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Research review paper

Human iPSC-derived cardiomyocytes and tissue engineering strategies for disease modeling and drug screening

Alec S.T. Smith [a], Jesse Macadangdang [a], Winnie Leung [a], Michael A. Laflamme [b], Deok-Ho Kim [a,c,d,*]

[a] Department of Bioengineering, University of Washington, Seattle, WA 98195, USA
[b] Toronto General Research Institute, McEwen Centre for Regenerative Medicine, University Health Network, Toronto, ON, Canada
[c] Center for Cardiovascular Biology, University of Washington, Seattle, WA 98109, USA
[d] Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, WA 98109, USA

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ABSTRACT

Improved methodologies for modeling cardiac disease phenotypes and accurately screening the efficacy and toxicity of potential therapeutic compounds are actively being sought to advance drug development and improve disease modeling capabilities. To that end, much recent effort has been devoted to the development of novel engineered biomimetic cardiac tissue platforms that accurately recapitulate the structure and function of the human myocardium. Within the field of cardiac engineering, induced pluripotent stem cells (iPSCs) are an exciting tool that offer the potential to advance the current state of the art, as they are derived from somatic cells, enabling the development of personalized medical strategies and patient specific disease models. Here we review different aspects of iPSC-based cardiac engineering technologies. We highlight methods for producing iPSC-derived cardiomyocytes (iPSC-CMs) and discuss their application to compound efficacy/toxicity screening and *in vitro* modeling of prevalent cardiac diseases. Special attention is paid to the application of micro- and nano-engineering techniques for the development of novel iPSC-CM based platforms and their potential to advance current preclinical screening modalities.

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* Corresponding author at: University of Washington, Department of Bioengineering, N410G William H Foege Building, 3720 15th Ave NE, Box 355061, Seattle, WA 98195, USA.
E-mail address: deokho@uw.edu (D.-H. Kim).

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1. Introduction: Induced pluripotent stem cells

Advances in bioengineering and *in vitro* culture technologies have led to a rapid expansion of myocardial model development for use in drug efficacy/toxicity testing (Navarrete et al., 2013), disease modeling (Moretti et al., 2010; Wang et al., 2014), and mechanistic studies of cardiac development (Paige et al., 2012). However, the widespread adoption of such techniques for generating engineered human cardiac constructs that accurately model the *in vivo* tissue is predicated on the establishment of reliable sources of human cardiomyocytes. To that end, a number of recent studies have been performed assessing the suitability of a variety of different cell sources, including bone marrow-derived stem cells (Valarmathi et al., 2011), embryonic stem cells (ESCs) (Clements and Thomas, 2014), and induced pluripotent stem cells (iPSCs) (Mathur et al., 2015) for use in producing cardiac cells that accurately recapitulate the phenotype of their native counterparts. This review article will focus on iPSCs for potential cardiac engineering strategies, due to the significant advantages they offer over alternative cell sources. Specifically, induced pluripotent stem cells are capable of differentiating down multiple disparate lineages, easy to expand, readily available, and do not require the destruction of embryos, reducing ethical concerns and criticisms associated with their use in research. Furthermore, the isolation of cells from patients opens the door to the potential development of patient specific disease models and individualized medicine applications, which will be discussed in more detail later.

The production of iPSCs from somatic cells began with the groundbreaking work of Dr. Shinya Yamanaka's research group, who used a gammaretrovirus to randomly express four transcription factors responsible for pluripotency (*OCT4*, *SOX2*, *KLF4*, and *c-MYC* (OSKC)) in mouse and human fibroblasts (Takahashi et al., 2007; Takahashi and Yamanaka, 2006). Since the publication of these landmark papers, multiple methods have been developed for producing iPSCs more efficiently. The reprogramming process to convert somatic cells to iPSCs can be performed using cells from multiple different tissue sources, including skin fibroblasts (Takahashi et al., 2007), extra-embryonic tissues from umbilical cord and placenta (Cai et al., 2010), mononuclear cells from peripheral blood (Loh et al., 2009), and even urine-derived cells (Xue et al., 2013; Zhou et al., 2012). Following the establishment of iPSCs as a viable cell source, a number of methods have since been developed to improve the efficiency of iPSC generation, including viral and lentiviral integration, non-integrating viral vectors, and protein- and small molecule-based reprogramming (Table 1). An in-depth discussion of the different methods for deriving iPSCs is beyond the scope of this review, but has been discussed in detail elsewhere (Malik and Rao, 2013; Raab et al., 2014; Sommer and Mostoslavsky, 2013).

Despite the potential for integrating transgene sequences to negatively impact translational studies, integrating retro- and lentiviral reprogramming methods remain commonplace in cardiac modeling studies (Davis et al., 2012; Itzhaki et al., 2011; Lee et al., 2015; Malik and Rao, 2013; Sun et al., 2012; Wang et al., 2014). This is in part due to the fact that viral reprogramming technologies has been available for several decades, which means the expertise is relatively widespread and the methods are well characterized and reliable (Rao and Malik, 2012). Other methods used for cardiac modeling so far include the use of Sendai virus vectors (Churko et al., 2013), which obviate translational issues associated with transgene integration into the host cell's genome. Additionally, the use of episomal plasmids (Burridge et al., 2011), co-MIP (Diecke et al., 2015), microRNAs (Li et al., 2011), and direct protein delivery (Zhou et al., 2009) have all been shown to be capable of producing iPSCs that can be differentiated into beating cardiomyocytes.

Enthusiasm for the use of iPSC lines in advancing clinical and basic science research, concerns have been raised regarding whether such cells are identical to ESCs. On the one hand, it has been argued that

Table 1
Examples of methods to reprogram somatic cells into induced pluripotent stem cells.

Method	Notes on method	Advantages and disadvantages	Reference
Viral integration	• Gammaretroviral vectors for introducing transcription factors (Oct4, Sox2, Klf4, and c-Myc): -11–25 days of reprogramming -reprogramming efficiency of 0.001–0.01% (efficiency can be improved with mouse receptor for retroviruses) • Lentiviral vectors for introducing transcription factors (Oct4, Sox2, Klf4, and c-Myc) combined with 2A peptide and internal ribosome entry site: -16 days of reprogramming -15% reprogrammed iPSCs -reprogramming efficiency of 0.5%	• Low efficiency with gammaretroviral vectors • Higher efficiency with lentiviral vectors • Safety concern with viral integration • Potentially tumorigenic	(Romli et al., 2013, Sommer et al., 2009, Takahashi et al., 2007, Takahashi and Yamanaka, 2006)
Non-integrating viral vector	• Adenoviral system introducing Sox2, Klf4, and c-Myc to adherent Oct4 cells in presence of doxycycline: -required 24 to 30 days of culturing infected cells -yielded reprogramming efficiency of 0.0001% to 0.1%	• Extremely low efficiency • No viral integration necessary • Potentially tumorigenic	(Stadtfeld et al., 2008)
Recombinant reprogramming protein	• Cell-penetrating protein fused with reprogramming factors (Oct4, Sox2, Klf4, and c-Myc) and 9 arginines delivered to cells: -reprogramming process took 8 weeks -reprogramming efficiency of 0.001% • Inclusion body proteins from *E. coli* fused with 11 arginines and reprogramming factors (Oct4, Sox2, Klf4, and c-Myc): -30–35 days of reprogramming -valproic acid can significantly improve reprogramming efficiency	• Low efficiency • Repeated protein treatment required	(Kim et al., 2009, Zhou et al., 2009)
	• Single transfer of embryonic stem cell-derived proteins: -28 days of reprogramming	• Only require a single transfer of protein extract • Ethical issues	(Cho et al., 2010)
Small molecules	• Chemicals can be used to replace core reprogramming factors or increase reprogramming efficiency: -VPA histone deacetylase inhibitor increase reprogramming efficiency 100 fold with Oct4, Sox2, Klf4, and c-Myc -AZA DNA methyltransferase inhibitor increase reprogramming efficiency with Oct4, Sox2, Klf4, and c-Myc 4 to 10 folds -BIX-01294 G9a histone methyltransferase inhibitor can replace Sox2 and increase reprogramming efficiency 5 times more than Oct4 and Klf4	• Avoids genomic integrations of exogenous sequence • Enhance reprogramming efficiency • Chemicals might promote genetic aberrations	(Feng et al., 2009, Huangfu et al., 2008, Ma et al., 2013b, Mikkelsen et al., 2008)