Project title:	Using Human Induced Pluripotent Stem-cell (hiPSC) Technology to Model Endothelial Dysfunction In Patients with Chest Pain and Non-Obstructive Coronary Artery Disease
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Reflective report

This elective grant has allowed me to spend 8 weeks in Dr Wu's laboratory at Stanford to study cardiovascular diseases using stem-cell models under Dr Nguyen's supervision. During the elective I have learnt various study approaches, both theoretical and experimental, to tease apart the complex mechanisms involved in cardiovascular disease such as radiotherapy induced cardiomyopathy. I became acquainted with laboratory equipments and learnt invaluable experimental techniques in stem-cell biology, including stem-cell culture, cardiomyocyte differentiation, viability assays, molecular characterization and flow cytometry. These are all transferable skills that I can certainly apply in the future. More importantly, this elective has given me an invaluable insight into the prospects of using induced pluripotent stem-cells as to simulate and study pathological mechanisms.

The burden of cardiovascular diseases

It is estimated that non-communicable diseases (NCD) kill more than 36 million people each year. Cardiovascular diseases account for most of the non-communicable diseases, with 17.3 million deaths annually worldwide. [1] The World Health Organisation has therefore developed a Global NCD Plan 2013-2020 to provide a roadmap to tackle this problem. However, most of the resources were focused on optimising care pathway for coronary artery disease such as lifestyle changes. However, many patients with chest pain do not have significant stenosis of the coronary artery. Recent studies have found that endothelial dysfunction, an early manifestation of coronary artery disease, is a primary cause of chest pain in these patients. [2] The lack of access to endothelial cells from these otherwise healthy patients hampers characterisation of this disease. Similarly, the limited access to human cardiomyocytes makes it difficult to study heart failure, such as those caused by genetic defects (hypertrophic cardiomyopathy, dilated cardiomyopathy), or as an adverse effect of medications/radiation therapy.

Modelling disease process

A potential strategy of better understanding these diseases and identifying therapeutic targets is the use of embryonic stem cells (ESCs), which have the potential to differentiate into endothelial cells and cardiomyocytes. However, there are huge controversies and ethical issues surrounding the access to embryonic stem cells. There has been an appeal to the US Supreme Court against the use of human embryonic stem cell. [3] In Europe, there were bans on patents based on embryonic stem cells. [4] The emergence of induced pluripotent stem cell (iPSCs) technology, whereby adult peripheral cells such as skin cells and adipocytes are converted to pluripotent state, has provided a new source of pluripotent stem-cells without the need for human embryo. (Figure 1a and 1b) Techniques of differentiating stem-cells into cardiomyocytes have also improved over the recent years. Non-viral techniques have evolved with high efficiency in cardiomyocyte differentiation. [5] During this elective placement, I became familiar with culturing and maintaining various ESCs and iPSCs lines, as well as differentiating them into cardiomyocytes and endothelial cells. (Figure 1) By generating cardiomyocytes from patients with genetic disorders such as hypertrophic cardiomyopathy and dilated cardiomyopathy, cellular pathologies could be characterised. Parameters such as contractility, electrophysiology and calcium handling could be measured in vitro and compared with cardiomyocyte derived from healthy volunteers. Using this approach, it was found that iPSC-cardiomyocytes (iPSC-CMs) from dilated cardiomyopathy patients have abnormal sarcomeric α-actinin distribution, and treatment with β-blocker significantly improved sarcomeric organization and contractility. [6]

Clinical trial and personalised medicine

In vitro-derived iPSC-CMs have also been assessed as potential screening platforms for drug discovery and toxicology studies, where it was estimated that 40% of all drug withdrawn from the market is due to cardiac toxicity [7]. Several cardiovascular drugs, such as adrenergic receptor blockers, have been shown to exert chronotropic and inotropic effects on iPSC-CMs. By phenotype profiling the iPSC-CMs that were exposed to the drugs, high throughput cardiotoxicity screening in drug discoveries could be achieved. An added benefit of this approach is to elucidate the toxic effects that are specific to human but not other animals. Indeed most of the withdrawn medications did not observe toxicity in preclinical studies with animals. iPSC-CMs also provide a useful tool for drug screening while avoiding some of the limitations of conventional clinical trials such as cost and ethical concerns. Taking this further to personalising medicine, iPSC-CMs generated from patients' own skin cells/adipocytes could be used for therapeutic trial. This could help to evaluate variations in pathology and response to medications due to genetic diversity, hence clinical decisions could be made to maximise therapeutic benefit while avoiding unpredicted adverse effects.

Regenerative medicine

One of the most exciting prospects for iPSC-CMs is the potential for autologous transplantation with the added benefit of not needing immunosuppression. Transplantation of iPSC-CMs has been shown to improve cardiac function in rat and guinea pig models of acute myocardial infarction. [8,9] However, poor stem cell survival after transplantation remains the main limitation [10], as well as generation of arrhythmias. Further understanding of stem cell biology is needed to overcome these challenges in the future.

Reflections

Equipped with advanced facilities and a strong academic environment, the Cardiovascular Institute is a fantastic platform for biomedical research. The experience at Stanford has certainly broadened my insight into studying cardiovascular disease, and provided an indispensible opportunity for me to apply my knowledge acquired in the medical course into research. As much as I enjoyed the project work, I have also derived huge pleasure of meeting other researchers in the department. They were amicable, approachable, and attentive in clarifying my queries. Working alongside established researchers has been an invaluable experience. In addition, there were ample opportunities for me to attend seminars that gave me an insight into latest research works from other laboratories. Completion of this project gave me immense satisfaction and pride, having contributed to furthering the understanding in the cardiovascular diseases. I am greatly indebted to my supervisors Dr Wu and Dr Nguyen, and would like to thank the Society for the opportunity to allow me to spend an extremely interesting and educational period in a high quality laboratory at Stanford.

References

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Figure 1 a) human embryonic stem cell b) human induced pluripotent stem cells generated from skin cells c) cardiomyocytes derived from human induced pluripotent stem cells.

A.

B.

C.