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Engineered heart tissues and induced pluripotent stem cells: Macro- and microstructures for disease modeling, drug screening, and translational studies☆

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ABSTRACT

Engineered heart tissue has emerged as a personalized platform for drug screening. With the advent of induced pluripotent stem cell (iPSC) technology, patient-specific stem cells can be developed and expanded into an indefinite source of cells. Subsequent developments in cardiovascular biology have led to efficient differentiation of cardiomyocytes, the force-producing cells of the heart. iPSC-derived cardiomyocytes (iPSC-CMs) have provided potentially limitless quantities of well-characterized, healthy, and disease-specific CMs, which in turn has enabled and driven the generation and scale-up of human physiological and disease-relevant engineered heart tissues. The combined technologies of engineered heart tissue and iPSC-CMs are being used to study diseases and to test drugs, and in the process, have advanced the field of cardiovascular tissue engineering into the field of precision medicine. In this review, we will discuss current developments in engineered heart tissue, including iPSC-CMs as a novel cell source. We examine new research directions that have improved the function of engineered heart tissue by using mechanical or electrical conditioning or the incorporation of non-cardiomyocyte stromal cells. Finally, we discuss how engineered heart tissue can evolve into a powerful tool for therapeutic drug testing.

Contents

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1. Introduction

1.1. Cardiovascular disease

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the United States [1]. Cardiomyopathies are a prevalent and serious class of CVD in which the contractile strength of the heart is compromised. Furthermore, although patients may present with similar symptoms, and hence are classified as suffering from a single disease, their underlying disease mechanisms are not uniform. Due to the variability in disease mechanisms, medications and dosages may vary among patients, making it difficult for caregivers to provide universally efficacious treatment, thus creating a tremendous burden on the healthcare system. Furthermore, the development of a new drug is expensive and inefficient, requiring on average over a decade of research and development and up to ~$5 billion per drug [2–6].

1.2. Drugs withdrawn from the market due to cardiac toxicity

Withdrawal of drugs from the market due to previously unobserved toxic effects in animals (or false negatives), which have been attributed to off- and on-target toxicity including cardiotoxicity, is an unfortunate reality [7–10]. As examples, Terfenadine (trade name Seldane) was withdrawn in 1998 for inducing cardiac arrhythmias; Grepafloxacin (trade name Raxar) was withdrawn in 1999 for prolonging the QT interval; Rofecoxib (trade name Vioxx, Ceoxx, and Ceeoxx) was withdrawn in 2004 for the risk of myocardial infarction; and Rosiglitazone (trade name Avandia) suffered from a drastic decrease of sales due to reports of increased risk of heart attack [3,9,122]. Typically, drug safety and efficacy are evaluated using non-human animal models followed by costly clinical trials. Human models are difficult to establish because human heart cells or tissue from patients cannot survive long-term culture [11] and are difficult to obtain [12,13]. The existing preclinical testing paradigm relies heavily on the use of *in vitro* cell lines such as Chinese hamster ovary (CHO) and human embryonic kidney 293 (HEK293) cells overexpressing the human ether-à-go-go-related gene (hERG) channels, *ex vivo* tissue preparations such as isolated arterially perfused left ventricular rabbit wedge preparations, and *in vivo* studies such as chronic dog atrioventricular (AV) block models [10]. However, there are several challenges with these models, including their high costs and their poor predictive capacity owing to inter-species differences in cardiac electrophysiology and human biology [14,15]. In addition, CHO and HEK293 cells are not ideal models for cardiotoxicity because ectopic expression of a cardiac ion channel does not always recapitulate function in human cardiomyocytes [16]. Models with poor predictive power lead to a high probability of discarding new chemical entities (due to false positives) that otherwise might have become safe and effective drugs. Hence, there is a need for immediate attention from all stakeholders involved in the drug discovery process to address these concerns and to better evaluate drugs before clinical trials.

1.3. Induced pluripotent stem cells for disease models

A new approach towards reducing inefficacious drug treatment is precision medicine, and this endeavor is increasingly feasible with the advent of induced pluripotent stem cells (iPSCs) [17,18]. Unlike other cells, iPSCs reflect a person's unique genotype because they are derived from a patient's somatic cells (*e.g.*, peripheral blood mononuclear cells or skin fibroblasts). They have the capacity to differentiate into all cell types, including cardiomyocytes (CMs), the force-producing cells of the heart [19,20]. Patient- and disease-specific models are being developed to provide unprecedented multi-dimensional information on the individual's disease and a system to evaluate innovative therapeutic options.

Patients carrying known mutations for a disease are able to contribute to the generation of disease-specific iPSC lines. For example, some of the first iPSC-derived cellular models were developed for LEOPARD syndrome [21], long QT [22,23], familial dilated cardiomyopathy (DCM) [24], familial hypertrophic cardiomyopathy (HCM) [25], Timothy syndrome [26], and aldehyde dehydrogenase 2 genetic polymorphism [27]. Channelopathies, caused by specific mutations in cardiac ion channels, can also be modeled using iPSC-CMs. One example is long QT syndrome, which is characterized by prolonged ventricular repolarization that can lead to sudden cardiac death [28,29] and is caused by mutations in potassium channels [30].

The quality of the disease model can be determined by the disease phenotype of the iPSC-CM as compared to the physiological disease phenotype. For example, DCM iPSC-CMs carrying the TNNT2 mutation [31] displayed disorganized sarcomeric structures, abnormal calcium handling, and decreased contractile function similar to the cardiomyocyte phenotype in DCM patients. Likewise, iPSC-CMs from patients with an HCM mutation in the myofilament myosin heavy chain 7 (MYH7) [25] recapitulated phenotypic features of abnormal calcium handling, increased myofibril content, and cellular hypertrophy at baseline and upon stress [25].

A disease model must also recapitulate physiological drug response in order to accurately evaluate drugs before treatment administration. For example, iPSC-CMs derived from DCM patients respond to β agonists and β blockers in a similar manner to patients. It is important to understand patient-specific β blocker response because although β1-specific adrenergic blockers are beneficial to patients with DCM [32,33], the use of β-adrenergic agonists can lead to increased morbidity and mortality in patients with heart failure [34]. Upon exacerbation by β-adrenergic agonists such as norepinephrine (a drug which physiologically activates the fight-or-flight response and increases heart rate), the DCM iPSC-CM model recapitulates the DCM phenotype [31]. After adding the β-blocker metoprolol, the phenotype was rescued, recapitulating results from previous β-blocker trials [32,33]. In a recent mechanistic study using the DCM iPSC-CM model, a mechanism of compromised β-adrenergic signaling was identified as nuclear localization of mutant TNNT2 and epigenetic regulation of phosphodiesterases 2A and 3A [35].

In another example, iPSC-CMs with long QT mutations had a similar drug response as patients with long QT. Arrhythmias were induced upon treatment with β-adrenergic stimulants and arrhythmias were rescued upon subsequent addition of β blockers [22,36]. In a drug response study, an iPSC-CM model derived from long QT patients showed shortened repolarization upon treatment with the experimental potassium channel enhancers nicorandil and PD118057 [36]. Based on these results, the use of potassium channel enhancers might be considered in patients with long QT.

1.4. Human iPSC-CMs as compared to neonatal and adult cardiomyocytes

The quality of human iPSC-derived cardiomyocytes (iPSC-CMs) is determined by the maturity of their structure and function as compared