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Highlights

Level up for culture models - How 3D cell culture models benefit SARS-CoV-2 research

*Sophia Julia Häfner**

University of Copenhagen, BRIC Biotech Research & Innovation Centre, Anders Lund Group, Copenhagen, Denmark

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ABSTRACT

Welcome to a new decade and a new issue of the *Biomedical Journal* - casting a sorrowful look onto a year that will go down in history as a tombstone etched by the COVID-19 pandemic, but also a hopeful glance into the future, now that multiple vaccination programs against the SARS-CoV-2 virus have started. This issue is dedicated to the continuous effort by researchers all around the globe to understand and counter the pathogen, as well as to be better prepared for future threats. Therefore, we learn about the advantages of complex 3D cell culture models for studying host–virus interactions, and the disease course of COVID-19 in children. Moreover, we discover how neutralising monoclonal antibodies and peptide-based vaccines against SARS-CoV-2 are developed, and the therapeutic potentials of lopinavir/ritonavir, mesenchymal stem cells, as well as plant and algae extracts. Finally, we ponder over the lessons to be learnt from SARS-CoV and MERS, and hear about differences between nucleotide-based SARS-CoV-2 detection methods.

Spotlight on reviews

Level up for culture models - how 3D cell culture models benefit SARS-CoV-2 research

Although the COVID-19 pandemic has blown the candles of its macabre one-year anniversary, there is finally some light at the end of the tunnel, as the first vaccination campaigns against the SARS-CoV-2 virus are deployed at full power [1]. Nonetheless, until enough individuals will have received the vaccine, we will need to continue to contain the crisis as much as possible for a considerable amount of time. As such, there is to respite for the research to decipher the disease mechanism, monitor its evolution, and hunt for efficient therapies. This issue of the *Biomedical Journal* fully focuses on the most essential facets of fighting this global threat, comprising detection methods [2], vaccine design [3], drug development [4–8], and model systems.

Here, De Melo et al. have put together an exciting review about the latest updates of cell culture models for an accurate study of infectious diseases [9].

The art of cultivating living cells is over a century old and has beyond all doubt been a game changer for the emergence of modern biology, medicine, and pharmaceutics [10]. Virology is no exception to the rule, and the *in vitro* testing phase on cell culture models is an obligatory step between *in silico* bioinformatics predictions and cost- and labour intense animal models. However, for various reasons, the classical

* *Corresponding author*. University of Copenhagen, BRIC Biotech Research & Innovation Centre, Anders Lund Group, Ole Maaløes Vej 5, 2200 Copenhagen, Denmark.
E-mail address: sophia.hafner@bric.ku.dk.

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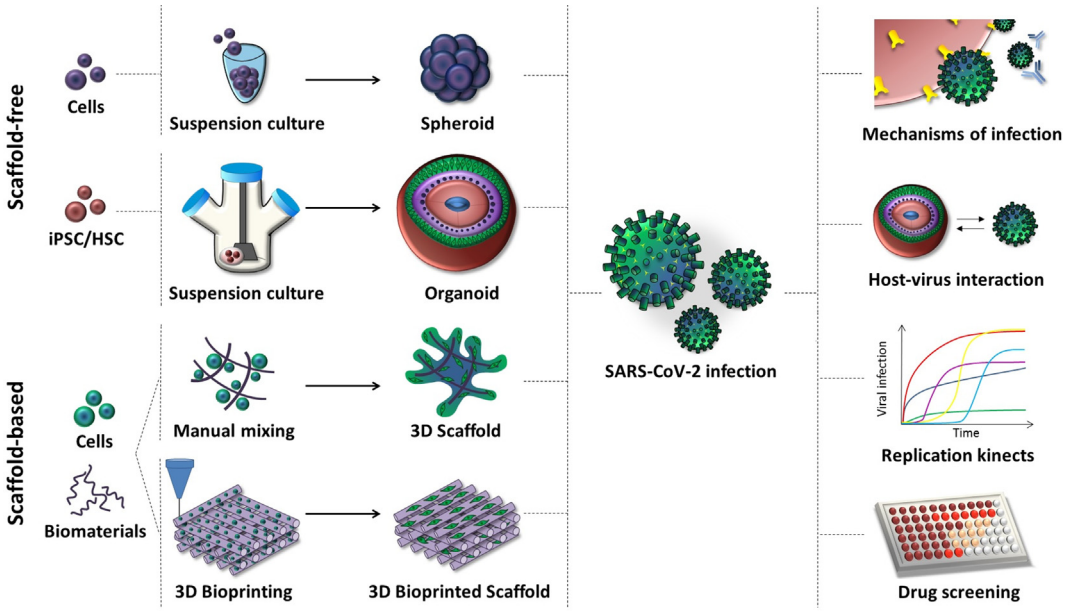

Fig. 1 **The different levels of 3D cell culture models and their usefulness to study SARS-CoV-2 infection.** 3D cell culture models range from spherical agglomerations of one cell type to complex mimics of organs obtained through the colonisation of bioprinted extracellular matrices with stem cells, which recapitulate the chemical, mechanical, and physical properties of infectious diseases. Figure kindly provided by de Melo et al. [9]. See main article for details.

monolayer comprised of a single cell line is a poor model for infectious diseases [11].

Two dimensions are amply sufficient, as long as the main focus is on events taking place inside one cell, like everything related to the decoding of signalling pathways. In contrast, infection is globalised warfare not against a single cell type, but organs as a complex organisation, and suddenly 3D architecture, cellular composition, the area of exposed surface, the tightness of cellular junctions, intercellular communication, and mechanical parameters all matter in determining the outcome of the invasion [12]. Moreover, for the sake of convenience, most established cell lines are either immortalised or directly of tumour origins, thus not especially prone to mimic physiologically relevant sensitivities to pathogens. Conscious of these limitations, over the past decades, considerable effort has been spent on the establishment of more realistic models [13]. With the help from modern technologies and the flourishing field of stem cell research, cell culture has literally levelled up into the third dimension.

De Melo et al. introduce us here to the different levels of complexity of 3D cell culture, as well as their benefits for the study of infectious diseases in general, and the understanding of SARS-CoV-2 in particular [9] [Fig. 1].

To begin with, the authors describe the two-dimensional cell culture models currently in use for SARS-CoV-2 studies, given that they still deserve some fair credit. In particular, the discovery that the angiotensin-converting 2 enzyme (ACE2) is hijacked by the virus as a receptor and entrance ticket into the host, took place in the simian Vero E6 cell line, an essential tool in the field.

Level one of the third dimension consists in spheroids, cellular agglomerates of usually a single cell type [14]. They present a very good trade-off between the simplicity of generation and the noticeable gain in authenticity of accessible surfaces and cell–cell contacts, enough to improve already the sensitivity of several cell types when confronted with viruses. One important feature of SARS-CoV-2 not to be neglected is its ability to infect many different cell types due to the widespread presence of ACE2. A direct consequence is that multiple organs are damaged upon severe COVID-19 disease, including heart, kidney, and liver [15]. Hence it is of critical importance not to restrict the investigations to a single cell type, but to ascertain the specific modalities of host–virus interaction for all susceptible tissues. In particular, substantial evidence has started to accumulate regarding the long-term sequelae affecting the central nervous system (CNS) caused by severe COVID-19, such as long-term fatigue, memory issues, or irreversible anosmia [16,17], a characteristic as puzzling as concerning. This explains without doubt why neurospheres were added to the set of spheroids confronted experimentally with SARS-CoV-2, leading to some interesting observations [9].

Nevertheless, spheroids still lack the presence of more than one cell type, as well as an organised cellular architecture, qualities that can be found instead in “organoids” [14].

Organoids have sprouted on the fertile soil prepared by the massive progress in the stem cell field since 2006. Cell culture of mouse and human embryonic stem cells (ESCs) has been possible since 1998 [18], but the sector received its main boost thanks to the discovery of induced pluripotent stem cells (iPSCs) – terminally differentiated cells reprogrammed into

ESC-like cells through reactivation of key stem cell transcription factors - by the teams of Shinya Yamanaka and James Thomson [19,20].

Not only did iPSCs circumvent the delicate ethical issues linked to ESC research, but sparked major hopes for autologous cell therapy and tissue repair [21]. Although the path from bench to bedside is still long, the finding resulted in the 2012 Nobel Prize in Medicine for Yamanaka [22,23], and an exponential rise in stem cell research, with entire companies emerging uniquely around the business of stem cell maintenance and directed differentiation.

Intestinal organoids have already attained the status of a classic in the field. The tiny tubular structures faithfully recapitulate the intestinal anatomy including villi and provided valuable information of their intricate spatially directed cell differentiation process [24]. Under discussion for transplants, and used to enquire into numerous diseases from cancer to cystic fibrosis, intestinal organoids are also excellent models for host-microbiome-nutrition and host-pathogen studies. Importantly, they can be made for different sections of the digestive tract and from other species, notably bats, the presumed zoonotic origin of SARS-CoV-2 [25]. De Melo et al. give us a good overview on how intestinal organoids have benefitted the research of the virus, as well as human bronchial and kidney organoids [9]. Furthermore, SARS-CoV-2 research has been the impetus to increase the arsenal of available organoid models, as the first liver ductal organoids were successfully developed specifically for this purpose [26]. More recently, the generation of brain organoids has caused quite some excitement amidst both the scientific community and the broad public. These "mini-brains" displayed a remarkable similarity to the complex regionalisation of the mammalian brain, including the spatiotemporal stratification of the cortex [27,28]. There is no doubt that they will be very helpful for investigating COVID-19-caused damages to the CNS.

Finally, another technology has become immensely popular outside the biological field recently: 3D-printing. Although the concept evolved in the 1980s, only the computational power and material range developed over the past decade made it really take off and democratised the system outside the realms of industry and Science [29]. Nowadays, 3D-printers of decent quality which require less space than a microwave can be purchased for a couple hundred dollars, complemented by popular websites such as *thingiverse.com* that offer countless open-source model files for literally anything - from torque wrenches to figurines of Bernie Sanders with mittens. At the same time, 3D printing has found its way not only into the nerd household, but also the medical field [30]. Customised "biodevices" that react to stimuli such as temperature or light can be easily integrated into "lab-on-chip" systems [31], just as much as tailored replacement pieces into human body parts [32]. In there, cellular architecture, homeostasis, and behaviour are greatly shaped by the extracellular matrix (ECM). This complex network of collagen, glycoproteins, minerals, and enzymes influences cell–cell communication, accessibility, survival, and polarisation [33]. Its role in various pathologies, notably cancer and infection, is uncontested [34,35]. Thereby, De Melo et al. conclude their review with an overview on how 3D-bioprinting perfects biological models both in organisation and behaviour.

Spotlight on original articles

No PG-13 for COVID-19

From the very beginning of the pandemic, the risk of developing severe COVID-19 and the mortality rate have been associated with advanced age. Although the picture has been complicated since by the identification of numerous comorbidities [36], this holds still true. As a direct consequence, most countries have decided to start their vaccination programs against SARS-CoV-2 with the elderly, alongside the healthcare personnel and high-risk individuals [37]. Children seem indeed fairly exempt from severe forms of COVID-19 illness [38], although there have been reports on cardiac abnormalities that should not be underestimated [39].

Altogether, there is no reason not to monitor the disease course in this age group. In the present study, Jiang et al. contribute to increasing the amount of available data with the case reports of ten paediatric patients with COVID-19 from the Huangshi Maternity and Children's Health Hospital, located in the Hubei province of China [40]. They detail the clinical features, laboratory, imaging, and test results for all ten cases. Conforming to the observation that COVID-19 tends to be mild in children, the clinical classifications range from asymptomatic to moderate, with symptoms no worse than a slight fever and a running nose. Regardless, half of the patients displayed abnormal chest imaging results, notably the ground-glass opacities frequently also described in adults [41].

Furthermore, Jiang et al. detect some minor deviations from the norm upon the examination of the patients' blood tests, adding some weight to the suggestion that lactate dehydrogenase (LDH) and hydroxybutyrate dehydrogenase α-HBDH might function as COVID-19 biomarkers in children [42].

An interesting point raised by the authors is the apparent longer shedding of virus *via* the faecal route [43], an observation speaking in favour of either adding or even favouring rectal swabs for nucleic acid tests before deciding on patient discharge.

Knowing about COVID-19 dynamics in children is as a matter of fact important for major political decisions, notably the prolonged closure of schools, as it has been the case in most countries. Not only does this add a substantial burden onto the shoulders of parents having thus to juggle remote work and home-schooling, but might also have a negative neuropsychiatric impact on the children [44].

Several voices plead for the re-opening of schools based on studies that ruled out children as super-spreaders due to lower infection and transmission rates [45]. On these grounds, further studies on COVID-19 cases specifically in children are definitively required.

Also in this issue

Reviews

Locked out

Through an unprecedented effort, vaccines against SARS-CoV-2 have been developed in a record time, and many nations have begun to deploy vaccination programs [1]. Nonetheless, it will take at least half a year or more until a sufficient proportion of any country will have been vaccinated. Hence, therapeutic strategies that can bridge the transition period are still urgently required in order to minimise the amount of fatalities. The administration of neutralising monoclonal antibodies (nMAbs), which interfere with host–cell interactions and entry of the virus by capping docking proteins, ranges among the most promising strategies developed so far to counteract severe COVID-19 [46].

Here, Juan Jaworski provides us with an exhaustive account on the state of the art regarding the development of nMAbs against SARS-CoV-2, with a detailed description of their molecular targets and level of (pre)clinical trial [4]. Advantages and promising results are debated just as much as possible adverse events and the main hurdle consisting of high manufacturing costs.

Home-schooling the immune system

After a year of intense turmoil, vaccination against SARS-CoV-2 has finally begun, compiling a number of "first time in history" events. Not only have the different vaccines been developed in record time, but also is it a premiere for a large-scale use of DNA- and RNA based vaccines [47], and probably the interest of the large public in the inner workings of the vaccination process.

Fittingly, Lim et al. have put together an exceptionally informative review about vaccines made of synthetic peptides based on pathogen epitopes [3]. The authors lay the groundwork with an accurate account on the characteristics of epitope recognition by the different members of the adaptive immune system, and the challenges of mimicking these *via* peptide-based vaccines. Subsequently, they present their own thorough *in silico* analysis of the SARS-CoV-2 genome in the search of B- and T-cell epitope candidates, meticulously explaining the broad arsenal of available informatics tools. In addition, they intersect and enrich it with multiple studies performed by other groups, in a synergistic effort to promote the soonest testing of the best candidates.

New tricks for old drugs

There are two recurrent themes to the frenetic scramble for treatment of COVID-19, one of them being the attempt to repurpose known drugs and molecules in order to shorten clinical trial time and limit safety issues [48], the other one being the investigation of natural compounds for similar reasons. Several articles in this issue of the *Biomedical Journal* tackle these themes [5,6,8].

For instance, Magro et al. focus on the available literature concerning the effectiveness and safety of treating COVID-19 patients with the drug Lopinavir/ritonavir (LPV/r), a combination of protease inhibitors [5]. So far, they have been used as a second line treatment upon Human Immunodeficiency Virus (HIV) infection, and showed some potential against SARS-CoV and MERS-CoV. The authors here review elaborately all available studies from *in silico* to clinical trials. Although the overall benefit seems marginal, they nonetheless encourage further studies, as the combination with other treatments or the exact timing of LPV/r might matter substantially for the patient outcome.

Help in the wake of Poseidon

Everyone pictures luscious trees when the conversation shifts to saving the planet and halting climate change, completely overlooking the tiny organisms swimming though our oceans and responsible for fixing 40% of CO_2 from the atmosphere [49].

Sami et al. have decided that algae deserve better visibility by dedicating their review to them and their antiviral powers [6]. After a brief, concise reminder on the main features of SARS-CoV-2, the authors provide the reader with a detailed list of all algal-derived polysaccharides and lectins harbouring anti-viral properties, as well as the associated molecular mechanisms. Despite the fact that most studies stem from cell cultures, clear potential seems to show through all results combined. Moreover, we discover that two groups are developing algae-based edible vaccines for SARS-CoV2, presenting multiple advantages in terms of production cost and stability.

Factotum cells

The popularity of mesenchymal stem cells (MSCs) has been steadily increasing over the past decades. Derived from various sources including bone marrow, adipose tissue, umbilical cords, and tooth buds, these multipotent cells can be readily expanded and differentiated into a large panel of bone, muscle, and fat tissues [50,51].

As a consequence, these cells have been primarily considered for cell therapy and tissue, repair, as covered by previous issues of the *Biomedical Journal* [52–55].

However, MSCs have more than one string to their bow, and it is becoming increasingly clear that their true potential might actually reside in their paracrine signalling activity. Mahajan and Bhattacharyya present a compelling set of arguments regarding the potential of MSC treatment for severe COVID-19 cases [7]. The authors provide first a comprehensive description how SARS-CoV-2 infection, immune invasion, and subsequent hyper-inflammation take place at the cellular level, then summarise all current therapeutic efforts to treat severe COVID-19 and their limitations, and finally detail how MSCs can oppose SARS-CoV-2 at multiple levels. Most interestingly, MSCs kill two birds with one stone by wielding both anti-viral and broad anti-inflammatory properties. Moreover, they partake directly and indirectly in tissue repair by cell differentiation and stimulation of the local stem cells respectively, and provide a broad selection of aid packages in form of secreted molecules, including mitochondria. The main bottleneck for a pharmaceutical use of MSCs is obviously the standardisation of cell material, culture conditions, and administration protocols. Nevertheless, although more studies are required to reach statistical significance, the comprehensive compilation of clinical studies in the review condense into a highly promising outlook.

Plant power

Confronted with the bottleneck of synthetic drug development, the interest in active natural compounds has been steadily rising [56]. In this context, Swain et al. attempt to promote the investigation of phytochemicals to fight SARS-CoV-2 *via* a structure-based *in silico* analysis [8]. Based on a literature compilation, the authors focus on the SARS-CoV-2 cysteine-like protease, crucial for the maturation of viral proteins, as a target. 78 phytochemicals with anti-SARS-CoV-2 activity are subjected to a thorough structural analysis and docking studies in order to evaluate their inhibition potential and the underlying molecular mechanisms. Several clusters of structurally similar inhibitors are identified, including compounds with theoretical potentials similar to antivirals currently in use, which could be further explored or used as templates for drug design.

Lessons from the past

Coronaviruses have probably been around for eons, but until recently, only four variants were known to infect humans, causing merely mild infections. For unknown reasons however, the past two decades have seen the emergence of three strains causing severe respiratory disease in humans, starting with the SARS-CoV and the MERS-CoV outbreaks in 2002 and 2012 respectively [57].

The COVID-19 pandemic that currently keeps the world on tenterhooks is caused by SARS-CoV-2, sharing nearly 80% of genetic similarity with SARS-CoV. Hence, Abdelghany et al. suggest to draw further knowledge from the closest relatives of the virus, in order to predict the evolution of the ongoing crisis [58]. Although written still under the premise of no available vaccine against the virus, the authors list several compelling points of similarity between the three strains, such as their zoonotic origins, molecular features, clinical symptoms, and treatment options, leading them to forecast a three-year duration for the COVID-19 pandemic. Despite the fact that vaccines against SARS-CoV-2 are available by now, the review's call for a rigorous scrutiny of all three coronaviruses dangerous to humans remains as topical as ever, considering the odds that similar threatening strains will emerge in the foreseeable future.

Brief communication

Testing the tests

Rapid and extensive testing has proven absolutely key to fight the COVID-19 pandemic, with a country's testing capacity being closely proportional to their success in managing the outbreak through contact tracing, enforcing self-isolation, and early treatment onset [59]. While reverse transcriptase polymerase chain reaction (RT-PCR) based tests, able to detect even very low levels of viral RNA in sputum, have proven the best method to date, differences in protocols and material exist. Hence, You et al. have undertaken here the comparison of the automated Roche cobas 6800 SARS-CoV-2 kit and the Taiwan Centers for Disease Control (CDC) protocol [2]. They find an overall very good agreement between the two methods, with the system by Roche displaying a slightly higher detection sensitivity and less need for manual handling.

Conflicts of interest

The author declares no conflict of interests.

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To Jan Schley, my wonderful proofreader.

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