Defining Cell Culture Conditions to Drive Cell Identity and Scalability in Cell Therapy

Using epigenetically predicted factors to mimic *in vivo* microenvironments and systematically define *in vitro* culture conditions for optimal cell maintenance and differentiation

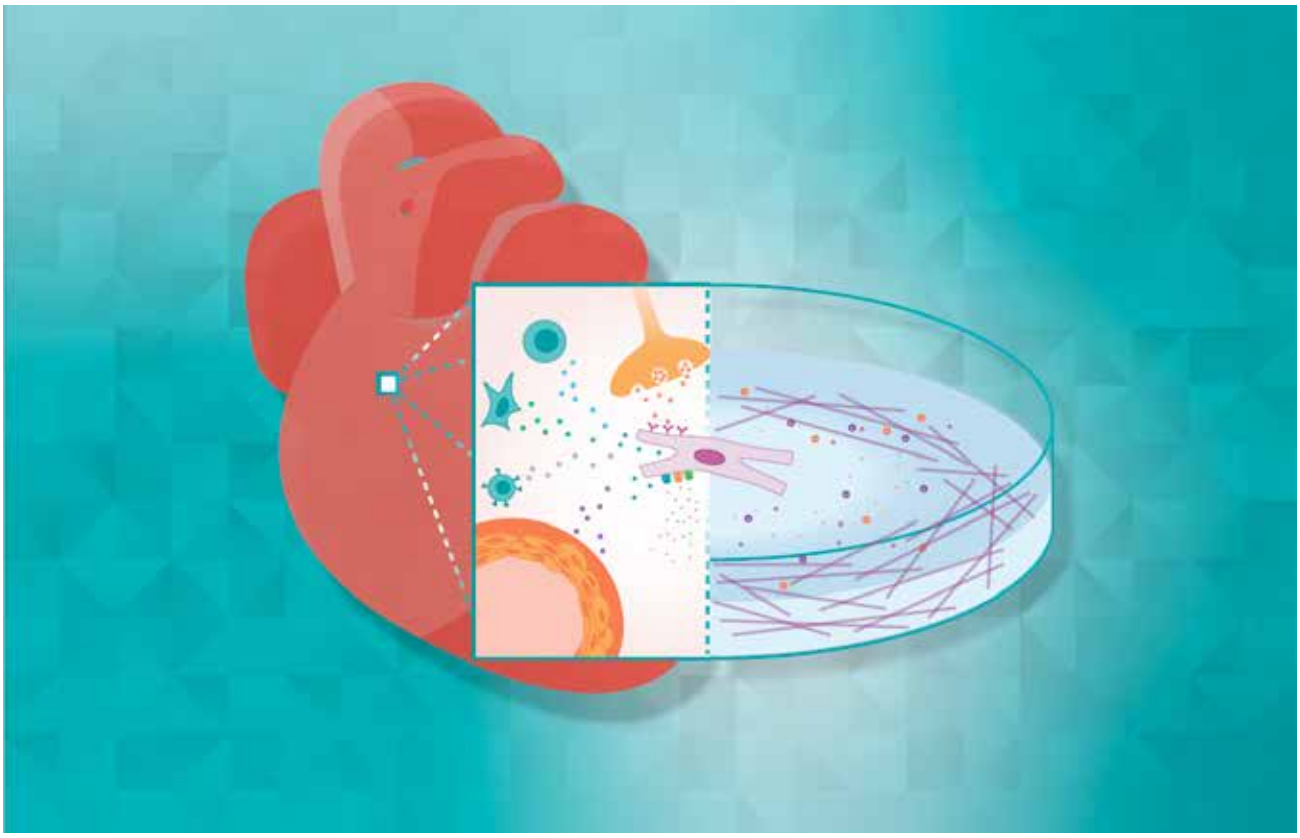
Dr Aida Moreno-Moral and
Dr David Anderson at Mogrify

Cell therapy is a powerful strategy to treat and cure diseases that have been untreatable to date. For many diseases, including heart disease, diabetes, and liver failure, cell replacement remains the only option for curative therapy. Pluripotent stem cells (PSCs) represent a valuable resource for the generation of cell types for treating these diseases. Protocols have been developed and refined, allowing the differentiation of human embryonic stem cells and induced pluripotent stem cells (iPSCs) to numerous cell types *in vitro*. These include cardiomyocytes, pancreatic beta cells, and hepatocytes, which have therapeutic potential to treat heart disease, diabetes, and liver failure. Many studies have shown that PSC-derived cardiomyocytes, pancreatic beta cells, and hepatocytes can be transplanted in animal models, and are capable of engrafting and providing a functional benefit.

However, the development of cell therapies is tightly linked to our ability to culture cells in artificial environments outside of the body, i.e., *in vitro* conditions. In our bodies, cells live in highly regulated and specialised microenvironments, also known as niches. These niches provide adequate conditions for cells to perform their function, maintain their cellular identity and, whenever required, divide and respond to their surroundings. In these *in vivo* conditions, there is also an active and dynamic communication to nearby cells. This communication acts via the exchange of specific signalling molecules, or in some cases via physical, mechanical, or even electrical stimulations, such as in the heart. Unfortunately, cells lose their characteristics whenever they are taken out of their natural *in vivo* conditions or when they are grown *in vitro*.

It is very difficult to generate cells that resemble the characteristics of primary cells via *in vitro* cell differentiation or maintenance laboratory protocols. Different cell types have different chemical, environmental, and cell attachment requirements, and, therefore, the development of cell therapies comprises significant cell manipulation and complex manufacturing processes. Current state-of-the-art cell culturing protocols heavily rely on the use of poorly defined materials and animal-derived products, for example, the use of mouse embryonic fibroblasts as feeder layers, or the use of Matrigel, a mouse sarcoma protein extract. Using these undefined products can pose significant risks as they pose batch-to-batch variation and, in some cases, can even be tumourigenic. The development of high-quality cells for therapeutic applications requires good manufacturing practices (GMP) that are reliable and have highly regulated quality controls. Hence, there is a strong need for cell culture protocols that are chemically defined and free from the use of undefined animal serums, i.e., xeno-free.

In addition to the lack of chemical definition and despite the therapeutic promise of cell transplantation, several other technical issues exist that hinder further progress. Some of



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these issues are the low level of engraftment of transplanted cells and their potential for teratoma formation and immune rejection. Other key factors to be addressed are the maturation state and purity of the cell population used. Evidence suggests the maturation state of PSC-derived cardiomyocytes at the point of transplantation is a critical determinant of the efficiency of engraftment and level of increase in heart function observed. For example, it was demonstrated that the level of functional improvement observed in a mouse model of myocardial infarction varied dependent on the age of PSC-derived cardiomyocytes used (1). Where for PSC-derived cardiomyocytes, obtaining the optimal maturation state is key, the use of PSC-derived pancreatic beta cells and hepatocytes for transplantation has been restricted by their incomplete functional maturation *in vitro*. Despite this, there have been encouraging transplantation studies, reporting that PSC-derived pancreatic beta cells can ameliorate the phenotype of diabetic mice and PSC-derived hepatocytes can improve survival rates of mice following acute lethal liver injury (2-3). These studies indicate the potential of PSC-derived therapeutic cell types, if the highlighted issues, including maturation state and purity, can be overcome.

The functional maturity and purity of many PSC-derived cell types can be altered by manipulation of *in vitro* cell culture conditions. Various cell culture media additives have been proposed to maintain PSC-derived cardiomyocytes in a progenitor-like state, improve their functional maturation, and direct their differentiation into specific cardiac lineages (4-6). Modulation of the extracellular matrix for PSC-derived cardiomyocyte differentiation can also influence the efficiency and maturation state of cardiac differentiation (7-8). For both PSC-derived pancreatic beta cells and hepatocytes, culture media additives and substrate modifications, to mimic the cell-specific niche, have each been important for enhancing the maturation state of cells *in vitro* (9-10). However, the discovery of cell culture conditions that can support cells with niche-like properties is often achieved through trial and error, partially informed by the study of developmental biology and animal models – processes that often extend across years or even decades of experimental work.

In recent years, there have been major breakthroughs in our ability to profile cells at high resolution, which, in turn, will speed up the ability to develop robust cell culture protocols. For instance, with the current high-throughput technologies, it is possible to understand what genes are being transcribed, translated, or even to profile the state of the chromatin at a given time point. This is all contributing to our understanding of the cellular features underlying cell identity, how cells behave, respond to their environment, and communicate in a broader cellular context. To get further insights into the cellular niche, it is even possible to study the transcriptomic state of each cell and its physical localisation at the same time. An example of this is the work by Baccin *et al*, in which they inferred the 3D organisation of the bone marrow

from single-cell spatial transcriptomics data (11). In their study they found specific cell subsets acting as specialised cytokine-secreting cells that establish micro-niches (11). Recent efforts have also led to the inference of cell-cell signalling networks, which are of great value to uncover the signals that cells are naturally engaged with, also as part of their niche. To uncover molecules that can provide improved growth conditions, screening approaches have also been developed that take advantage of combined high-throughput cellular profiling (12).

These recent advances have brought us unique opportunities to create powerful computational platforms that can leverage high-throughput cellular profiling to unbiasedly predict optimum growth conditions to enhance and maintain cell identity, such as the recent method published by Kamaraj *et al* (13). In their work, high-throughput epigenetic profiling was used to build a framework to predict the matrix and media ligands required for cell culture. This method was validated on both cardiomyocyte and astrocytes maintenance and differentiation. The platform was used to predict media and matrix components that replaced Matrigel and provided xeno-free and chemically defined culture conditions, saving on years of experimental trial and error. Computational approaches like this are key to the successful development of cell therapies, especially as the demand for high-quality cells under GMP increases, to tackle major diseases that have been untreatable to date.

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Dr Aida Moreno-Moral is Director of Cell Informatics at **Mogrify**. Aida completed her PhD in Cardiovascular System Genetics at Imperial College London, UK. In her PhD she carried out high-throughput omics data integration across species to uncover novel targets for cardiovascular disease. This work led to a National Medical Research Council grant at Duke-NUS Medical School, Singapore, where Aida worked in the discovery and development of therapeutic targets for fibrotic, autoimmune diseases, and cell therapies.

Dr David Anderson is Principal Scientist at **Mogrify**. David completed his PhD training in Pluripotent Stem Cell Biology at the University of Nottingham, UK. During his PhD and post-doctoral training, David worked to define the differentiation of PSCs to cardiovascular lineages for disease modelling and drug toxicology screening purposes. David has also held a number of scientific roles at healthcare and biotech companies, including GE Healthcare, Horizon Discovery, and Empyrean Therapeutics, and has developed expertise in gene editing of primary cells and PSCs.