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FACULTAT DE FARMÀCIA

FINAL DEGREE PROJECT
DEGREE IN PHARMACY

Physiology and Pathophysiology
Biochemistry and Molecular Biology
Cell Biology

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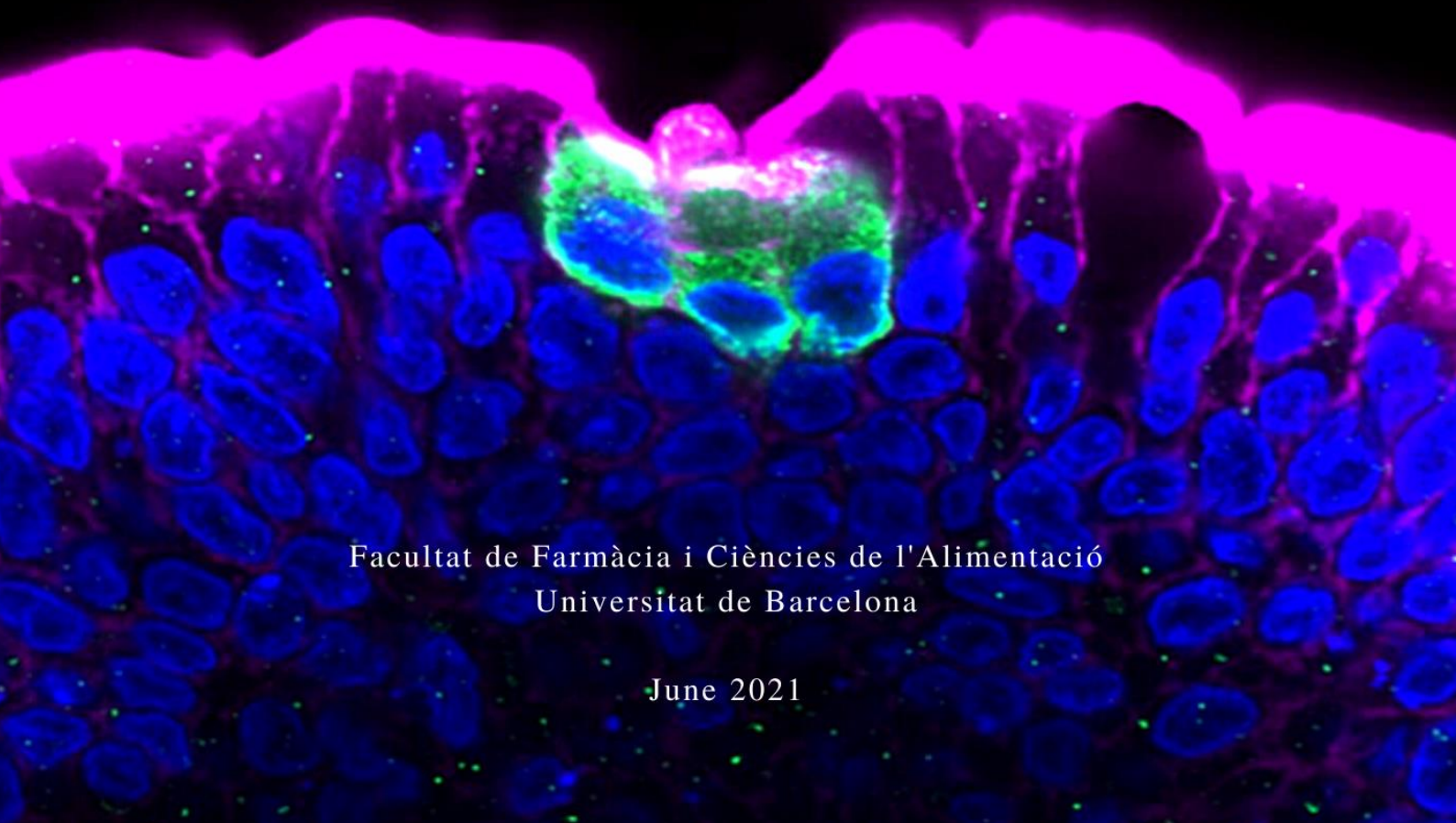
ORGANOIDS
in COVID-19 research



Facultat de Farmàcia i Ciències de l'Alimentació
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Figure 1. Productive infection and cellular tropism of SARS-CoV-2 in human intestinal organoid. The human intestinal organoid was fixed after a low multiplicity of infection (MOI) inoculation and subjected to immunostaining to identify the viral N protein (green)-positive cells. From: Zhou J, Li C, Liu X, Chiu MC, Zhao X, Wang D, et al. Infection of bat and human intestinal organoids by SARS-CoV-2. Nature Medicine. 2020;26(7):1077–1083.

"Organoids of individual patients are like avatars: they predict which drug will work best for the patient from which they derive."

– Hans Clever (Stem Cell Biologist)

INDEX

ABBREVIATIONS

(+)ssRNA: single-stranded positive-sense RNA

2D: two-dimensional

3D: three-dimensional

ACE: angiotensin-converting enzyme

ACE2: angiotensin-converting enzyme 2

ASC: adult stem cells

AT2 cells: alveolar epithelial type 2 cells

CASP3: caspase-3 (cysteine-aspartic acid protease 3)

CNS: central nervous system

COVID-19: Coronavirus disease 2019

CYP3A: cytochrome P450-3A

dnCFR: DeepNEU Case Fatality Rate

EMC: extracellular matrix

ESC: embryonic stem cells

E protein: Envelope protein

GOF: "gain of function" mutation

hrsACE2: human recombinant soluble ACE2

INF: interferon

iPSC: induced pluripotent stem cells

LOF: "loss of function" mutation

MPA: mycophenolic acid

M protein: Membrane protein

N protein: Nucleocapsid protein

PEG: polyethylene glycol

QNHC: quinacrine dihydrochloride

RAAS: renin-angiotensin-aldosterone system

RT-qPCR: real time quantitative PCR

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus-2

SC: somatic cells

S-RBD: S receptor binding domain

S protein: Spike glycoprotein

TMPRSS2: transmembrane serine proteinase 2

ABSTRACT

SARS-CoV-2 pandemic has caused that many of research teams around the world have had to focus on the study of its disease, the COVID-19. The knowledge of its infective mechanism and the search for effective treatments have been the main aim for many of them, and this is why they have had to work under pressure and with the latest technology. One of these methods has been the use of Organoids, also known as "mini-organs", on which this review will be focused. Organoids are three-dimensional structures composed of cells and originated *in vitro* from stem cells of different types, depending on the organ to be achieved. Once the structure is formed, it could be used for disease research, in this case for COVID-19 disease, by inoculating SARS-CoV-2 and studying the infective mechanism, the target molecules the virus uses to enter the host cell and spread, and the organs it affects, and thus, organoids could be applied to understand the disease and also to search effective therapies, which most of them will be involved with the angiotensin-converting enzyme 2 (ACE2), which participates in renin-angiotensin-aldosterone system (RAAS), and that it also turn out to be the receptor that virus uses to enter the cell. By blocking this enzyme, the researched drug is able to prevent SARS-CoV-2 infection.

La pandèmia per SARS-CoV-2 ha fet que molts dels equips d'investigació arreu del món hagin hagut de focalitzar-se en l'estudi de la seva malaltia, la COVID-19. El coneixement del seu mecanisme d'infecció i la recerca de tractaments efectius han estat el principal objectiu de molts d'ells, i per això han hagut de treballar a contrarellotge amb les més altes tecnologies. Un d'aquests mètodes és la utilització d'Organoides, també coneguts com "mini-òrgans", en els que ens centrarem en aquest treball. Els organoides són unes estructures tridimensionals formades per cèl·lules que s'originen de manera *in vitro* a partir de cèl·lules mare de diferents tipus, segons l'òrgan que es vol aconseguir. Una vegada formada la estructura, es poden utilitzar per a la investigació de malalties, en aquest cas la COVID-19, inoculant el SARS-CoV-2 i estudiant el mecanisme infectiu, les molècules diana que utilitza per entrar a la cèl·lula hoste i disseminar-se, i els òrgans als quals afecta, i així poder aplicar aquests organoides per entendre la malaltia i també per buscar teràpies efectives, la majoria de les quals tenen a veure amb l'enzim convertidor d'angiotensina 2 (ECA2), que participa en el sistema renina-angiotensina-aldosterona (SRAA), i que, a més, resulta ser el receptor que utilitza el virus per entrar a la cèl·lula. Bloquejant aquest enzim, el fàrmac subjecte a estudi és capaç d'impedir la infecció del SARS-CoV-2.

Key words: | Organoids | Stem cells | SARS-CoV-2 | COVID-19 | | S protein | ACE2 | TMPRSS2 | Drug screening |

FIELD INTEGRATION

The present review encompasses several fields due to its broad scientific spectrum. The main field is Physiology and Pathophysiology since it studies the mechanism of COVID-19 disease, how it works at systemic level but also at organs: it is a multiorgan disease and this review will discuss the organs affected. Therefore, it also involves the area of Cell Biology, which will help to understand, through Organoids, the cellular effect that SARS-CoV-2 infection implicates, and the pathways involved. In addition, the understanding of organoid functioning and its stem cell-origin are included in this field. Finally, it integrates the area of Biochemistry and Molecular Biology since it allows a more concrete vision on a smaller scale showing molecular processes such as infective mechanism using ACE2 and TMPRSS2, that can be seen in the organoid, and the targets that possible therapies could be focused on.

INTRODUCTION

1. ORGANOIDS: History, formation, and applications

For the past decades, research has been limited to two-dimensional (2D) cells as *in vivo* methods for human biology and histology studies. These scientific advances have been useful for the knowledge of many processes, mechanisms, and functions of the human body and its pathologies. However, 2D cells models have a lack of spatially organization, proliferation, and differentiation, and therefore, they are unable to simulate vital aspects such as compartments, systems, functions, etc. and are restricted to cellular and molecular analysis due to their immaturity, usually monotype and they have no tissue architecture (1,2). 2D cells have been limited to their use in early diseases research as they mimic foetal cells rather than adult ones (1).

Thus, science has developed an alternative based on three-dimensional (3D) cells as an *in vitro* method to supply the gaps that 2D cells implied, so they could advance in pathologies research and their therapy, in toxicology, and even in a possible application in medical transplantation (3). This 3D cells system is called Organoid and it consists of small pieces of tissue created in the laboratory, which has the ability to self-organize, grow, and form a 3D structure simulating the architecture and functions of a determinate realistic tissue or organ which can also produce biomolecules, mucous, etc. (2,4).

The idea of these 3D structures started in 1910, when H.V. Wilson observed that a sponge divided into its cells and then joined randomly, has the ability to reorganize and be a new realistic sponge, demonstrating that cells have enough information to form complex structures without additional information (3). In 1950, many laboratories studied the same "disaggregation + reaggregation" method with vertebrate animal cells and also had the ability to self-organize: in 1952, A. Moscona and H. Moscona conducted an experiment with tubular epithelial cells and mesenchymal cells. Once aggregated and incubated, epithelial cells had formed tubules, and mesenchymal cells became into stroma that surrounded the tubules. That was the closest structure to a modern organoid of the time, and also demonstrated the self-organization as mentioned (5).

Mid-century, several scientists discovered that tissues from different species could aggregate and behave as one. In 1956, Moscona confirmed it by mixing mouse and chick

embryonic cells, and they formed one only organoid. Each type of tissue associated with the same type of cells, thus it concluded that cells are destined to make a determinate tissue and that the type of cells are more important than the species they come from (3).

In 1975, Howard Green and James Rheinwald set the idea of organoids in motion by mixing human keratocytes and mouse fibroblasts, and as result it was obtained squamous epithelium with cell division in the basal layer and differentiated keratinized cells in the external layers. It was the first project that cultivated realistic 3D tissue without transforming cells. Likewise, in 1987 Mina Bissel demonstrated the advance that entail cultivating 3D cells in a hydrogel by showing pathological state in mammary epithelial tissue, innovating the field of *in vitro* morphogenesis (2).

Afterwards in 2006, Hans Clever cultivated intestinal stem cells in an artificial tissue-like matrix. Stem cells were proliferating and finally formed a 3D organized structure which contained both stem cells and differentiated cells. This structure was considered the first organoid (2).

Nowadays, Organoids can proceed from different types of cells, such as embryonic stem cells (ESC), induced pluripotent stem cells (iPSC), adult stem cells (ASC), somatic cells (SC), or even cancer cells, depending on the required type of tissue. For instance, ESC are responsible for embryonic organ development due to their ability of unlimited proliferation, and ASC allow organ regeneration due to their undifferentiated pluripotent cells (4,6). Therefore, organoids from ESCs or iPSCs are acquired from foetus tissues that include the tools to produce a determinate organ.

From this point, organoids can be developed by differentiation and proliferation pathways producing a group of mixed cells so it would be creating different types of tissues and compartments. In this way, a kidney organoid would contain ureteric stem cells, nephrons stem cells, stroma stem cells, etc. so it would be an *in vitro* realistic renal structure with realistic histology (2,3,6,7). That is why organoids can also be called “mini-organs”.

The entire methodology described is shown in Fig. 2:

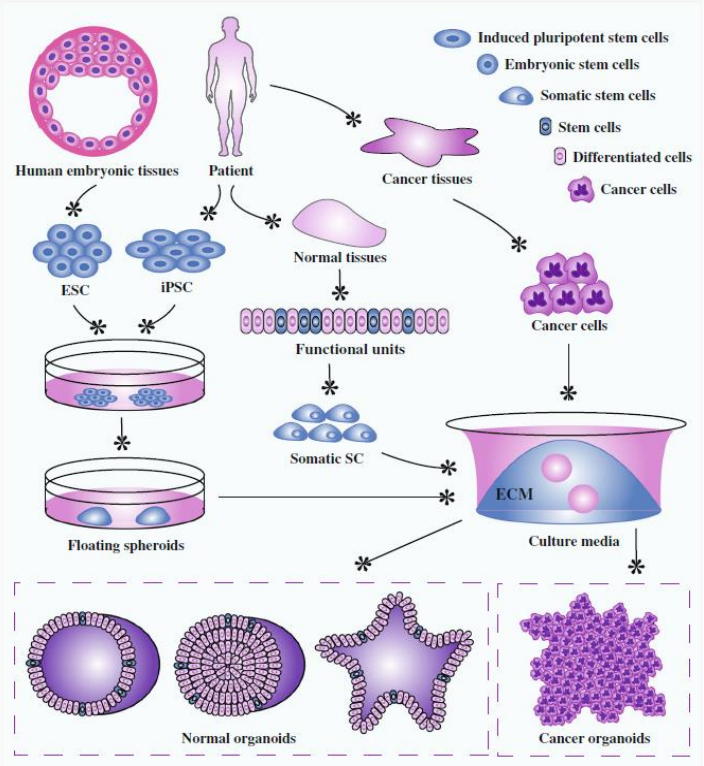

Figure 2. Organoid establishment from stem cells and cancer cells (7).

To proceed with the culture of the organoids, it is needed a hydrogel matrix where tissue will be able to grow, that is why culture medium must contain essential components that allow proliferation and differentiation of the cells and even signaling pathways, migration, and selection of molecules (2,6). Hydrogel consists of a synthetic highly hydrated polymer structure which comes from decellularized extracellular matrices (dECM) derived from mouse sarcomas. The most common polymer for hydrogel is polyethylene glycol (PEG). However, this structure has limitations in reproducibility and growth so it must be added some components such as type I collagen, gelatin, hyaluronic acid, laminin, fibrin, some ECM proteins, proteoglycans, growth factors and other adhesion components (8,9). Once sample and culture are prepared, the whole is taken to a bioreactor where cells will self-organize, and tissue will grow (2).

The formation of organoid structures is based on a thermodynamic hypothesis that Malcolm Steinberg expounded on in 1963 which consists in the adhesion system that cells have on their surface. Each cell has a different adhesion strength, and they group depending on that energy, therefore Steinberg saw that homotypic systems joined together stronger than heterotypic systems (3). That would explain the ability of movement and self-organization mentioned before.

In 2005, Steinberg and Ramsay A. Foty carried out an experiment based on clone cells which had the same adhesion strength and sorted into the different amounts of adhesive molecules they had in the core. This fact confirmed the organization theory and explained that it is more important the number of adhesion molecules than the type (3), hence heterotypic and homotypic systems can adhere with the same strength. However, nowadays there are not enough information about these adhesion mechanisms yet, that is why there is controversy among scientists.

Once cells have grouped, organoids can continue differentiating and proliferating by exogenous factors that promote signals to get the organogenesis (4).

Formation, differentiation, proliferation, maturation, and the complete process of the organoid creation lasts various months from the culture preparation to obtain the entire structure ready to research use (10).

Recently, organoid technology has been developing and covering more scientific and medical fields therefore nowadays they are useful for important studies on pathology, toxicology, therapy, even in transplantation. As it is known, organoids have a significant regeneration potential, and it could be an important method to replace diseased or non-functional tissues or organs. However, this technique is not widely developed yet due to the scant information available in this field, for instance organoids lack vascular system.

Organoids allow to evaluate drug development, efficacy, and pharmacokinetics due to the ability to accurately predict drug responses for each patient individually. In addition, it has helped to study drug toxicology without the need of animals due to the fact that “safety in animals does not mean non-toxicity in humans”. Furthermore, organoids allow to accomplish the desire to Refine, Reduce and Replace the use of animals in research (3,11).

The most important advantage is the application of organoids to study human diseases and the development of their therapies. Organoids are useful for modelling intestinal diseases, renal diseases, kidney diseases, brain diseases, genetic diseases, cancer, infectious diseases, etc. (2,6,11).

In particular, in this review we will focus on the way we could use organoids for the COVID-19 research.

2. SARS-CoV-2 AND ITS INFECTIVE MECHANISM

As we know, COVID-19 is a multiple organ disease caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2), responsible for the 2020 pandemic that still persists. COVID-19 manifests several symptoms such as fever, fatigue, headache, dry cough, dyspnoea, bronchitis, pneumonia, and other severe respiratory illnesses, also diarrhoea and other gastrointestinal manifestations, and some neurological symptoms such as loss of smell and taste (12–14). These symptoms appear after approximately 5.2 days of incubation and can last 41 days with a median of 14 days from the beginning of the symptoms, shorter in patients over 70 years old (14). In about 80 – 90% of cases the infection is asymptomatic or mild, but around 10% of patients have serious manifestations, and 5% can suffer a multiorgan failure that can cause death (15).

The virus is transmitted from person to person via aerosols through liquid droplets or contaminated surfaces. Therefore, it is important a well hand hygiene and wearing an individual equipment including mask. Social distancing of 1.5m and surface disinfection using ethanol, hydrogen peroxide or sodium hypochlorite can also be effective (15).

SARS-CoV-2 is a (+)ssRNA virus composed of 30-nucleotides which form four major structural proteins in its capsid: Spike glycoprotein (S), Envelope protein (E), Membrane protein (M) and Nucleocapsid protein (N) (Fig. 3) (16).

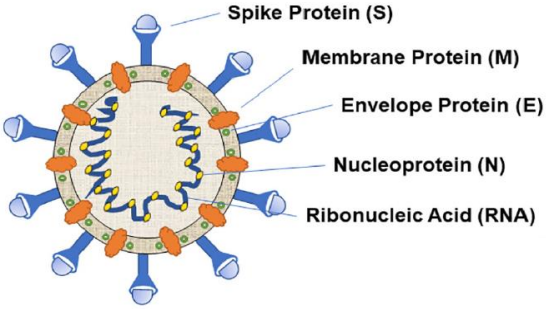

Figure 3. Schematic representation of the SARS-CoV-2 structure (16).

This virus needs cells' machinery to replicate and infect others. The process initiates with the entry of the virus into the host cell by binding the viral S protein to a cellular membrane enzyme which will act as a receptor: angiotensin-converting enzyme 2 (ACE2) (13). It occurs with the help of another enzyme called transmembrane serine proteinase 2 (TMPRSS2) which primes the S protein, and it is highly expressed in olfactory

epithelium, nasal goblet and ciliated cells, bronchial transient secretory cells, small intestine enterocytes, etc. (17,18).

ACE2 is present in many organs since it maintains homeostasis and negatively regulates the renin-angiotensin-aldosterone system (RAAS). To know the mechanism of SARS-CoV-2 infection it is necessary to understand the RAAS.

In the first place, Angiotensinogen is catabolized in plasma by Renin giving Angiotensin I, and then converted into Angiotensin II by ACE on the surface of many vascular endothelial cells. Angiotensin II interacts with AT1 receptor causing vasoconstriction and stimulating the secretion of Aldosterone, which increases blood pressure by favouring the sodium reabsorption. On the other hand, ACE2 degrades Angiotensin I and II to Angiotensin 1-9 and 1-7 respectively. Angiotensin 1-7 interacts with MAS receptor and induces vasodilation, balancing the vasoconstriction effect of Angiotensin II (Fig. 4) (16,19).

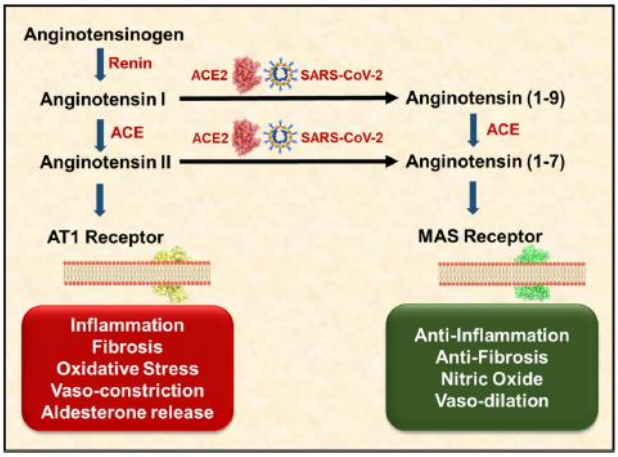

Figure 4. Showing role of ACE2 in SARS-CoV-2 infection (16).

As soon as SARS-CoV-2 enters the organism, S protein interacts with ACE2 and its expression levels diminish causing a decrease of the Angiotensin 1-7 effects, therefore a deterioration of the tissue and a worse evolution of the disease (19).

Due to the high presence of ACE2, SARS-CoV-2 affects many organs such as lungs, kidney, heart, brain, pancreas, gastrointestinal tract, and vasculature (Fig. 5). For this reason, patients with obesity, diabetes, heart diseases, asthma, etc. have worse prognosis due to COVID-19 since ACE2 levels are elevated to counteract the high levels of Angiotensin II that have these health problems (13,19).

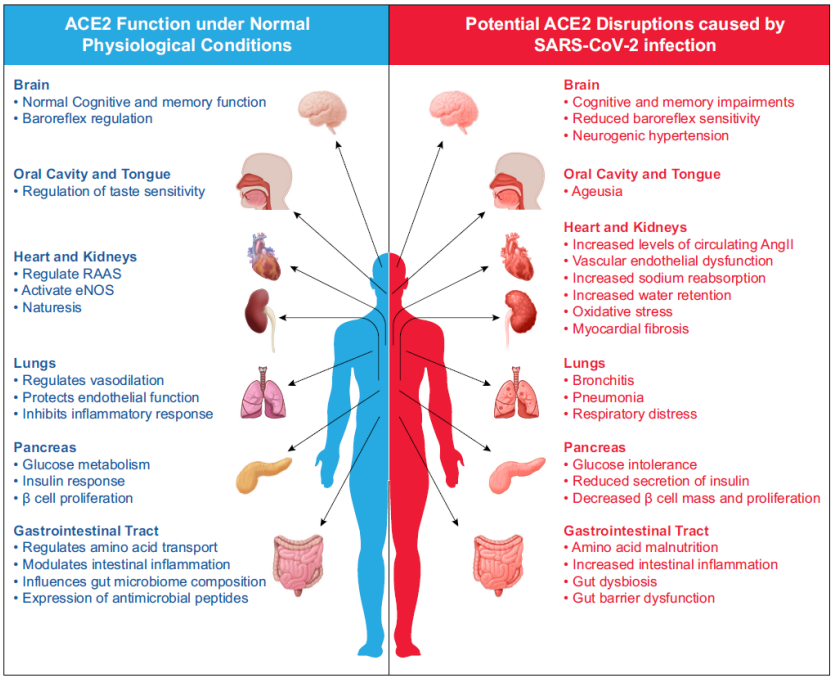

Figure 5. An organ review of normal ACE2 function versus potential pathophysiological consequences of ACE2 disruption caused by SARS-CoV-2 infection (13).

As mentioned before, SARS-CoV-2 needs cells' machinery to replicate, therefore the genome uses cellular ribosomes and enzymes, and it is rapidly replicated. At this moment there are new virions in the host cell that will leave and travel around the organism infecting other cells (Fig. 6) (16,20).

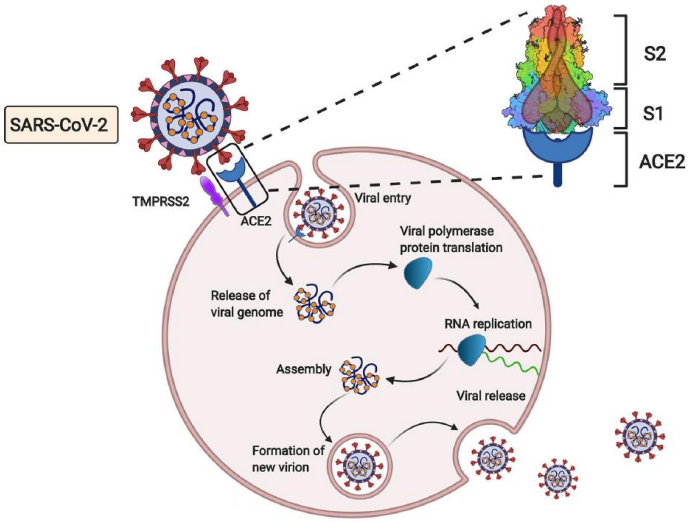

Figure 6. Simplified depiction of SARS-CoV-2 lifecycle (20).

The patient will experience an activation of their immune system and T cells, secondary messages and humoral antibodies will activate recognizing viral proteins (16).

OBJECTIVES

In this review, the main aim is to study the application of these Organoids in the SARS-CoV-2 research. To do so, it is necessary to establish the following objectives:

- To know in depth how these *in vitro* structures work.
- To analyse infection and replication mechanisms the current virus carries out.
- To be able to perform the infection in the organoid.
- To observe how SARS-CoV-2 works in organoid cells in order to study the disease.
- To search for possible therapies to battle COVID-19.

MATERIAL AND METHODS

For the development of this review, several databases such as *PubMed*, *Google Scholar*, *Scopus* and University of Barcelona's database '*Cercabib'* have been resorted to proceed with the research. Scientific journals such as *Nature* have also been consulted at some moments of the process.

For most of the bibliographic search, filters were restricted to recent documents, from the last 5 years maximum, ordered by number of citations and also by relevance.

First of all, search was based on finding general information about the studied method using keywords: "Organoids", "Stem cells", "Mini organs" or "3D cell method" in order to understand the technique and have a basis for further study. Likewise, the next step was to look for information about "SARS-CoV-2" and its "COVID-19" disease. When some specific information about some mechanism or enzyme was needed, the keyword was the interested one accompanied with the infection implicated, for example "ACE2 SARS-CoV-2".

Once the information had been collected separately and a knowledge basis had been established, it was time to go in depth. The next step was to conduct a bibliographic search about organoids technique used for COVID-19 research, thus it was used "SARS-CoV-2 organoids", "COVID-19 organoids" or "COVID-19 drugs organoids".

When articles, journals or books of interest were found, they were entered into *Mendeley* library, or even some of them were taken directly from this database using the same keywords. Once all the documents were added to the library, the last step was to read them and synthesise for a proper understanding of the method and then proceed to the compilation and transcription of the information of interest for this review.

Regarding the figures and tables that are in this review, they all are taken from articles with Open Access which let the reader use them with no permission required.

RESULTS

Due to the impact COVID-19 pandemic has had, many scientists have thrown themselves into its research. To understand how SARS-CoV-2 works has become priority, and organoid technique offers considerable advantages to study viral agents and their diseases. This review focuses on the applications of organoids for the COVID-19 research. They help to study which organs SARS-CoV-2 affects, how, the targets involved, and the effectivity of the treatment. To do so, a human structure-like organoid is essential to replicate the model of the real viral infection.

To achieve de ideal conditions for the SARS-CoV-2 infection, organoids must have high levels of ACE2 and TMPRSS2 on their cells so the virus can enter (21). ACE2 is highly expressed in most of the organoids and TMPRSS2 is present in all of them. In addition, each organoid has different type of cells and each type of cells has different levels of ACE2 and TMPRSS2 expression (22).

Therefore, many organoids are useful since SARS-CoV-2 infects variety of cells: bronchial organoids, lung organoids, kidney organoids, liver organoids, intestinal organoids or blood vessel organoids can be used (Tab. 1) (23). That is the main reason to use organoids as an excellent method to study this viral mechanism and for research therapies and analyse drugs effectivity. Organoids can also help to a better understanding of the molecules and targets implicated in SARS-CoV-2 pathogenesis (22).

Table 1. Organoids currently being used in COVID-19 research (23).

Type	Origin	Key points
Human bronchial organoids	Generated from commercially available human bronchial epithelial cells	After SARS-CoV-2 infection, not only the intracellular viral genome, but also progeny virus, cytotoxicity, pyknotic cells, and moderate increases of the type I interferon signal can be observed.
Human lung organoids	Generated from human embryonic stem cells	The lung organoids, particularly alveolar type II cells, are permissive to SARS-CoV-2 infection.
Human kidney organoids	Generated from human embryonic stem cells	Human kidney organoids produce infectious progeny virus.
Human liver ductal organoids	Generated from primary bile ducts isolated from human liver biopsies	Human liver ductal organoids are permissive to SARS-CoV-2 infection, and SARS-CoV-2 infection impairs the bile acid transporting functions of cholangiocytes.
Human intestinal organoids	Generated from primary gut epithelial stem cells	Human intestinal organoids were readily infected by SARS-CoV-2, as demonstrated by confocal and electron microscopy. Significant titers of infectious viral particles were detected.
Human blood vessel organoids	Generated from human induced pluripotent stem cells	SARS-CoV-2 can directly infect human blood vessel organoids.

In contrast with the organoids in Table 1, many scientists agree that brain organoid is not the best option to study SARS-CoV-2 due to the low ACE2 expression in its cells. The virus will have difficulties to enter and replicate. Thus, despite brain organoids may help to understand the central nervous system (CNS) effects of SARS-CoV-2, they are not entirely useful. An available option to study neurodegeneration effects would be using transgenic mice cells with higher levels of ACE2 on them (12). However, SARS-CoV-2 do have CNS effects and it has two possible reasons: low levels of ACE2 are sufficient for viral entry, and also since there still are viral entry factors unknown (12).

On the other hand, ACE2 and TMPRSS2 are highly expressed in airway epithelia, therefore bronchial organoids and lung organoids can be an excellent option to study SARS-CoV-2. Furthermore, airway organoids have been used for studying the immunological responses to the virus by co-culturing with immune cells, and observe the secreted molecules in response (Fig. 7). Airway organoids are also useful to research antiviral therapies for COVID-19 disease by laboratory methods such as RT-qPCR to evaluate viral load, immunofluorescence and electron microscopy to recognize infected cells and to observe cytopathy, and microarray analyses which can identify mechanism and targets of the researched drug (18).

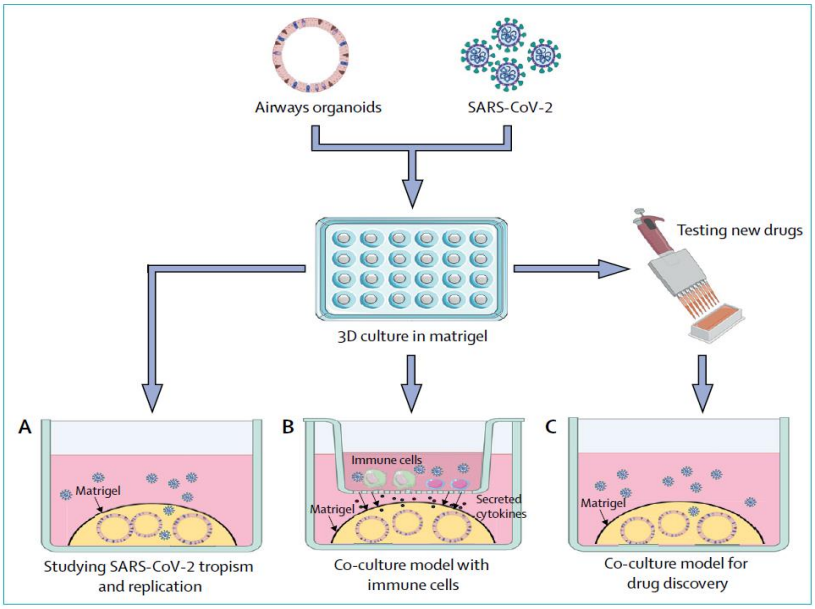

Figure 7. Co-culture of airway organoids (18).

As a restriction, organoids lack immune components and blood vessels, thus it is necessary to include these cells in the culture of development (10,18).

Once the appropriate organoid is chosen and created, it is transferred into a tube and exposed to a viral inoculum and then incubated for 24h at 37ºC so the virus can enter the cells by ACE2 and S protein binding. 48h later, S protein expression increases 57% which means that the virus has replicated, and infection has spread (24). This method must be done at a P3 safety laboratory on account of the viral particles handling (12).

HUMAN ORGANOID APPLICATIONS FOR COVID-19 RESEARCH

Organoids have lots of applications for the research of COVID-19 disease. They can be useful for studying SARS-CoV-2 biology, its infective mechanism, its cellular tropism, the epithelial responses as well as immune system response, and most important, organoids help to study drug effectivity for therapy development (Fig. 8). Organoids also allow to study viral responses individually (25).

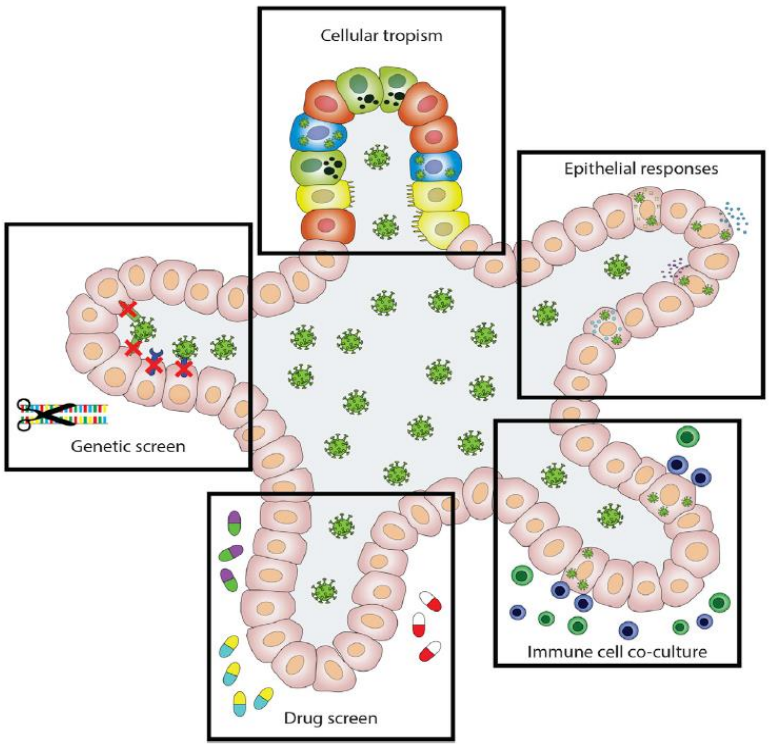

Figure 8. Overview of the Potential of iPSC and ASC-Derived Organoids in Virology Research (25).

As mentioned, one of the most important applications of organoids is the drug screening to analyse which therapy is the best candidate to treat COVID-19 disease:

COVID-19 THERAPY SCREENING IN HUMAN ORGANOIDS

Recent studies have focused on therapy research to fight the current pandemic. Many of these studies have been developed in organoids as an effective method to simulate human organisms: vaccines, antivirals and other drugs have been evaluated in order to improve COVID-19 symptoms (10).

Most of the research projects focus on blocking ACE2 receptor, which is the main target for viral entry, and also TMPRSS2 (17). Several alternative targets could be RAAS inhibition (16) or AT1 receptor block (19), for instance.

Some of these drug screenings are described hereunder:

A. REMDESIVIR, FAMOTIDINE AND EK1 ANALYSIS

It has seen that intestinal organoids can serve as a method to study therapies against SARS-COV-2. There is an experiment where Remdesivir, EK1 and Famotidine were compared as effective drugs to inhibit viral infection and replication (24).

Some of the COVID-19 symptoms are diarrhoea and nausea since SARS-CoV-2 affects the gastrointestinal tract. ACE2 and TMPRSS2 are highly expressed in intestinal cells and the virus can easily enter, infect, replicate and disseminate. That is why intestinal organoids are useful to observe antiviral effects. They will mimic real intestinal structure, thus the therapy effectivity can be analysed.

The experiment consists in creating an intestinal organoid and then inoculate with SARS-CoV-2. An increase of RNA replication will be detected by marking viral S protein, and a deterioration of the tissue will be observed. During this study, an increase of CASP3 (Caspase-3) was detected in infected cells, which means a first stage of apoptosis. Only goblet cells lacking ACE2 are not affected (24).

Remdesivir is an ATP analogue which acts as antiviral drug by interfering with new viral RNA in the replicate process and avoiding its binding with the natural ATP (26). Remdesivir was administered into the organoid in different doses: 25nM had no results, 125nM did low effects, but at a dose of 500nM had an 86% decrease of infection. At 5mM the infection was almost eliminated, likewise viral copies were decreased. The

study suggested that Remdesivir has a successfully dose-dependent inhibitor effect of viral infection and replication (24).

On the other hand, Famotidine is an antagonist of histamine-2 receptor and thus, it is used for diseases that are associated with stomach acid (27), It acts as an immunomodulator since it counteracts the histamine effects by favouring the immune system activation. Famotidine was tested at a dose of 2.5mM with no effects, unlike Remdesivir, which had visible results at a dose of 125nM. Therefore, in spite of its gastrointestinal use, Famotidine did not produce a reduction of viral infection (24).

Lastly, it was observed the effects of EK1, a peptidic pan-coronavirus fusion inhibitor, which inhibit viral infection (28). EK1 had effects at a dose of 10 mM decreasing a 38% the levels of S-positive cells (24).

The study concluded that Remdesivir and EK1 were effective against SARS-CoV-2 whereas Famotidine did not block viral infection (Fig. 9). Hence, Remdesivir is a proper option to battle gastrointestinal infection of COVID-19 and relieve its symptoms by rescuing cell morphology.

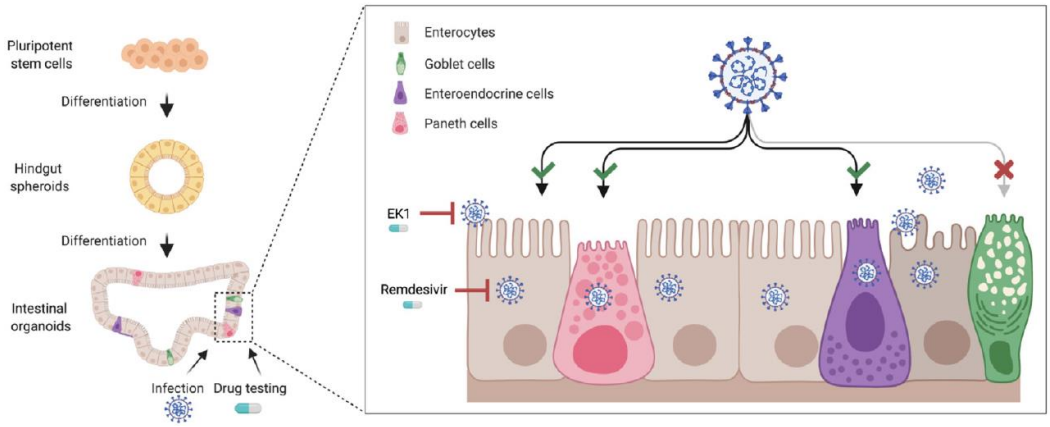

Figure 9. Infected intestinal organoid and drug testing against SARS-CoV-2 (24).

B. REMDESIVIR + COBICISTAT

Cobicistat is an inhibitor of Cytochrome P450-3A (CYP3A) and also blocks viral replication by preventing the binding between S protein and ACE2. On the other hand, Remdesivir is an ATP analogue as described, effective at inhibiting SARS-CoV-2 replication, and also it is a CYP3A target. Therefore, it could be a synergistic effect

between Cobicistat and Remdesivir. To confirm this fact, a study used colon organoids, which has a high expression of CYP3A corresponding the real tissue levels (29).

Colon organoids were infected with SARS-CoV-2 and incubated for 2h. Afterwards, it was performed a treatment with Remdesivir + Cobicistat. 48h post-treatment it was observed that Cobicistat favours antiviral effect of Remdesivir by blocking its metabolizing enzyme CYP3A. Therefore, this combination causes an increase of Remdesivir concentration and a higher effect (29).

Also, it was confirmed the Cobicistat effect of blocking viral replication by avoiding membrane fusion between SARS-CoV-2 and host cell (29).

Hence, Cobicistat is an effective drug for the COVID-19 treatment separately and in combination with Remdesivir.

C. HUMAN RECOMBINANT SOLUBLE ACE2 (hrsACE2)

HrsACE2 is a recombinant protein which interacts with viral S protein mimicking ACE2, therefore the binding between the virus and cells is blocked (Fig. 10) (30,31). This study has been conducted in blood vessel and kidney organoids since ACE2 is highly expressed. In addition, SARS-CoV-2 can be detected in urine and in blood (31).

The virus was inoculated, and the organoid was monitored during 6 days after the exposure. Thus, an increase of viral RNA was detected. Therefore, it proceeded to hrsACE2 administration. After 3 days, it was demonstrated that this treatment lessens viral propagation by blocking the viral entry (30,31).

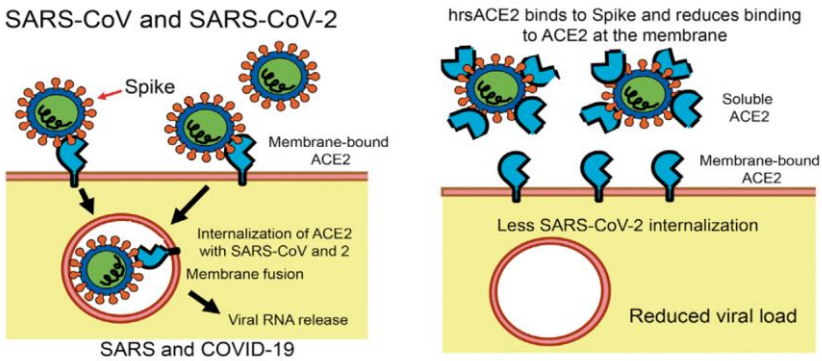

Figure 10. SARS-CoV-2 replicates in human blood vessel and kidney organoids which is inhibited by hrsACE2 (31).

However, there is a limitation in this trial: hrsACE2 study focused on the early stages of infection since the protein blocks viral entry in host cells, but there is no evidence of this treatment in later stages of the disease (31).

D. INTERFERON LAMBDA 1

In lungs, ACE2 is mainly expressed in ciliated cells, alveolar type II cells and also alveolar type I cells. When the virus attacks alveoli, it mostly affects alveolar type I cells disrupting the oxygen transport between alveolus and blood and causing pneumonia. However, ciliated cells are the main target as it contains the highest levels of ACE2 (32).

This study consists in a bronchioalveolar organoid to observe effectivity of a particular COVID-19 therapy: interferon lambda 1 (IFN-λ1) (32). IFN are proteins secreted by organism in adaptative response of the immune system against viral infections, therefore they have been used as antiviral drugs. In addition, SARS-CoV-2 blocks INF-λ receptor, disrupting the immune system pathway (33).

In the experiment, it was used a lung organoid, and a dose of IFN-λ1 was administered. It was observed that viral replication was reduced demonstrating that IFN are an effective option to battle COVID-19 (32).

E. IMATINIB, MPA AND QNHC

This experiment was tested in lung and colonic organoids. It consists in studying the effects of three drugs that belong to the group of antineoplastics and immunomodulators: Imatinib, mycophenolic acid (MPA) and quinacrine dihydrochloride (QNHC).

Imatinib is an antineoplastic inhibitor of the proliferation and induces apoptosis of abnormal cells in some types of cancer (34). MPA is a selective immunosuppressor which acts avoiding guanosin incorporation into DNA by blocking new guanosin synthesis, and it is used as a prophylaxis method in organ transplants rejection and to treat autoimmune diseases (35). And finally, QNHC is an antineoplastic used as antiparasitic (36).

Three lung organoids were pre-treated with 10µM Imatinib, 3µ MPA and 4.5µM QNHC respectively. Then, each organoid was infected with SARS-CoV-2. After 24h, qRT-PCR confirmed a reduction of viral RNA replication in the three organoids (37).

Now, same drugs and same doses: 10µM Imatinib, 3µ MPA and 4.5µM QNHC, but administered in colonic organoids, prior to SARS-CoV-2 infection. Then incubated for 24h and a reduction of RNA levels were detected (38).

Additionally, Imatinib not only affects virus replication but also maturation since it disturbs fatty acid biosynthesis. Fatty acids participate in membranes fusion, virion maturation and replication. Therefore, Imatinib is an effective drug for SARS-CoV-2 which disturbs viral entry by blocking the S protein and preventing membrane fusion, and also affects virus replication and maturation (37).

In the two cases, both lung and colonic organoids, an upregulation of inflammatory factors were detected, such as interferon and cytokine (38).

These experiments suggested that Imatinib, MPA and QNHC are effective drugs to battle COVID-19 (38).

ORGANOID STUDIES FOR COVID-19 RESEARCH

Besides the drug screening, organoids can also be useful for many other studies such as SARS-CoV-2 biology, its infective mechanism, its targets, its cellular tropism, the COVID-19 pathogenesis, or the epithelial responses as well as immune system response.

In this review many of these investigations have been consulted and their projects have been analysed to know the progress and contribution to this research.

To conclude, a summary-table (Tab. 2) will compile the organoids that have been used for the last year and the projects developed:

Table 2. Organoid studies for COVID-19 research.

ORGANOID	STUDY	RESULTS
Lung organoid (18)	Infectivity and cytopathy Immune system responses Drug screening	RT-qPCR detection of viral replication kinetics and variations in gene expression due to viral infection. Flow cytometry observation of cell variations after infection. Immunological profiling recognition of signalling pathways and cytokine secretion to detect cytokine release syndrome in COVID-19 patients. Microarray analysis of molecular mechanism of drugs and their targets.
Lung organoid (39)	COVID-19 pathogenesis New therapies and vaccines research	Determination of the most easily infected cell in the lungs by detecting expression levels of ACE2 and TMPRSS2: Alveolar epithelial type 2 (AT2) cells and ciliated cells. Consideration of SARS-CoV-2 reproducibility, propagability and scalability with drugs or vaccine, and also cost-effectiveness of the therapy.
Lung organoid (40)	COVID-19 pathology	Detection of AT2 cells, AT1 cells, club cells and ciliated cells. Identification of SARS-CoV-2 target cells with higher ACE2 expression: AT2 cells. Detection of RNA replication. Confirmation of pneumonia association with SARS-CoV-2.

Lung organoid (41)	Drug screening	Confirmation of cell function. Chloroquine, Hydroxychloroquine, Umifenovir, Lopinavir, Ritonavir, Favipiravir efficacy and safety
Lung organoid (42)	Prediction and evaluation of mutations on SARS-CoV-2	Infection of AT1 and AT2 cells. DnCFR detection of mutated S receptor binding domain (S-RBD), M protein and N protein. Identification of Gain of function (GOF) mutation or loss of function (LOF) mutation. Evaluation of drugs, antibodies or vaccines that target these mutations.
Bronchioalveolar organoid (32)	Alveolar type 2 (AT2) cell infection	Lower ACE2 expression in healthy AT2 cells than in infected AT2 cells. Ciliated cells are more easily infected than AT2 cells. Ciliated cells are the principal target. Effective treatment with Interferon λ1 to reduce viral replication and dissemination.
Brain organoid (12)	SARS-CoV-2 targets	SARS-CoV-2 modelling and its preference for cortical neurons soma over neural stem cells. Confirmation of CNS infection but inability to replicate due to the lack of replication factors in neurons. Detection of ACE2 low expression.

Liver organoid (43)	COVID-19 pathogenesis Liver damage Drug discovery	Verification of SARS-CoV-2 infection and replication. Higher ACE2 expression in cholangiocytes than hepatocytes. Alteration of barrier function and bile acid transport of cholangiocytes. Bile acid accumulation and liver damage.
Intestinal organoid (44)	Enteric infection of SARS-CoV-2	Identification of different types of cells: enterocyte, goblet cell, Paneth cell and enteroendocrine cell. Evaluation of SARS-CoV-2 tropism and enterocyte infection.
Intestinal organoid (24)	Drug inhibition of SARS-CoV-2 replication	Verification of non-infection in goblet cells. Remdesivir and EK1 effectivity for COVID-19 compared to Famotidine. Detection of morphology rescue by Remdesivir.
Blood vessel and kidney organoid (17)	SARS-CoV-2 infection Drug screening	Confirmation of ACE2 entry target. Human recombinant ACE2 effect against SARS-CoV-2 entry.
Lung organoid (37) Colonic organoid (38)	Drug screening	Demonstration of COVID-19 model in human cells. Evaluation of Imatinib, Mycophenolic acid and Quinacrine dihydrochloride effectivity for COVID-19 disease.

DISCUSSION

Despite the revolution and the importance that the discovery, development and application of organoids has had for the research of diseases in recent years, and due to the high economic cost but also the time involved, there are many scientific teams that have not been encouraged to use them for their studies yet. In addition, in case of this COVID-19 disease, it is so recent that for now there are not many teams that have opted for this technique to understand SARS-CoV-2 infection or to search for its therapy.

To develop this review, practically all the sources available today for the research of COVID-19 in organoids have been consulted, and they all agree that it is a revolutionary technique, extremely useful for the investigation of any type of disease by choosing the appropriate organoid for each of them, and although it is a very recent method, its use and knowledge will continue to grow in the coming years.

It has been observed the number of applications organoids have for the research of COVID-19, from the study of SARS-CoV-2 infective mechanism to the type of cells affected or the therapies that can be effective to battle the disease. There are not many official data for now, there has not been time for studies with many types of drugs or vaccines, but many scientists are involved and will continue to be results in the coming months.

Therefore, as disadvantages, it should be kept in mind that this is a very new technique, of which many scientists have not total certainty to develop these structures yet, due to their elevated complexity and also their high cost, it takes weeks or months to develop the organoid, and more components must be added for their growth due to the lack of immune cells and blood vessels that they have.

On the other hand, as advantages it can be mentioned that organoids are the existing structures closest to an *in vivo* organ, which allows a very important approximation to reality, and thus an investigation of what really happens in the original organism without need to affect a living being, besides a capacity of individualized research. As living beings also refers to animals, which means that organoids become the best technique to avoid the excessive use of laboratory animals.

In addition, it has been demonstrated that stem cells are the future of science, a great revolution that will allow great advances in the coming years and will improve medicine

and thus people's health. It has already seen with creation of organoids that it will be possible to continue being used for the research of diseases, as well as in organ transplantation, placing these organoids as substitutes for damaged organs. Therefore, stem cells will contribute to further advance in science.

CONCLUSIONS

Organoids are a great advance in science, research and medicine for the study of diseases as seen in this review. Likewise, the following statements can be concluded:

- Organoids can be applied to many uses and to investigate many diseases.
- They are complex structures but with today's resources they can be developed easily.
- Organoids are an excellent technique to study COVID-19 disease.
- They have confirmed the SARS-CoV-2 entry method into host cell as well as its pathogenesis.
- They get to have the appropriate targets for the virus to infect cells.
- Lung organoids are the best option to investigate this disease due to their elevated expression of ACE2 and TMPRSS2.
- There are therapies that have been demonstrated its effectivity thanks to the good results in organoids: they have achieved to determinate the appropriate drug candidates to treat COVID-19 as well as discard those that had no effect.

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Author	Source of stem cells	Propagability	Cell types						SARS-COV-2 infection	Demonstrated reproducibility using more than one patient	Cost-effective (use of condition ed media)	Notes
			AT1	AT2	Club	Basal	Ciliated	Goblet				
Zhou et al PMID: 29891677	Small pieces of normal lung tissue adjacent to the diseased tissue from patients undergoing surgical resection for clinical conditions.	Long term culture >1 y	✗	✓	✓	✓	✓	✓	✗ Infection with H1N1 pandemic Influenza virus	✓	✓	Proximal differentiation (PD) of human Adult Stem Cell-derived airway organoid (AO) culture. Differentiation conditions (PneumaCult-ALI medium) increase ciliated cells. Serine proteases known to be important for productive viral infection, were elevated after PD.
Sachs et al PMID: 30643021	Generation of normal and tumor organoids from resected surplus lung tissue of patients with lung cancers.	long term culture for over 1 year	✗	✗	✓	✓	✓	✓ Not clearly mentio ned	✗	✓	✓	airway organoid (AO) expressed **no mesenchyme or alveolar** transcripts. Strongly enriched for bulk lung and small airway epithelial signature limited to basal, club, and ciliated cells Withdrawal of R-spondin terminated AO expansion after 3–4 passages similar to the withdrawal of FGFs
Duan et al PMID: 32839764	hPSC derived lung cells and macrophages	✗	✓	✓	✓	✓	✓	✓ Low	✓ SARS-CoV-2 infection mediated damage onset by macrophages.	✓	✓	Co-culture of lung cells and macrophages. Protocol followed enables alveolar differentiation process, although described presence of almost all lung cell types.
Salahudeen et al PMID: 33238290	Cells sorted from human peripheral lung tissues.	Distal Lung organoid with possibility of long-term culture	✓ From diff of AT2	✓	✓ After diff of basal cells	✓		✓	✓ Infection and presence of dsRNA and nucleocapsid	✓	✓	No RNA seq of infected samples to compare with COVID Differentiation to different cell types SARS CoV2 infection in apical-out organoids (not polarized monolayers). The combination of EGF and the Noggin was optimal, without any additional growth-promoting effects of either WNT3A or R-spondin
Han et al BioRxiv doi: https://doi.org/10.1101/2020.05.05.079095 PMID: 33116299	hPSC-derived lung organoids	Organoids were generated by 50 days of differentiation	✓	✓	✓	✓	✓	✓	✓ SARS-CoV-2 and SARS-CoV-2-Pseudo-Entry Viruses.	✓	✓	AT1, AT2, stromal cells, low number of pulmonary neuroendocrine cells, proliferating cells, and airway epithelial cells were reported. Mostly AT2 based ACE2 receptor was used for virus infection. High throughput screen using hPSC-derived lung organoids identified FDA-approved drug candidates, including imatinib and mycophenolic acid, as inhibitors of SARS-CoV-2 entry.
Youk et al PMID: 33142113	Adult alveolar stem cells isolated from distal lung parenchymal tissues by collagenase, dispase and sorting	Multiple passages upto 10 months	✓ From AT2 Lost in higher passag es	✓	✗	✗	✗	✗	✓ In the organoid form	✓	✓	Single cell transcriptomic profiling identified 2 clusters and type I interferon signal pathway are highly elevated at 3 dpi
Mulay et al doi.org/10.1101/2020.06.29.174623	a) Alv organoids with distal lung epithelial cells with lung fibroblast cells	✓	✗	✓	✗	✗	✗	✗	✓ In the organoid form	✓	✓	Infection of AT2 cells trigger apoptosis that may contribute to alveolar injury. Alteration of innate immune response genes from AT2 cells
	b) Proximal airway ALI with heterogenous cells	✓	✗	✗	✓	✓	✓	✓	✓	✓	✓	Infection of ciliated and goblet cells Two separate models for SARS-CoV2 infection
Huang J PMID: 32979316	iPSC derived AT2 cell ALI model	✓	✗	✓	✗	✗	✗	✗	✓	✓	✓	Bulk RNA seq after day 1 and day 4 infection. The infection induces rapid inflammatory responses.
Tindle et al [Current study]	Deep lung tissue sections surgically obtained from patients undergoing lobe resections for lung cancers.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	RNA Seq and cross-validation of COVID-19 model. Single model with all the cells types and infection of SARS-CoV2 in the 2D form with Apical accessibility that close to physiologic state.

Table I. A comparison of current versus existing lung organoid models available for modelling COVID-19 (39).

INTERVIEW WITH SANDRA ACOSTA

Sandra Acosta is a postdoctoral researcher in Evolutionary Systems Biology Laboratory in the *Instituto de Biologia Evolutiva* (*IBE*) of *CSIC* and *UPF* at *PRBB*, who is developing lung, brain and intestine organoid models for the identification of new treatments against COVID-19.

Buenos días y gracias por atenderme. Me puedo imaginar lo complicados que deben estar siendo estos meses trabajando a contrarreloj, así que agradezco que me dedique estos minutos. La verdad que fue una buenísima sorpresa descubrir que había un equipo en Barcelona que se estaba dedicando a la investigación de la COVID-19 a través de organoides. Como sabe, estoy realizando el Trabajo de Fin de Grado sobre este tema, así que me gustaría hacerle una serie de preguntas.

Sabemos que las células madre y los organoides están revolucionando la ciencia, esta técnica se ha empezado a utilizar hace relativamente poco, unos 5 – 10 años. ¿Desde cuándo están trabajando con ellos? ¿Se pusieron en marcha nada más empezar la pandemia o ya tenían desarrollados los modelos con anterioridad para otro tipo de estudio? De ser así, ¿en qué otros proyectos los han podido utilizar?

Vale, vamos por partes. La tecnología de los organoides es algo más vieja que 5 o 10 años, yo empecé en el 2009 trabajando con modelos derivados de iPS (células madre pluripotentes inducidas) que funcionaban de manera parecida a los organoides. Más tarde estuve en uno de los laboratorios pioneros en Bruselas, el Pierre Vanderhaeghen Lab., y utilizábamos organoides de células madre embrionarias de ratón y humanas. Luego estuve en Estados Unidos como Post Doc, y cuando volví a Barcelona ya llevaba 10 u 11 años haciendo "bolitas" que crecían y recapitulaban las enfermedades. Incorporé esta técnica al laboratorio con una beca Beatriu de Pinós en la Universidad Pompeu Fabra. En ese momento desarrollé varios proyectos como investigadora principal y trabajábamos con organoides de cerebro estudiando enfermedades del SNC que afectan a etapas del desarrollo, concretamente probábamos medicamentos que pudieran servir para tratar epilepsias farmacorresistentes.

En cuanto nos encerraron, muy desde el principio de la pandemia ya sabíamos que el SARS-CoV-2 generaba algunos efectos en el SNC, contacté con Javier Martínez-Picado, mi colaborador en este proyecto, y a finales de marzo, Javier y yo nos pusimos manos a la obra y volvimos a la Pompeu junto con virólogos, sólo 3 o 4 personas estuvimos trabajando en la fase más dura del confinamiento.

Según he leído sobre el estudio que están llevando a cabo, están trabajando con organoides de pulmón, intestino y cerebro. ¿Es debido a que son los órganos más afectados por el SARS-CoV-2? Tengo entendido que a pesar de la evidencia de que el virus afecta a las células cerebrales, no está del todo claro que tenga suficientes cantidades de ECA2 para entrar con facilidad como podría hacerlo, por ejemplo, en los pulmones.

Los organoides de intestino son una de las partes que no nos han financiado, nosotros trabajamos con los de cerebro, y hemos incorporado los de pulmón por motivos obvios: la COVID-19 es una enfermedad principalmente pulmonar. Trabajamos en colaboración con dos investigadoras del EMBL (Laboratorio Europeo de Biología Molecular), que están en el Parc Científic de Barcelona.

Respecto a los organoides cerebrales, sí que es cierto que el cerebro no expresa masivamente la enzima ECA2 como en otros tejidos como por ejemplo el de riñón. Las diferentes regiones del cerebro, los diferentes tipos de neuronas y los diferentes tipos celulares que tiene el cerebro humano, no todos expresan de la misma manera la proteína ECA2. Además, el cerebro es un órgano diferente al resto en los que la difusión con vasos sanguíneos es permeable. La vasculatura del cerebro no lo es tanto, el cerebro tiene la barrera hematoencefálica que es extremadamente conservadora a la hora de permitir el paso de las diferentes substancias, y esto evidentemente afecta a los virus. Pero hay que recordar que el principal mecanismo de entrada del SARS-CoV-2 es a través de ECA2 de las células endoteliales. El cerebro es el órgano más vascularizado de todo el cuerpo, los vasos en el cerebro son capilares que están distribuidos de manera que cada 50 micras, es decir, cada 2 células hay un capilar que pasa por alrededor. Está extremadamente vascularizado.

Por tanto, sí que hay una perfusión del virus a las células endoteliales del cerebro, sí que tiene la capacidad de unirse a estas células y afectarlas, aunque todavía no se sabe muy bien el mecanismo, si es transcitosis, si es por necrosis, si es por las hemorragias que producen los pequeños trombos que se forman, pero hay una perfusión de suficiente carga viral al parénquima cerebral que permite infectar las neuronas.

Nosotros lo que hemos visto en el laboratorio con nuestros organoides es que cuando hay carga viral suficientemente grande, el virus infecta a neuronas y además produce patología en estas, citopatologia. El virus tiene la capacidad de reducir el tamaño de los axones en los organoides y, por tanto, hay una retracción de la red neuronal que tiene el cerebro.

¿Cuánto tiempo les ha llevado la creación de cada organoide?

Depende del tipo de organoide y del tipo de células del que procede. Tenemos células madre que son intrínsecas de los tejidos, por ejemplo los tejidos con alta capacidad de regeneración como el epitelio respiratorio o el intestinal, y estos tienen una población bastante prevalente de células progenitoras, entonces se pueden coger esas células progenitoras y hacer organoides con ellas. En muy pocas semanas tendríamos organoides suficientemente maduros.

Nosotros no trabajamos con este tipo de tecnología, lo que hacemos son organoides que derivan de células madre embrionarias o células pluripotentes que vienen de pacientes. Es un proceso mucho más largo porque tenemos que generar los progenitores a partir de una célula extremadamente indiferenciada, por ejemplo para generar un pulmón, tardamos 2-3 semanas hasta conseguir tener esos progenitores maduros y a partir de ahí son aproximadamente 6-9 semanas hasta tener el organoide.

En el caso del cerebro, para este tipo de estudios con el SARS-CoV-2 queremos tener una población neuronal lo más madura posible para que toda la red de dendritas y axones, que es lo que denota maduración neuronal y actividad cerebral, esté bien representada, por lo que hay que dejar madurar los organoides en las placas de cultivo alrededor de 4-5 meses antes de poderlos infectar con el virus. Además, el cerebro

adulto no tiene progenitores, no podemos hacer neuroesferas y no tenemos manera de poder baipasear ese tiempo como lo haríamos con el pulmón, del que sí podemos coger progenitores. En el cerebro no es el caso, siempre tenemos que partir de células indiferenciadas como las iPS.

En la obtención de células madre para el desarrollo de estos organoides, ¿han seguido algún criterio? ¿Son extraídas de paciente ya infectado? Y el virus, ¿lo han inoculado una vez formado el organoide?

Las células que utilizamos para generar los organoides son de pacientes, pero nosotros no podemos trabajar con el SARS-CoV-2, somos un laboratorio normal con nivel de bioseguridad bajo, no estamos autorizados a trabajar con patógenos. Lo que hacemos es que diseñamos y maduramos los organoides y los llevamos a IrsiCaixa, uno de los pocos centros que hay en España con nivel de biocontención suficientemente alto para trabajar con el virus, y son ellos los que infectan con el SARS-CoV-2 comercial. La variante con la que nosotros trabajamos es la variante alemana que se pudo aislar en las primeras semanas de pandemia.

Están enfocados a buscar tratamiento efectivo para combatir la COVID-19, ¿con qué fármacos están trabajando? ¿Han obtenido algún resultado concluyente o aún están en proceso?

Aún estamos en proceso, pero los fármacos no los puedo decir porque son confidenciales, trabajamos en colaboración con algunas farmacéuticas. Lo que sí puedo decir es que tenemos algunos fármacos que son antivirales "clásicos" por así decirlo, y también tenemos otros fármacos que actúan sobre otro tipo de vías de señalización, como por ejemplo los corticosteroides, que no solamente actúan en la inflamación sino también en otras vías de señalización no proinflamatorias, especialmente en las membranas de células que no son inflamatorias, pero sí tienen la capacidad de responder. Por tanto, estamos trabajando en varias tipologías de fármacos.

¿Cree que en un futuro estos organoides ayudaran a encontrar tratamiento definitivo para enfermedades que aún a día de hoy son difíciles de controlar o no tienen cura como, por ejemplo, el Cáncer o el Alzheimer?

¡Sí, desde luego! No solo lo pienso, sino que estoy trabajando para conseguirlo.

En el cáncer queremos llegar a los tumores lo suficientemente pronto para matar todas las células tumorales. En el caso del SNC, no queremos matar las células, queremos: o bien recuperar las funciones perdidas, o bien reestabilizar la función celular. Por ejemplo, en la epilepsia farmacorresistente los niños nacen con mutaciones que en muchos casos no están descritas, y no responden a ningún tratamiento. Esto genera un peligro para la vida, además de que las epilepsias producen una toxicidad que conlleva a la muerte neuronal, lo que provoca un deterioro cognitivo bastante grande. Nosotros probamos nuevos compuestos, pero también fármacos comercializados para otras indicaciones: hacemos un "repurposing". A los pacientes se les da estos tratamientos para ver si hay recuperación de las funciones perdidas, pero se tardan años en saber si ha sido efectivo, tiempo en el deterioro cognitivo del niño ha avanzado. Con los organoides, en cuestión de meses podemos probar si el fármaco es efectivo, y podemos baipasear las funciones que no podemos ver correctamente en el paciente.

En resumen, sí, creo fervientemente que los organoides tienen la capacidad de proporcionar nuevas terapias y de ver mucho mejor si los fármacos que tenemos ahora mismo funcionan o no. Y uno de los puntos más relevantes es que se puede hacer de forma personalizada. No todas las personas respondemos igual a un medicamento, siempre hay un "A mí me va mejor un ibuprofeno que un paracetamol para el dolor de cabeza". Es una cuestión intrínseca de cada individuo y los organoides, al poderse derivar de células del propio paciente, dan muchísima información que no podemos tener de otra manera.

Muchas gracias por dedicarme su tiempo y por el avance científico que supone trabajar con estos organoides que espero sirvan en el futuro para cambiar la visión de la ciencia y para mejorar la calidad de vida de todo el mundo.