Quantifying functional phenotypes in human pluripotent stem cell derived cardiomyocytes for disease modelling and drug discovery

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Summary

Studying diseases or the effect of new drugs on the human body is challenging, not least because of the lack of proper testing models that recapitulate human physiology. Most research is done using animal models but these often show differences with humans in disease manifestation and responses to drugs. For example, drugs that had no effect on the hearts of animals later turned out to cause lethal arrhythmias in some humans. This – and the ethical issues around animal testing – is why, as in this thesis, there is increasing interest on making human heart models based on pluripotent stem cells (hPSCs). Aside from the heart, hPSCs can form all other cell types of the human body. hPSCs are normally only present in early embryos but can be isolated in culture as 'embryonic stem cells'. Since 2006, however, they can also be created artificially by genetic reprogramming of cells from the adult body. These hPSCs are referred to as 'human induced pluripotent stem cells' (hiPSCs). Many protocols are now available to direct 'differentiation' of hPSC to different types of tissue and organ specific cells, including lung, retina, blood vessel, brain and heart cells. This now means small numbers of cells can be collected from a patient (e.g. from blood, urine or skin), reprogrammed to hiPSCs, and then differentiated to heart muscle cells (cardiomyocytes). Since the genetics of the patient are maintained during reprogramming, the phenotype of a genetic disease affecting cardiac function can also be captured. The focus of this thesis has been developing methods to measure these cardiac phenotypes robustly and with sufficient complexity to reflects drug responses and disease of the heart.

Arguably, the heart is the most important organ of the human body and is thus a life-threatening target of toxic compounds and disease. Side effects of drugs designed to treat cancer (e.g. doxorubicin) often lead to impaired cardiac function. Using cardiomyocytes derived from hPSCs (hPSC-CMs) pharmaceutical companies are expected to be able to identify and possibly mitigate cardiotoxicity in early stages of their drug development pipeline.

To study the effect of drugs but also to investigate disease phenotypes, it is not only important to have hPSC-CMs but also the measurement tools used to evaluate cell behaviour. In this thesis, different tools to study the function of hPSC-CMs were developed, validated and tested. Firstly, the state-of-the-art on measurement methods in current use were reviewed (Chapter 1 & 2). We noted that quantification of cardiomyocyte contractility was mainly being done in research groups dedicated to physiology using complex measurement tools. This inspired us to develop MUSCLEMOTION, an open source software tool that enabled every biologist to quantify contractility simply using standard laboratory equipment (Chapter 3 & 4). Moreover, we found that we could quantify cardiac contractility not only *in vitro*, but

also *in vivo* in zebrafish and human echography recordings.

Cardiomyocyte contraction is not a simple process but the result of changes in the level of free intracellular calcium ions which is in turn regulated by changes in the electrical potential of the cell membrane. This process is called the excitation-contraction coupling (EC coupling) and cardioactive drugs typically induce changes in different stages of this process resulting in an increase or decrease of contraction force and/or beat rate. In Chapter 5, we developed for the first time a measurement method that provides insight in the different stages of the EC coupling. The microscope we built is capable of quantifying the electrical activity, calcium flux and contraction of muscle cells simultaneously. In addition, using a prediction algorithm based on previously published knowledge we could not only detected drug-induced changes in EC coupling but also identify mechanisms of action of these drugs. In other words, we could not only identify the resulting changes in contraction force and/or beat rate but also the mechanism that was targeted by those drugs. In Chapter 6 we validated the use of hPSC-CMs in combination with complex measurement systems through a blinded, multi-center study. We found that overall, hPSC-CMs were similarly predictive – if not better – than isolated rabbit cardiomyocytes for the compounds tested. Nevertheless, hPSC-CMs were not 100% accurate compared to clinical data and one important underlying reason for the discrepancy is that hPSC-CMs are typically immature, resembling fetal cardiomyocytes rather than muscle cells from the adult heart.

Further advance in methods to induce cardiomyocyte maturation is an important research area. While approaches such as development of multi cell type 3D tissues are among the biological routes to enable maturation, microengineering of a more physiologically relevant environment may achieve the same goal. These engineered microenvironments containing cells are often called micro physiological systems or Organs-on-Chips (OoCs). They can also accommodate sensors and actuators. In Chapter 7, a chip containing such a micro environment was developed and tested. This OoC device was modular to accommodate different cell types (e.g. heart, skin) and include different sensors (e.g. electrodes, force transducers). One of the key components in this device and used widely in the field is poly-dimethylsiloxane (PDMS) even though it is known to absorb compounds. We quantified this in detail for cardiac relevant drugs in Chapter 8 and investigated methods to mitigate this problem. Importantly, we found that it was not hydrophobicity as generally claimed but topological polar surface area that seemed to predicted compound absorption in PDMS.

Finally, Chapter 9 the results of these thesis and future perspectives are discussed.

Our results supported the notion that hPSC models will become human avatars and accurate measurement models able to recapitulate essential human-specific processes. Evidence suggests that it will also be possible to include inflammatory triggers of disease and the immune system. Nevertheless, for application in the drug development pipeline, disease modelling or personalized medicine, validation and development of robust, reproducible, scalable technology will be key.