onset of the COVID-19 pandemic by pivoting towards development and the release of a wide range of research tools to help in the fight against COVID– all of which can be found here. The ongoing pandemic has seen Bio-Techne rapidly develop and manufacture nearly all known spike protein variants – being one of the first companies to upscale and release a full version of the Indian Delta variant.

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