Getting wired

Chapter 9

Valorization

When it comes to research, conflicting thoughts come to my mind. On one side, I firmly believe that research should focus on real world problems and work collaboratively to find efficient solutions in an economical and sustainable manner. This entails working from day one towards a goal to minimize tangential tasks that often arise when the direction is unclear. On the other hand, I also believe that uncompromised fundamental research is needed to expand the base knowledge. Oftentimes, in the quest for nothing, a serendipitous outcome results in an extraordinary and impactful finding. Thus, it seems to me that a balance between translational and innovative research is necessary to achieve an optimal valorization path. The research shown here commenced with a mildly defined goal/concept and it took some trial-and-error and some tangential exploratory work to finally arrive at the present stage.

In this chapter, the valorization potential of the research described in this thesis is discussed, specifically how it fits within societal needs and how it can be commercially explored.

The need for organ models

The drug development process is a long and expensive path that lasts, on average, 10 to 12 years and uses hundreds of millions of dollars to generate a single clinically applicable product[1]. Most of the budget is spent during the clinical trial phase, and a large fraction of it is wasted since several drug candidates prove to be inefficient or unsafe[1,2]. To reduce the time and costs, it is imperative to improve the predictive power of the pre-clinical phase, which means eliminating ineffective drug candidates as soon as possible and detecting compounds with potential benefits early on. To this end, 3D *in vitro* organ models can provide a tremendous help and revolutionize the pharmaceutical industry, with organ-on-a-chip technologies representing the lion's share of this emerging field. By combining tissue engineering strategies with microfluidic/microfabrication technologies, miniaturized versions of an organ functional element can be fabricated and analyzed in a convenient manner. The goal is to provide a better testing system than current *in vitro* culture models, which do not recapitulate several aspects of native organs and thus produce unreliable data. Compared to animal models, these devices would also provide a superior testing platform, by permitting a direct, real-time and more focused analysis with overall reduced costs[3]. However, to produce a true replica of an organ

that presents identical characteristics in terms of gene expression, protein production, and physiological activity is an immense task, and validation studies will be required to define what are the benchmarks. Furthermore, it will be necessary to evaluate the pharmacokinetic behavior within the organ model and compare it with the ADME-TOX (adsorption, delivery, metabolism, excretion/toxicity) database[1].

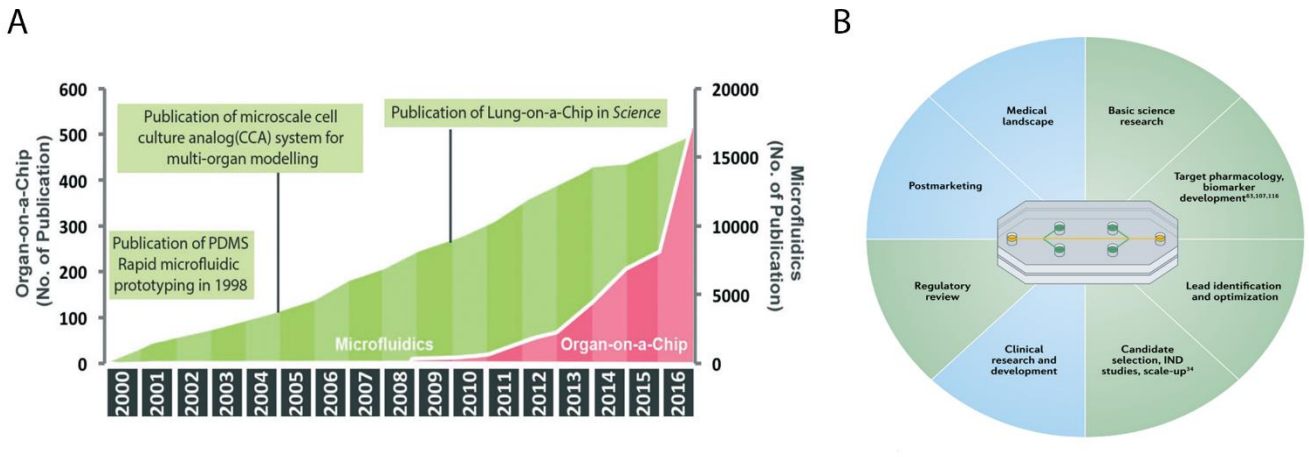

Figure 1. Organ models in research. A) Rise in publications in the organ-on-a-chip space from 2000 to 2016. Data extracted from Zhang et al., *Organ-on-a-chip devices advance to market* (2016)[1]. B) Utility of organs-on-a-chip on drug development phases. Green components represent the current or shortly predicted use of devices and blue components represent possible and predictable utility. Diagram extracted from Low et al., *Organs-on-chips: into the next decade* (2020)[4].

The market of organ models

Due to the long developmental road ahead, it will still take some years until organ-on-a-chip platforms become standard within the drug development pipeline. Despite this, several companies have already emerged in the scene and are commercializing organ models. In some cases, the commercial product is behind the biotechnological state-of-the-art, but the creation of these companies allow them to be pioneers and colonize the marketplace[1]. The type of product depends obviously on the type of tissue/organ in question, but also in the type of function analysis that is of interest. Currently, organ models can be categorized in:

- **Interface models** – example: lung-on-a-chip, gut-on-a-chip, kidney-on-a-chip (from Emulate, Inc)
- **Multi-organ models** – example: Human-on-a-chip (TissUse GmbH), Multi-organ chip (Hesperos, Inc)

- **Parenchymal tissue models** – example: AngioChip (TARA Biosystems, Inc), 3D cardiac systems (Myriamed GmbH)

To date, most devices only manage to model a single or a small-range of tissue functions, and the device in itself is often a simplistic representation, far from representing the biological anatomy, of the complex and hierarchical organ microenvironment. Thus, the current market is still developing and pursuing the creation of improved *in vitro* organ models that also replicate anatomical functionality.

Neural models: the existing competition

With peripheral nerve (PN) models in mind, there are a few companies commercializing compartmentalized microfluidic devices for neural cultures, such as Xona Microfluidics® (selling the XonaChips®) and ANANDA Devices (selling the Neuro-HTS™). Such devices permit a simple segregation of soma and axons, which is convenient to analyze axonal damage resultant from injury or disease-related imbalances and to screen therapeutic drug candidates. However, these platforms are composed of two-dimensional (2D) simplistic nerve tissue replicas that are very different from actual PNs. The only existing company that has a realistic PN model and whose product is closely related to this research is AxoSim, Inc that sells the NerveSim™ platform. This platform is composed of a plastic mold with a key hole-like design where a hydrogel (Matrigel) and cells are placed. Prior to implantation in the platform, human induced pluripotent stem cells (iPSCs)-derived neurons and human primary Schwann cells are formed into a spheroid. The cells are cultured in the platform up to 4 weeks to produce long anisotropic and myelinated neurites that can be probed for electrical activity. Beyond this, the platform has no other validated applications, as also described in their reference paper[5].

Applicability of this research

In this research, we describe the development of *in vitro* PN and tissue innervation platforms. Due to the high worldwide incidence of PN damage caused by traumatic injuries, such as motor vehicle accidents or sports injuries, or because of a pathophysiology, such as diabetes-related hyperglycemia, it is imperative to have PN *in vitro* models for preclinical research. The innervation of tissues is also critical for their function and homeostasis, and thus for an accurate *in vitro* modelling, it is necessary to include a neural component. Nerve presence can also have nefarious consequences such as propagating cancer[6] and transmitting high levels of pain in patients with endometriosis[7]. Therefore, this research falls upon a broad range of clinical needs that are highly relevant for the medical and pharmaceutical industry.

1) Peripheral nerve models

In chapter 3, we developed our first version of a PN model, based on a scaffold/hydrogel hybrid platform and using a cell line (PC12) as neuron population. The scaffold/hydrogel system constitutes a novel approach towards forming three-dimensional (3D) and anisotropic neural tissue in a simple and affordable manner. Using low technological and cheap culture methods, we were able to generate PN tissue with high-level of maturity and high-level of versatility regarding its contents (protein composition and cellular presence). We demonstrate the use of this model in neurotoxicity, drug screening and disease modelling studies, all of which are highly relevant within the neurobiology field. Despite its discrepancies from actual human neurons, PC12 cells constitute an important and useful tool because of their low-cost/low-maintenance requirements and wide range of applications, such as in neurotransmitter release tests. Therefore, we envision the use this method across labs for a "do-it-yourself" PN model fabrication, via full protocol replication or customized adaptation. Additionally, the major model components such as the scaffold and silicone platform could be commercially explored to facilitate the assembly tasks of potential clients.

In chapter 4, we developed an improved version of the PN platform, by substituting the PC12 cell line with human induced pluripotent stem cells (iPSCs)-derived nociceptors. These neurons are of human origin and we demonstrated that they behave as actual nociceptors, exhibiting electrical activity and reacting to noxious stimuli. These characteristics makes them suitable to construct an *in vitro* pain model that can be used to assess the safety and potential painful effects of a certain drug. In literature there are already a few *in vitro* nociceptor models such as the work of Wainger et al.[8] and Jones et al.[9] However, none of these models achieved the level of PN biomimicry and maturation that we attained which makes our model the most representative sensory PN model. Additionally, we developed a method that generates thousands of nociceptor spheroids, with uniform sizes, and in a short time frame. This confers a higher level of reproducibility and robustness to our method in comparison to existing models. When co-culturing the nociceptor spheroid with Schwann cells (SCs) in the scaffold/fibrin hydrogel system, we were able to generate 3D, long, anisotropic and stable myelinated tissue. Compared to the NerveSim™, our platform is larger (in fact the largest ever shown), thus showing a better approximation to the actual human dimensions. Rather than using Matrigel, our model is built from a less expensive and more defined hydrogel material (fibrin gel). Additionally, we show the ability to conduct disease modelling and drug screening experiments. In sum, due to the large range of applications and low competition, this platform is endowed with high commercial appeal. We contemplate the establishment of the 3D platform as a product that can be purchased ready-made, in order for laboratories to conduct their own investigations. Alternatively, the model components such as the agarose mold and scaffold could be sold individually or in a package, to allow researchers to assemble the platform

themselves. Finally, a testing service could be set up to provide, upon client (e.g. pharmaceutical companies) request, a detailed assessment of a compound's action on nerves (and nociceptors in particular). We demonstrate an example of the latter in chapter 5, where we investigate the action of phosphodiesterase-4 (PDE4) inhibitors on SCs and neurons. PDE4 inhibitors are a family of compounds with high research interest within neurobiology/neurosciences, due to their various proved benefits. New formulations of these drugs were synthesized by a collaborator based in Maastricht and Hasselt University, and were tested in our facilities using our system.

2) Innervation models

As previously mentioned, innervation models are highly desired within biomedical research, and besides some literature reports (using motor rather than sensory neurons[10,11]), there are no products in the market yet. In chapter 4, we demonstrate that our platform is also suitable to innervate target tissues, showing examples with pancreatic and endometrial tissue models. Both of these tissues are associated with neural pathologies, and thus the existence of innervation models will provide a very useful and needed research tool to investigate pathophysiological mechanisms and screen therapeutic drug candidates. In chapter 7, we maintained the same platform components, but altered its assembly process to include vasculature and generate a neurovascular model. The simultaneous presence of a vascular and neural network constitutes a step forward to achieve higher organ modelling complexity, necessary to improve the current state of organ-on-a-chip platforms. Therefore, this model can work as supporting unit for target tissues that require both vascular and neural supply.

Lastly, in chapter 6 we described the formation of a human innervated skin model. Advanced skin models are coveted within the biomedical field due to the large incidence of skin-related conditions. Additionally, because of the European Commission ban on the use of animals for cosmetic testing, there has been an increased interest by cosmetic companies, such as L'Oreal, in developing their own skin models. Currently, several companies, such as Phenion, LabSkin and Episkin, already commercialize skin equivalents. However, none of these products contains the innervation component that is critical for skin function and regeneration. Furthermore, the presence of nociceptors within a skin equivalent will provide the ability to detect if topically applied products can produce an adverse/unwanted reaction, such as irritation or burning sensation. Therefore, we envision that this model has a high commercial appeal, particularly for the pharmaceutical and cosmetic industry.

Future improvements

Despite the mentioned achievements, we recognize that in order to succeed in extracting the most valorization of this research, a number of improvements are still required. These improvements fall upon a number of parameters that due to the nature of this fundamental research do not yet meet the necessary criteria for commercialization. The suggested modifications are:

1) **Automated and clean fabrication** – The current scaffold production and assembly method is heavily reliant on manual labor. Automation would reduce the fabrication time and improve reproducibility. This could be achieved using an industrial setting electrospinning apparatus with precise control of all parameters for scaffold production, followed by an automated scaffold-cutting tool. These operations would be undertaken in a cleanroom facility to minimize contamination.

2) **Inclusion of microfluidic channels** – The addition of a perfusion system would greatly enhance the platform potential, by permitting a regulated perfusion of the vascular network or controlled drug delivery. This could be achieve with the microfabrication of an encasing structure made from a non-leachable/non-cytotoxic material (e.g. glass) that would permit to template channels (via removable rods, ambient-responsive materials, etc.). The platform could then be coupled to a perfusion pump for delivery of medium at a physiological shear stress value.

3) **Physical/Chemical parameter modelling** – Computational models (using for instance the COMSOL Multiphysics software) backed up by “real-life” experiments to describe some relevant physical and chemical parameters, such as oxygen concentration, which have an influence in several biological processes (e.g. angiogenesis).

4) **Precise cell positioning** – Automated methods for cell seeding to improve the reproducibility of the tissue-engineered constructs. This could be achieved using robotic spheroid dispensing techniques. Alternatively, formation of cell compartments within microfluidic platforms, where cells can be immobilized.

5) **In-line sensing devices** – Development of sensors that could be incorporated within the platform to acquire parameter information (pH, oxygen level, neuron activity, etc. in real-time and non-destructively.

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