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Maturing human pluripotent stem cell-derived cardiomyocytes in human engineered cardiac tissues☆

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ABSTRACT

Engineering functional human cardiac tissue that mimics the native adult morphological and functional phenotype has been a long held objective. In the last 5 years, the field of cardiac tissue engineering has transitioned from cardiac tissues derived from various animal species to the production of the first generation of human engineered cardiac tissues (hECTs), due to recent advances in human stem cell biology. Despite this progress, the hECTs generated to date remain immature relative to the native adult myocardium. In this review, we focus on the maturation challenge in the context of hECTs, the present state of the art, and future perspectives in terms of regenerative medicine, drug discovery, preclinical safety testing and pathophysiological studies.

Contents

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1. Introduction

Cardiovascular disease (CVD) is currently the leading cause of death in the world [1], with ischemic heart disease accounting for the majority of deaths over the past 10 years [2]. Unfortunately, that number is projected to continue to rise in the years to come [3]. In part, this is because damage to the myocardium due to ischemic heart disease (myocardial infarction) does not currently have a curative treatment aside from left ventricular assist devices (LVADs) and/or organ transplantation, which are an option for a limited number of severe cases; and because over 50% of heart disease patients are nonresponsive to the currently available drug therapies [4]. As a consequence, there is a large number of individuals experiencing the debilitating and ultimately fatal effects of heart failure. Hence, there is a need for novel and individualized therapeutic strategies, *e.g.* disease-specific or patient-specific drugs, and cardiac tissues for regenerative medicine [5].

Cardiotoxicity is one of the principle forms of drug toxicity. It accounts for the majority of drug recalls and regulatory approval delays. Recently, numerous non-cardiac drugs, *e.g.* terfenadine, have had to be withdrawn from major markets because of concerns of cardiotoxicity. Still others have been withdrawn prior to marketing, while another subset have required label changes that significantly restricted their use [6]. This phenomenon is attributable to the fact that the current drug testing strategies have inherent limitations. They rely upon animal testing, however there are fundamental differences in the electrophysiological properties of animal and human cardiomyocytes (CMs) that limit the relevance of preclinical animal studies [7]; and human clinical trials have limited applicability due to the necessarily small sample size and the frequent lack of genetic and phenotypic variability. Thus, there is a need for improved preclinical drug screening assays, specifically in discerning cardiotoxic effects and evaluating the efficacy of drug candidates.

Twenty years ago, the successful engineering of a cardiac tissue from embryonic chicken CMs[8] gave birth to three-dimensional (3D) cardiac tissue engineering. This field developed with the objectives of producing *in vitro* surrogates of cardiac tissue for *in vivo* repair and preclinical drug development, and of advancing *in vitro* models of heart function and disease [9]. To date, 3D engineered cardiac tissues (ECTs) have not entered the clinical arena nor have they found wide application in target validation and preclinical drug screening. However, a stacked (non-cardiac) cell sheet patch has been used in patient treatment [10] and there are 3D assays ready for application in automated drug testing. Moreover, recent cardiac cell therapy studies have had only limited success in restoring myocardial function, suggesting the need for alternative cell delivery methods or else a novel regenerative approach. In addition to challenges associated with cell retention, injecting dissociated cells into the injured myocardium can induce anoikis [11] and markedly reduce CM function [12]. Conversely, delivery of an intact ECT – cardiac cells seeded in a 3D biodegradable scaffold – to the damaged myocardium could restore ventricular function if the cells become mature CMs that can beat synchronously with the heart. Proof-of-concept experiments using rodent ECTs have demonstrated the utility of ECTs in elucidating basic principles of myocardial biology and in the development of organ-specific *in vitro* models for drug candidate evaluation, as well as the potential of ECTs as a regenerative therapy to partially or fully restore cardiac function [13–19].

Recent progress in stem cell biology has enabled the widespread availability of human pluripotent stem cell-derived CMs (hPSC-CMs). Human PSC-CMs are generally differentiated by timed application of cardiogenic growth factors or small molecules with cells cultivated as either embryoid bodies (EBs) [20,21] or in monolayers [22–25], and yield primarily CMs of the ventricular subtype. To produce ECTs the cells are typically dissociated between days 12 and 21 of differentiation and seeded into hydrogels or biomaterials followed by the application of a specific physical stimuli.

However, hPSC-CMs have a markedly immature phenotype at the end of the hPSC differentiation stage. In terms of morphology, gap junction expression, contractile apparatus, spontaneous automaticity, electrophysiology and calcium handling properties, hPSC-CMs are a better approximation of fetal CMs than adult [26]. The utility of ECTs as a model for myocardial development or as a surrogate for adult tissue in drug development depends on its close resemblance to bona fide heart muscle, *i.e.* its ability to reproduce the morphological and functional properties of mature adult cardiac tissue [27–29]. Signs of hPSC-CM maturation have been demonstrated through various means, including electrical stimulation [13,30,31], mechanical stretching [13,32–34], construct stiffness and topography [30,35], and chemical manipulation [29,34]. The lack of robust methods to promote the functional maturation of hPSC-CMs is currently one of the critical obstacles in the clinical application of ECTs and their use in preclinical drug development.

We review here the challenges in the field of human cardiac tissue engineering, the present state of the art of human engineered cardiac tissues (hECTs), and future perspectives. We will focus on the definition of CM maturity and the properties used to assess CM maturity as it applies to hECTs, and describe the level of maturation achieved in hECTs to date.

2. Cardiac cell maturity

Human PSC-CMs resemble human fetal CMs based on gene expression [36], electrophysiology [37] and morphology [38]. Relative to adult CMs, hPSC-CMs are small in size, have reduced electrical excitability [39,40], impaired excitation–contraction coupling [41,42] and incomplete adrenergic sensitivity [29,43]. In the stem cell field, there is no current consensus as to what constitutes a mature adult CM or which markers can be used to accurately and specifically track the maturity of hPSC-CMs. This is primarily because isolated primary CMs can re-express embryonic/fetal isoforms when cultured *in vitro*, and various structural or physiological markers – *e.g.* Ca^{2+} handling, cell morphology/striation pattern, and beating – undergo developmental reversion under conditions of pathological hypertrophy or disease [44]. The majority of hPSC cardiogenic differentiation protocols generate primarily CMs with a ventricular phenotype. Therefore in the following section, we will outline the different parameters used to assess the maturity of ventricular CMs in hECTs. For the sake of simplicity, only the non-failing human heart will be considered for comparison.