Mind the gut

CHAPTER IX

IMPACT PARAGRAPH

In this chapter, we discuss the potential impact of the research described in the context of this thesis. Specifically, we deliberate over how these intestinal organoid models fit within the societal needs and what the possible scientific and commercial applications are.

The digestive system is one of the most complex organ systems. It contains diverse cell types that are responsible for a wide range of functions that are crucial for life. Apart from digestion, absorption, secretion and excretion, the gastrointestinal (GI) tract acts as a barrier to harmful compounds and microorganisms, thus contributing significantly to the defense mechanisms of the body. Trillions of bacteria and other microorganisms reside within the GI tract that collectively make up the microbiota. GI tract cells communicate also with the central nervous system and the endocrine system, thus its function is regulated and affected by multiple factors. The complexity of the system along with the variation exhibited among different individuals entangle not only basic research related to the understanding of mechanisms underlying the normal and diseased state of the GI tract, but also translational research related to the treatment of these disorders. Thus, currently disorders of the digestive system (e.g., irritable bowel syndrome, Crohn's disease, GI cancers) are a major cause of morbidity in the elderly population. Every year, up to 370 million people are diagnosed with a digestive disorder globally[1,2]. This raises the need for the development of more advanced and representative *in vitro* models. 3D organoid systems can be tremendously beneficial for studying such complex tissues *in vitro* and are expected to revolutionize the conventional paradigm in pharmaceutical industry and drug discovery in the future. Currently there are 70 organoid research model companies globally, which focus on drug discovery using organoid models[3]. Till November 2020, there were globally 21 ongoing organoid clinical trials, a number which is expected to increase in the next few years, owing to the progress in the scientific field and improvements in infrastructures[4,5]. The sales of organoids had an increase of 5% in the period between 2016 and 2020 and investments in organoid products are constantly rising[4]. Solely the National Institute of Health in the U.S. awarded research grants, which exceeded $251 million between 2015 and 2019[4]. The global organoid market size was evaluated at $516.6 million in 2021 and is expected to reach $1.2 billion by 2031[5]. Among organoid models, intestinal organoids are expected to exhibit the highest sales in the market[4]. All these data indicate the importance and great potential of organoid models.

In the past decade, numerous 3D organoid models recapitulating different tissues have been developed. These mini-organs mimic structural and functional

characteristics of the *in vivo* tissues with great fidelity and they have been widely used to study physiology and disease. However, their use in drug development and clinical applications is still restricted because of certain limitations such as the use of animal-derived extracellular matrices substitutes, the low throughput and the lack of surrounding tissue microenvironment. With the research performed here, we managed to overcome some of these issues and broaden the applicability of organoid technology in basic and translational research.

Currently, drug discovery is a long and expensive process, which often turns out fruitless upon reaching the clinical trial phase. Specifically, it can take between 12 and 15 years from the discovery of a drug until its approval and requires an investment of $1 billion[6]. Furthermore, out of a million molecules screened, only a single one will reach the clinical trials[6]. This urges the need for improvements in the predictive power of the preclinical phase. Personalized drug testing with organoids could be particularly useful to bridge the gap between preclinical drug development and clinical trials. In the Netherlands, cystic fibrosis patients that would respond well to a certain treatment were identified after a drug screening in patient-derived organoids with different mutations[7]. This was a first step towards extending the application of organoids for personalized medicine purposes. The use of organoids can also reduce drug/compound testing in animals, which is currently an ongoing ethical debate topic. All these may also result in financial benefit, since experiments with organoids are cheaper than *in vivo* studies; therefore, the budget spent on unsuccessful clinical studies may be minimized since drug candidates will be better "filtered" during the preclinical phase. The microwell-based intestinal organoid model developed in chapter III allows for a better-controlled organoid culture with limited use of Matrigel and it facilitates downstream applications, such as drug-screenings, in high-throughput. Such systems could be adopted by pharmaceutical companies in the future to improve, reduce the cost of and accelerate preclinical drug testing.

To improve the drug discovery process, it is particularly important to understand the mechanisms underlying the physiology and disease. Although organoids represent multiple tissue features, they lack the surrounding microenvironment. Tissue communication plays a crucial role among others in homeostasis, in the development of certain diseases and in the responses to drugs/treatments. For instance, the immune system includes multiple components (innate and adaptive) in all tissues and is crucial for the host defense against harmful agents. With that in mind, in chapter IV we incorporated macrophages in our

microwell-based intestinal organoid culture system in two different ways. In the first configuration, organoids are in very close proximity and even direct contact with macrophages, mimicking chronic inflammation states. In the second configuration, the communication between organoids and macrophages is performed only by paracrine signaling and this situation mimics closer acute inflammation. These two systems could immensely benefit research related to the complex mechanisms underlying the immune responses in the intestine. For example, inflammatory bowel disease (IBD) has become a global disease with accelerating incidences and its pathogenesis is not fully understood yet. Consequently, there is no cure for this disease and current treatments only aim to reduce symptoms and prevent complications. Currently, the annual costs of care for IBD patients are approximately $23,000, which could reach up to $37,759 if a single visit to the emergency department is required[8]. Considering that up to 70,000 new IBD cases per year are diagnosed only in the U.S, there is a tremendous burden on healthcare. Using these intestinal organoid-macrophage models, studies focusing on the mechanisms underlying such diseases could be facilitated. Subsequently, therapeutic agents could be tested in a high-throughput manner in order to develop more efficient treatments.

For the proper validation of drug and other nutrition compounds, the characterization of absorption, distribution, metabolism, and excretion (ADME) properties is pivotal. In the intestine, orally administered nutrients and drugs are taken up via the apical surface. Currently, around 60% of the drug products that are commercially available are administered via the oral route[9]. However, in intestinal organoid models the apical side is facing the enclosed lumen, thus access to it is challenging. To facilitate ADME studies, we developed intestinal organoids with reversed epithelial polarity, where the apical surface is facing outwards to the culture medium (chapters V and VI). This scalable organoid model could facilitate nutrient and pharmaceutical studies, since it mimics more closely the *in vivo* situation where there is direct contact between the apical surface and the substances. Apart from that, organoids with apical-out orientation could be used to study the gut barrier function. This is important for the assessment of permeability, which again is connected to nutrient and drug uptake. Barrier dysfunction has been associated with several diseases such as food allergies, microbial infections, irritable bowel syndrome and diabetes, thus the use of such advanced intestinal organoid models could pave the way for unravelling unknown mechanisms underlying the relationship between barrier dysfunction and disease and later on, for testing new therapeutic agents.

The apical surface of the intestine is also exposed to the complex gut microbiota, which mainly consists of anaerobic microorganisms. To recapitulate closer this *in vivo* situation, we developed apical-out organoids in a hypoxic environment and performed co-culture with two of the most dominant probiotic bacteria species of the intestine; Lactobacillus and Bifidobacterium (chapter VII). Probiotics are known for their health-promoting benefits (e.g. epithelial barrier integrity and host immune response) and in the past decade, there is growing demand for probiotic supplements. Specifically, the amount of consumers taking probiotics increased by 66% in the U.S., 188% in Italy and 108% in China between November 2019 and May 2020. The global probiotics market was $58.17 billion in 2021 and is expected to expand tremendously in the upcoming years[10]. Thus, such advanced 3D *in vitro* models could benefit food industry (global market amount to $8.66 trillion[11]), since they can be used as bacteria testing platforms to develop new, efficient probiotic supplements.

Overall, the research described in this thesis advanced our understanding in organoid models and provided powerful platforms to study intestinal physiology and disease, and conduct high-throughput nutrient and drug screenings. These models could be mainly used by researchers for fundamental research or for nutrient/ drug screening applications. Overall, these are critical steps towards novel treatment options for digestive disorders, which affect almost 40% of the adult population worldwide with varying severity.

References

1. GI Alliance. Digestive disease continues to rise among Americans digestive disease continues to rise among Americans. *GI Alliance: Nation's Premier Gastroenterology Practice* (2021). at https://gialliance.com/gastroenterology-blog/digestive-disease-continues-to-rise-among-americans.
2. New report reveals Major Health and economic impact of digestive diseases across Europe. *UEG* (2022). at https://ueg.eu/a/306.
3. Top Organoid Research Models Startups. *Tracxn.com* (2022). at https://tracxn.com/d/trending-themes/Startups-in-Organoid-Research-Models.
4. Organoids Market. *Futuremarketinsights.com* (2021). at https://www.futuremarketinsights.com/reports/organoids-market.
5. Vikita, T. & Onkar, S. Organoids Market | Spheroids Market Statistics | Forecast - 2031. *Allied Market Research* (2022). at https://www.alliedmarketresearch.com/organoids-and-spheroids-market-A17036.

6. Deore, A., Dhumane, J., Wagh, R. & Sonawane, R. The Stages of Drug Discovery and Development Process. *Asian Journal of Pharmaceutical Research and Development* **7**, 62-67 (2019).
7. Kingwell, K. 3D cell technologies head to the R&D assembly line. *Nature Reviews Drug Discovery* **16**, 6-7 (2016).
8. Park, K. et al. The Cost of Inflammatory Bowel Disease: An Initiative From the Crohn's & Colitis Foundation. *Inflammatory Bowel Diseases* 26, 1-10 (2019).
9. Alqahtani, M. S., Kazi, M., Alsenaidy, M. A. & Ahmad, M. Z. Advances in oral drug delivery. *Frontiers in Pharmacology* **12,** (2021).
10. Probiotics Market Size | Industry Report, 2021-2030. *Grandviewresearch.com* at https://www.grandviewresearch.com/industry-analysis/probiotics-market.
11. Food - Worldwide | Statista Market Forecast. *Statista* (2022). at https://www.statista.com/outlook/cmo/food/worldwide.