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Chapter

Organoids Models for the Study of Cell-Cell Interactions

Margarita Jimenez-Palomares, Alba Cristobal and Mª Carmen Duran Ruiz

Abstract

Organoids have arisen as promising model systems in biomedical research and regenerative medicine due to their potential to reproduce the original tissue architecture and function. In the research field of cell–cell interactions, organoids mimic interactions taking place during organogenesis, including the processes that conduct to multi-lineage differentiation and morphogenetic processes, during immunology response and disease development and expansion. This chapter will address the basis of organoids origin, their importance on immune system cell–cell interactions and the benefits of using them in biomedicine, specifically their potential applications in regenerative medicine and personalized therapy. Organoids might represent a personalized tool for patients to receive earlier diagnoses, risk assessments, and more efficient treatments.

Keywords: organoids, cell-interactions, disease development, regenerative medicine, personalized therapy

1. Introduction

Most multicellular living organisms, especially vertebrates, develop from a single totipotent cell to a multicellular complex adult organism, reflecting an outstanding coordination and organization capacity. Furthermore, in some cases, after organ dissociation, cells can recombine and reconstruct the original structure. Researchers have used that feature to create organ-like structures from stem cells or tissues samples, leading to the formation of structures currently known as organoids [1].

Thus, organoids are self-organizing 3D structures derived from stem cells highly similar in structure and function to actual human organs. The different cell types and interactions guide and make possible this organization process. These structures resemble crucial aspects of the tissues from which derived and thereby organoids allow for biological relevant cell–cell and cell-matrix interactions. Those attributes make organoids technology a valuable tool in multiple applications such as developmental biology, molecular biology, and health studies like pharmacology, disease development and therapy, among others [2, 3].

The organoids field has exponentially accelerated in the last years, mainly after the application of appropriate culturing conditions that allow stem cells to differentiate and participate in cell–cell interactions responsible of the community effect required for optimal resembling of self-organized tissue-like structures.

For instance, the use of Matrigel, a gel protein mixture that mimics the complex extracellular environment found in many tissues [4], has allowed the establishment of the right culture conditions required to achieve 3D cell cultures *in vitro*.

Organoids technology constitutes a step-forward approach for conventional cell-based research, full-filling the gap between 2D cultures and *in vivo* mouse/human models. Organoids are physiologically more relevant than monolayer culture models, and allow easier manipulation of niche components, signaling pathways and genome editing than *in vivo* models [5].

Therefore, organoids represent a needed and also an advantageous approach in many senses. The organoids technology brings the opportunity to work with 3D-tissue models at a "bench-side" level, opening a wide range of opportunities in basic and clinical research. Moreover, organoids also overcome the problems derived from using animal models to study human physiology and related-diseases. Although many results obtained in animal models can be easily extrapolated, some biological processes are specific to humans [6].

2. Organoids origin, structures and culture

Organoids can be derived from either [1] pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) or [2] multipotent organ-specific adult stem cells (AdSCs). Both approaches take advantage of the endless expansion potential of stem cells in culture. Also, when PSCs and AdSCs are allowed to differentiate in culture, they display a remarkable capacity to self-organize into structures that reflect similar characteristics of the organ they attempt to mimic [7].

PSCs can be differentiated into different cell types and grown *ex vivo* as organoid models by the treatment with defined developmental stimuli. PSCs isolated both from mouse and human tissues have given rise to brain, retina, inner ear, stomach, intestine, thyroid, lung, liver, and kidney organoids. ESCs or iPSCs can be derived *in vitro* into endoderm, mesoderm and ectoderm, with specific procedures involving multiple differentiation steps. Thus, human iPSCs are sequentially exposed to a progression of differentiation signals in order to simulate the stages of a human developmental process. Once the initial germ layer has arranged, cells are transferred into 3D systems [8], where differentiated iPSCs aggregate to form an organ bud and, later on, organoids. These organoids contain multiple cell types and faithfully mimic the mature organ structure, and the interactions between them.

As an example, embryoid bodies (EBs), 3D aggregates of PSCs, originate cerebral organoids and develop into a forebrain region in the presence of growth factors (i.e., hFGF basic, ROCK inhibitor, N2, Heparin, MEM-NEAA, etc.). For other organs, the addition of Activin A to PSCs specifies them towards an endodermal fate. These cells are further cultured as 3D organoids in Matrigel with medium containing tissue-specific growth factors [9].

On the other hand, AdSCs-organoids can be originated from isolated adult stem/progenitor cells or from isolated tissue fragments of the corresponding organ (e.g. intestinal crypts, liver or pancreas ducts) [8]. These structures can be generated from biopsies isolated directly from the organ of interest or from diseased patient tissue without the complicated process of reprogramming and differentiation required in iPSC organoids. In general, human AdSCs-derived organoids are composed mainly of cell types found in the epithelium.

AdSCs were long believed to be unable to proliferate outside the body, but the culture with specific growth factor cocktails mimicking stem cell niches, has helped to sort out such obstacle. These niche factors are essential to support stem cell

activity and vary depending on the tissue of origin. Also, 3D Matrigel-based cultures have provided the appropriate culture conditions to generate AdSCs-derived organoids from various mouse and human tissues including the colon, stomach, liver, lung, prostate, pancreas, ovaries, taste buds, and lingual epithelium.

Thus, to generate AdSCs-organoids a tissue biopsy is cut into fine particles and then incubated with enzymes (i.e., collagenase, elastase, or dispase) to obtain a single cell suspension. Next, cells are grown in Matrigel and culture medium supplemented with specific tissue growth factors [9]. For example, intestinal organoids need Noggin, R-spondin, Epidermal growth factor (EGF), and WNT [10–12]; retina organoids need IWR1e and Smoothened agonist CHIR99021 [13, 14]; prostate organoids require Noggin, R-spondin and EGF [15], while pancreas organoids require Noggin, R-spondin, EGF, fibroblast growth factor (FGF) and Nicotinamine [16].

AdSCs organoids do not require genetic transduction with transcription factors, as it happens with those with PSCs. This situation makes organoids physiologically well-suited with the host tissue, leading to an improved stem cell transplantation. Moreover, molecular techniques such as clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated system (CRISPR-Cas9) genome technology and single-cell RNA sequencing, can be applied to organoids [7, 9]. On the other hand, the establishment of human AdSC-derived organoids is limited by the accessibility to the tissue and prior knowledge of the culture conditions for that tissue. However, an iPSC line, once established from a patient, can generate different tissue models without any time limit, beyond the patient's lifespan [17, 18].

3. Organoids in the immunology field

The knowledge concerning the interactions of the immune system with other tissues has been gained mainly from animal models and/or cell lines co-cultures. Nevertheless, some interactions between human cells cannot be addressed with murine models or cell lines which are usually transformed or genetically modified [19]. For instance, a specific immune cell morphology is required to maintain the tissue properties and, moreover, the immune system needs of multiple cell types interactions for appropriate functioning. Similarly, there are some aspects that cannot be extrapolated in mice due to, for example, different protein pattern expressions in human and mice. Thus, immunology researchers are starting to get the benefits of using organoids, for a better comprehension of the immune cell interactions with other tissues, its development, homeostasis and in the bout of disease. The organoids approach maintains those cells in a near-native state, mimicking more accurately its original state and environment, providing researchers with a new effective tool.

The main challenge in the use of organoids in immunology resides in the fact that the organoids technology cultures only epithelial cells. However, a more complete resource for immunological research can be developed by co-culturing these organoids with other elements.

The number of publications showing multiple co-cultures has spiked up in the past decade, particularly in the last five years [20–22]. In order to develop effective interventions to preserve health and defeat diseases it is necessary to know how immune cells coordinate their activities to initiate, modulate, and terminate inflammation. Immune cells and molecules released by immune cells promote inflammation processes that are mediating the interactions between these cells [23].

These studies have revealed not only the importance of the presence (or absence) of immune cell derived factors in the epitheliums in culture, but also the

need of the reciprocal communication with the immune system. A work concerning the role of macrophages and fibroblasts on myoblast proliferation and migration highlights the importance of multicellular communication [24]. Thus, co-culture of either macrophages or fibroblasts with myoblasts prompted a significant increase in myoblast proliferation. Conversely, in the triple co-culture, although macrophages continued promoting myoblast proliferation, they had a negative effect over the ability of fibroblasts to enhance myoblast migration [25]. Another study, using single-cell transcriptomics, highlighted that intestinal stem cells can function as non-classical antigen-presenting cells for CD4+ T cells. Moreover, these interactions, directly or through activated T cell-derived cytokines, seem to play a role in the intestinal epithelium differentiation [26].

The intestinal mucosal barrier function and the immune responses against invading pathogens seem to be regulated by the interaction between intestinal epithelial cells (IECs) and intraepithelial lymphocytes (IELs) [27]. IELs represent a heterogeneous population of activated and antigen-experienced T cells. A novel culture system of intestinal 'enteroids' has allowed the study of the complex interactions between IECs and immune peripheral T cells in long-term co-cultures. The development of these long-term co-cultures allowed the study of cell survival, proliferation, differentiation and IECs behavior. Moreover, IECs and T cells co-cultures revealed that peripheral T cells activated in the presence of enteroids acquire several features of IELs, including morphology, membrane markers and movement in the epithelial layer [27]. Similarly, mouse-derived enteroids co-cultured with intestinal myofibroblasts and macrophages boosted their growth and differentiation [28].

In the same line, another study with intestinal organoids underlined the importance of the interactions between immune cells and other tissues for optimal maturation. In this work, the inclusion of the immune component (co-cultured with human T lymphocytes) into the differentiation protocol to form human pluripotent stem cell-derived intestinal organoids (hIOs) from hPSCs, enabled hIOs maturation. hIOs co-cultured with human T lymphocytes displayed expression levels of mature intestinal markers equivalent to adult intestinal epithelium, as well as increased intestine-specific functional activities, retaining their maturation status even after their *in vivo* engraftment. This study has proven the needless for animal models and *in vivo* maturation when working with organoids [29].

Holokai, L. *et al.* were among the first researchers to successfully obtain a multiple organoid-co-culture involving cytotoxic T lymphocytes (CTLs) and Helicobacter pylori-infected gastric organoids. CTLs express programmed death 1 (PD1) on the surface. When PD1 interacts with its ligand, CTLs cannot induce apoptosis. Thanks to this approach they discovered that PD-L1 signaling induces cellular proliferation and survival, leading to an increased expression of PD-1, IL-2 and IFNγ in lymphocytes [30].

Overall, epithelial organoid cultures, whether derived from iPSCs or AdSCs, constitute a promising platform for immunological research for several applications, allowing, among others, to study immune cell–epithelial cell interactions in the context of pathogenic infections or sterile tissue damage [19]. In this sense, the vast majority of organoid studies about the immune system and its effects on epithelial differentiation and function have been performed on intestine-like structures. However, it would be useful to have similar works with different organoid systems such as skin or lung, which also interact with both immune cells and commensal microorganisms [31].

Despite the amount of work already accomplished regarding the immune system, there is still a long way to go in inflammation research, due to the current lack of optimal immune cells organoids cultures.

4. Organoids in the study and treatment of disease

Recent advances in the development of human patient-derived organoids have allowed a more accurate study of diseases. This technology has opened a new horizon in biomedical research, and provides unprecedented opportunities in translational medicine, and personalized therapy [32].

4.1 Disease modeling and drug screening approaches

Recent discoveries involving organoids as a disease model reflect that researchers have started to unravel the potential of this tool. To date, organoids have been mostly applied in cancer, cystic fibrosis and studies on host–microbe interactions. However, a growing interest in this field has promoted an exponential increase of publications using organoids technology to study many other diseases (**Table 1**). The fact that organoids are 3D structures originated from stem cells with similar architecture, multi-lineage differentiation and many of the original tissue functions, make them the perfect candidate for disease pathogenesis studies [33, 34].

Organoids can be designed to reproduce patient conditions of disease-relevant genetic and epigenetics. Thanks to the development of new techniques like the CRISPR/CRISPR -Cas9 genome engineering tool, is currently feasible to efficiently manipulate genomic sequences in hESCs and hiPSCs [35, 36]. In the case of host–microbe interactions, organoids can also reproduce the infection process allowing its study in more life-like manner.

Organoids can also be applied to study cellular dysfunction in diseased tissues, as well as to identify strategies for its restoration. For example, Dekkers *et al*. used organoids to study cystic fibrosis (CF), a disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, severely reducing the CFTR protein function [37]. Thus, rectal organoids from CF patients were used to evaluate CFTR function as well as the response to CFTR-modulating drugs. Their results demonstrated the pharmacological restoration of CFTR function in the rectal organoids of individual donors, suggesting that *in vitro* functional measurements of CFTR may be used to preclinically identify CF patients who would benefit from CFTR-modulating treatments, independent of their CFTR mutation [38].

A major challenge in clinical practice is the absence of appropriate models for drug screening and pre-evaluation of the pharmacological effects prior administration to patients. For cancer research, the development of tumor organoids, also known as tumoroids, represents an overwhelming step to be able to reproduce *in vitro* such a heterogeneous microenvironment. Tumor organoids can be generated *in vitro* for the analysis of cancer phenotypes [39], anticancer drug discovery, and to evaluate the response of patient cancer cells to a specific treatment [39, 40]. Lazzari *et al*. reported a triple co-culture of pancreatic cancer cells fibroblasts and endothelial cells. As a result, cells assembled in a hetero-type multicellular tumor-spheroid (MCTS) that reliably reproduced the impact of the surrounding environment, on the sensitivity of cancer cells to chemotherapy. This approach can be successfully applied as a predictive tool of various therapeutic strategies [41]. In this sense, the establishment of patient-derived tumor organoids (PDTO) biobanks provides exciting new insights into developmental biology. Different researchers have started to develop methods for generating and bio-banking PDTO. Among them, a non-profit organization called HUB (Hubrecht Organoid Technology) has initiated and established "Living Biobank", a collection of organoids representing different organs and disease models (huborganoids.nl). Overall, these biobanks maintain the key features to resemble the parental tumors and can be therefore used to evaluate patient-specific treatment approaches [42].

Tissue of origin	AdSC-derived		PSC-derived		Cancer biobank	Disease	References
	Human	Mouse	Human	Mouse			
Brain			✔	✔	✔	Autism,	[6]
						Microcephaly, Macrocephaly	[43]
						Neurodegenerative disorders	[44]
						Infectious diseases	[45]
						Psychiatric disorders	
						Cancer	
Retina/Optic cup			✔	✔		Leber congenital amaurosis (LCA)	[13]
						Retinitis pigmentosa	[14]
						Age-related macular degeneration	
						Retinal degeneration	
Salivary gland	✔		✔	✔		Hyposalivation	[46]
Thyroid			✔	✔	✔	Cancer	[47]
Lung	✔	✔	✔	✔	✔	Cystic fibrosis	[24]
						Cancer	[48]
Breast	✔	✔	✔	✔	✔	Cancer	[49]
Esophagus	✔	✔		✔		Barrett's esophagus	[50]
						Cancer	
Stomach	✔	✔	✔	✔	✔	Infectious diseases	[10]
						Cancer	[35, 51]

Tissue of origin	AdSC-derived		PSC-derived		Cancer biobank	Disease	References
	Human	Mouse	Human	Mouse			
Intestine	✔	✔	✔	✔	✔	Infectious diseases	[10–12]
						Cystic fibrosis	[26, 28, 29]
						Cancer	[52, 53]
Colon	✔	✔	✔	✔	✔	Infectious diseases	[52–54]
						Ulcerative colitis	
						Crohn's disease	
						Cancer	
Pancreas	✔	✔	✔	✔	✔	Cystic fibrosis	[16, 41, 55]
						Pancreatic ductal adenocarcinoma	
						Diabetes mellitus	
						Cancer	
Liver	✔	✔	✔	✔	✔	Alagille syndrome	[56–58]
						Nonalcoholic fatty liver disease	
						Cystic fibrosis	
						Lethal liver failure	
						Cancer	
Kidney	✔	✔	✔	✔	✔	Polycystic kidney disease	[59, 60]
						Cancer	
Female reproductive tract	✔	✔	✔	✔	✔	Cancer	[36, 61]
Prostate	✔	✔	✔	✔	✔	Cancer	[15, 62]

Table 1.
Use of organoids as disease models.

Jacob *et al.* reported the generation of patient-derived glioblastoma organoids (GBOs) biobanks [42]. The authors successfully transplanted the GBOs into adult rodent brains and performed personalized tests. Calandrini *et al.* have recently established the first pediatric cancer organoid biobank [59]. This biobank contains a collection of over 50 tumors matching normal kidney organoids and also covers a diversity of tumor subtypes. Similarly, a primary gastric cancer organoid (GCO) biobank was established by Leung and coworkers [63], including a total of 34 patients with different gastric cancer subtypes. In this study, whole-exome sequencing and transcriptome analysis were performed, as well as large-scale drug screening studies. Overall, the establishment of organoids biobanks provides a rich resource for cancer cell biology and drug-screening studies to test personalized therapies. Patient-specific drug sensitivities could be achieved as the organoids closely resemble the *in vivo* tumors. Furthermore, these biobanks could play a prominent role in biomarker discovery and represent a powerful tool to predict disease development, recurrence and progression [42, 51, 64].

4.2 Applications in regenerative medicine

Several of the most life-threatening diseases require organ transplantation in order to save patients life. Nevertheless, transplantations are not always an option due to the high cost, organ availability or potential organ rejection. Therefore, other alternatives needed to be explored in order to overcome this challenge. The development of organoids brought hope to the scientific community and patients themselves. This technology could potentially serve as an unlimited source for replacing damaged tissues. Furthermore, the transplantation of organoids derived from healthy tissue of the same patient would prevent immune responses related to non-autologous transplants. In this sense, diseases involving dysfunctional organs such as kidneys or the liver, can significantly benefit from the opportunity that liver-derived organoids bring. Researchers have already developed strategies to allow long-term *in-vitro* expansion of liver progenitors into "liver organoids" [56]. The huge expansion and differentiation potential of liver organoids cultures has facilitated the engraftment [56] and survival of livers in murine models, as it happened in a study with liver organoids transplanted to a tyrosinemia type I liver disease model, partially restoring the hepatic function [57]. Similarly, transplantation of human adult stem cell-derived liver organoids into chemically damaged-liver immune-deficient mice produced functional hepatocytes containing grafts [58]. Cultured organoids have also shown the potential to expand, engraft, reconstitute and recover the colon and intestinal epithelia as well as their function in several murine models [52–54].

Despite all the advances in the field, there is still a long way before organoids transplantation becomes a reality. Current resources and techniques do not provide a suitable organ niche, limiting the formation of optimal organ sizes and tissue structures *in vivo*, as well as the appropriate intercellular communication required for functional restoration. Thus, alternative approaches are required, such as the combination of organoids with gene therapy, to implement organ transplantation [65]. Experts on the field will still have to poise excitement with reality before organoid research can be successfully translated to clinical practice and become a real therapy option [66].

4.3 Personalized medicine

Over the past decades, medicine has discovered novel ways of changing the course of many human diseases [67–70]. Nowadays, researches all over the globe

are discovering new therapies which bring new options for previously untreatable diseases [71, 72]. However, the key challenge is that the efficacy of most of these new treatments will depend on the complex and unique nature of each individual human being. Lastly, the efficacy of a treatment is significantly determined by behavioral factor, environmental influences as well as genetic particularities.

Moreover, currently available therapies might cause a high impact on patient's quality of life due to the unpleasant side effects directly related to the treatment. Thus, research groups and pharmaceutical companies are developing strategies to personalize their treatments in order to predict the outcome of the proposed therapy and avoid unnecessary aggressive treatments. These aspects are key to achieve the ultimate goal of any therapy: to ensure patients´ health and integrity.

The concept of Personalized Medicine arose with the aim of tailoring the best response and highest safety standards to preserve patient's well-being. This optimized health care strategy would also lead to reduced treatment costs and shorter diagnosis times required for each patient [73–75].

Organoids have revolutionized personalized medicine due to their unique ability of simulate, even mimic, specific cellular microenvironments with remarkable similarity to *in vivo* organs/tissues under normal or pathological conditions [76]. Such models have started to be used in the clinic, mainly in cancer research, to evaluate the response to experimental therapies prior administration of certain drugs or other treatments to patients [77]. The possibility of using accessible models of organ diseases allows to understand the effect of experimental therapy in a deeper manner than in a traditional culture assay or "sphere" culture assays, applied over the last decades [78].

Personalized medicine could also be linked to regenerative medicine which is based on the capacity of the stem cells to derive into many different cell subtypes. Currently, this basic characteristic is key for the understanding of normal and abnormal cell behavior and organization, and is leading to improved tissue engineering approaches [60].

In this scenario, organoids constitute a solid foundation on which personalized and regenerative medicine is taking long steps forward.

One of the best examples of this input on current society is the novel application of organoids cultures to optimize treatment to cancer patients [55, 79]. Oncologic centers are developing translational procedures to understand as much as possible the specific characteristics of each tumor in order to optimize the therapy approach.

Once the tumor is detected, a biopsy of the mass is obtained to culture organoids derived from patient's tumor cells. A complete biological profile of the tumor could be developed combining this information together with histopathological analysis of primary tumor sample, histopathological analysis of the organoids, gene sequencing and cytotoxicity analysis from *in vitro* drug assays or studies using avatar mice.

This complete analysis only takes 2–4 weeks and it could provide physicians relevant information regarding the best treatment for the patient according to the characteristics of the tumor.

Furthermore, in cases of progressive disease or metastasis, new tumor biopsies could be collected, new organoids lines could be established, and new therapeutic strategies could be carried out giving a new opportunity and new hopes to the patient [80–82].

According to the website ClinicalTrials-gov, by 2019 there were 30 projects related to cancer organoids. Most of them (73%) focused on studying anti-cancer therapies, including among others T-cell immunotherapy, or evaluation of radiotherapy sensitivity. The rest aimed to generate patient-derived cancer organoid models (13%) or to evaluate the mechanisms related to cancer progression [83].

A large number of cancer patients are insensitive to immunotherapy due to the heterogeneity of the T cell repertoire [83]. Thus, the use of cancer organoids allows studying the effectiveness of combining immune therapy with specific anti-cancer drugs. To date, two clinical trials involving cancer organoids for immunotherapy have been registered (NCT03778814, NCT02718235). Overall, the inclusion of PDTO into clinic represents an enormous potential to understand the onset of diseases such as cancer and, moreover, to evaluate the individual response to specific therapies for personalized approaches.

5. Limitations and future perspectives

Regardless of the advances made in this emerging technology, a series of limitations still need to be addressed in order to fully exploit its potential. For instance, despite the development of specific culture conditions and growing techniques, there are still tissues that withstand to organoid derivation [84, 85]. Organoid technology requires further advances to achieve less laborious protocols as well as the establishment of standardized conditions for proper differentiation and maturation. A reduction of the heterogeneity seen in organoids size and shape should also be achieved [85]. In addition, it requires the co-induction of the essential cell types, the associated extracellular matrices and native microenvironment that will allow the recapitulation of the *in vivo* tissue sizes, structures, organization, inter-cellular communication and functionality. Also, shorter processes and more affordable culture conditions are required to ensure that the organoids system becomes accessible to a large number of academic and clinical researchers, thereby helping to maximize its potential [5]. Moreover, the protocols used for generating one specific type of organoid are usually not transferable to another organ system. Due to this drawback, scalable and cross-system parameters are challenging to generate via bioengineering tools. Computational prediction models are also difficult to design limiting the capacity to predict phenotypic, toxicological and drug screening results. Lastly, organoids technology requests the development of a complex vasculature network to provide not only oxygen, nutrient and waste exchange, but also an inductive biochemical exchange and a structural template for growth. The advances in microvascular patterning and organ-on-a-chip microfluidic technology would bridge this limitation supporting the use of perfusable organoids generation [86, 87].

In this context, different strategies are currently under research and new ideas have arisen to implement the potential use of organoids. As stated before, the development of organoid biobanks constitutes an important step in this direction. Currently, there are organoid biobanks with healthy organoids as well as patient-derived intestine, liver, pancreatic, lung and mammary gland organoids related to cancer, cystic fibrosis or inflammatory bowel disease [88]. Thus, organoid biobanks are becoming a demanding business and several companies worldwide have already started to commercialize organoids after the establishment of optimized organoid biobanks [88]. Advantages of organoid biobanks include immediate accessibility or cost-effectiveness, as well as the possibility to access a large repository of data related to patient's diseases [83, 88]. This, however, involves some ethical and regulatory challenges that need to be addressed such as donor confidential information or the organoid source itself [89].

The development of microfluidic organoid-on-a-chip platforms [90] and 3D bioprinting [91, 92] constitute two major advances in the last years that are contributing to speed up organoid manufacturing and commercialization [88]. Organ-on-a-chips are devices containing living cells, extra-cellular matrix (ECM)

and microstructures emulating key features of organs or issues, and their functions [83, 93]. These devices aim to provide continuous flow perfusion culture to simulate organ microenvironments. Nevertheless, most of these systems are made of primary cell lines or stem-cell-derived cells to mimic organs, but they are unable yet to imitate the cellular interactions taking place in the native sources [94].

Similarly, advances in 3D printing technology and biomaterials research have led to the creation of 3D bioprinting, with the aim to resemble *in vitro* the interactions between tumor cells, ECM and the 3D tumor microenvironment [83, 95]. With this technology, different cell types can be printed in hydrogels and mixed with other cells and/or specific factors to simulate a healthy or pathological microenvironment. Increasing evidence has pointed to the tumor microenvironment as a major modulator of the tumorgenic process [96]. Thus, in order to understand the mechanisms by which tumor cells become metastatic, different studies are benefiting with the use of 3D bioprinting strategy. For example, Grolman JM *et al.* designed a 3D environment with breast adenocarcinoma and macrophage cell lines printed in hydrogel to evaluate the effect of paracrine signals in the regulation of breast cancer metastasis [97]. In the same way, Pang Y *et al*. developed an *in vitro* cervical tumor model to demonstrate the epithelial-to mesenchymal transition (EMT), by mixing *HeLa* cells with hydrogel. These authors were able to evaluate the effect of different activators and inhibitors over the EMT in the 3D system designed [98].

Despite the benefits of using these techniques, there are still several factors that need to be optimized. For instance, biomaterials represent a limiting feature for 3D bioprinting, and the development of improved materials is required. A consensus in the best printing strategy (i.e. polymerization steps, light-based 3D bioprinting *vs* inkjet printing) should be also reached.

6. Conclusions

This chapter focused on the advantages of using organoids to expand our knowledge in the field of cellular interactions. We have focused specifically in immunology and disease-related research, going through some of the latest or more relevant publications involving organoids. Overall, organoids constitute an efficient tool to study immune cells´ interactions *in vitro* in 3D-tissue models that provide a closer view of the interactions taking place *in vivo*. Moreover, organoids represent a promising approach in the development of autologous tissue-based cellular therapies, especially in life-threatening diseases. Nevertheless, despite the organoids relevance, the growing interest in these structures and their potential applications, there is still a long way to go to achieve the translation of organoids into clinical practice. The development of bioengineering tools such as microfluidic organ-on-a-chip platforms or 3D bioprinting systems represents a huge step in this direction. These strategies could provide consistent nutrients and factors required to emulate 3D tissue physiology *in vivo*. The optimal conditions are not yet established and further research is required before results can be undoubtedly extrapolated and clinical applications implemented. Nevertheless, the growing interest in organoids commercialization will probably help to speed up the translation of organoids to the clinic.

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Conflict of interest

The authors declare no conflict of interest.

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