Mind the gut

EPILOGUE

SUMMARY
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Summary

The advent of organoid systems has revolutionized biomedical research by offering powerful three-dimensional *in vitro* models, which closely recapitulate the architecture, cellular heterogeneity and function of specific oragns. Even though such systems might be of great potential for translational applications, such as drug discovery and regenerative medicine, organoids are not without limitations. This thesis focused on identifying ways to overcome current limitations of organoids and expand their range of applications. To achieve that, we used both adult (ASC) and pluripotent (PSC) stem cell-derived intestinal organoids, since they are one of the most well-established organoid models and we developed different methods to culture them. In chapter I, we provide a general introduction to intestinal organoid models development and present a thesis overview. In chapter II, we review the current challenges of intestinal organoid models and discuss the available approaches to overcome them. Additionally, we envision the next-generation of gastrointestinal tract organoids as integrated models that recapitulate structural and functional characteristics of multiple regions of the digestive tube in a single *in vitro* model. In chapter III, we develop a microwell-based intestinal organoid model, where organoids cultured with limited amounts of basement membrane extract, demonstrate reduced variability and survive for prolonged time periods, compared to organoids embedded in hydrogels. This system facilitated the *in situ* monitoring of organoids during culture as well as the downstream processes, enabling the possibility of high-throughput screenings. In chapter IV, we increase the system complexity to more accurately model its *in vivo* counterpart, by integrating immune cells, which surround the intestinal epithelium. Using a combination of organoids and macrophages in tightly controlled spatial conformation, we were able to model both acute and chronic intestinal inflammation states. In chapter V, we develop a novel PSC-derived intestinal organoid model with reversed epithelial polar organization, where the apical surface of the organoid is directly accessible, facing the culture medium. This system facilitated nutrient and drug uptake and metabolism studies, as well as studies related to host-microbiome and host-pathogen interactions. In chapter VI, we present the functional aspects of the system by performing a polarity-specific nutrient uptake assay and testing barrier formation and integrity, metabolic functions, as well as the presence of functional apical and basal transporters and drug metabolizing enzymes. In chapter VII, we follow up with the applications of reversed polarity organoids and adapt the protocol described in chapter V to hypoxic conditions. More specifically, we establish hypoxia-tolerant apical-out intestinal organoids in order to study host-microbiome interactions. Most

of microorganisms in the intestine are anaerobic, suggesting that they survive and grow only in environments with low or no oxygen and therefore, the hypoxia-tolerant apical-out intestinal organoid system allows for the first time the study of the complex gut-microbiome interactions in low oxygen conditions using apical-out organoids. In chapter VIII, we provide a historical and critical perspective of preclinical intestinal *in vitro* and *in vivo* models, we contextualize our findings in this context and provide our vision on the future of intestinal organoids. Finally, in chapter IX, we explore the scientific and social impact of the organoid systems developed in the context of this thesis.

To conclude, this thesis provides valuable insights into the development of more advanced and representative *in vitro* organoid models. The new methods established here to culture intestinal organoids, can be a steppingstone to overcome current limitations and expand the range of applications of organoid models.