Advanced Drug Delivery Reviews 96 (2016) 135–155

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Electrical and mechanical stimulation of cardiac cells and tissue constructs☆

Whitney L. Stoppel [a], David L. Kaplan [a], Lauren D. Black III [a,b,*]

[a] Department of Biomedical Engineering, Tufts University, Medford, MA 02155, United States
[b] Cellular, Molecular and Developmental Biology Program, Sackler School of Graduate Biomedical Sciences, Tufts University School of Medicine, Boston, MA 02111, United States

ARTICLE INFO

Article history:
Received 22 May 2015
Received in revised form 16 July 2015
Accepted 25 July 2015
Available online 30 July 2015

Keywords:
Cardiac tissue engineering
3D cardiac tissues
Bioreactors
Mechanical stimulation
Electrical stimulation
Electromechanical stimulation

ABSTRACT

The field of cardiac tissue engineering has made significant strides over the last few decades, highlighted by the development of human cell derived constructs that have shown increasing functional maturity over time, particularly using bioreactor systems to stimulate the constructs. However, the functionality of these tissues is still unable to match that of native cardiac tissue and many of the stem-cell derived cardiomyocytes display an immature, fetal like phenotype. In this review, we seek to elucidate the biological underpinnings of both mechanical and electrical signaling, as identified via studies related to cardiac development and those related to an evaluation of cardiac disease progression. Next, we review the different types of bioreactors developed to individually deliver electrical and mechanical stimulation to cardiomyocytes *in vitro* in both two and three-dimensional tissue platforms. Reactors and culture conditions that promote functional cardiomyogenesis *in vitro* are also highlighted. We then cover the more recent work in the development of bioreactors that combine electrical and mechanical stimulation in order to mimic the complex signaling environment present *in vivo*. We conclude by offering our impressions on the important next steps for physiologically relevant mechanical and electrical stimulation of cardiac cells and engineered tissue *in vitro*.

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Tissue engineering of the heart: from in vitro models to regenerative solutions".

* Corresponding author at: Department of Biomedical Engineering, Tufts University, 4 Colby Street, Medford, MA 02155, United States.
E-mail address: lauren.black@tufts.edu (L.D. Black).

http://dx.doi.org/10.1016/j.addr.2015.07.009
0169-409X/

1. Introduction

Cardiovascular disease is the leading global cause of death, accounting for 17.3 million deaths per year, and is responsible for about one in every three deaths in the United States [1]. Following significant injury such as myocardial infarction (MI), the myocardium has limited regenerative potential and, over time, a dense collagenous scar replaces the contractile tissue, which not only cannot contribute to the pumping function of the heart, but also impedes long-term function. To compensate, the remaining viable myocardium hypertrophies, the infarct wall thins, scar tissue replaces the viable myocardium, and the left ventricle (LV) dilates, all ultimately leading to heart failure (HF). As medical management tools such as stents and pharmaceuticals advance, almost 85% of patients survive their first heart attack, yet the 5-year survival rate is only 50% [1], suggesting that further improvements in methods to manage and treat subsequent HF are necessary. Current state-of-the-art treatments for end-stage HF primarily include the application of LV assist devices (chronic use of an external device which was designed as a bridge to transplant [2–4]) and total heart transplantation (limited by donor organ shortage [1,5]), which both require invasive surgeries and some form of immunomodulation. Therefore, there is a pressing clinical need for new therapies to ameliorate the deleterious responses post-MI that lead to HF, including negative left ventricular (LV) remodeling, arrhythmias, and diastolic dysfunction [6].

Cell-based therapies for cardiac repair have taken two forms: 1) Cardiomyoplasty and 2) the generation of a functional cardiac graft for implantation on or in the heart. Cardiomyoplasty has demonstrated substantial promise *in vitro*, as well as in repair in animal models [7–16], but clinical results thus far have shown limited success, often due to poor cell survival and retention [14,17,18]. In addition, when using cell types such as skeletal myoblasts, poor electrical integration into the host tissue can lead to secondary complications such as arrhythmias [19]. The use of tissue-engineered grafts has also shown significant promise in small animal models [20–24], but concerns about cell/ graft viability and the level of cell maturity have limited the clinical potential of tissue-engineered grafts. Future success and development of cardiomyoplasty- and cardiac graft-based treatment strategies would benefit from optimized *in vitro* methods to evaluate cardiomyocyte (CM) viability, phenotype, maturation level, and contractility under varying conditions that mimic the *in vivo* cellular environment. In particular, significant effort should be made to understand the use of induced pluripotent stem (iPS) cell-derived CMs *in vitro*, focusing on methods that aid in maturation, as differentiated iPS cells are functionally closer to a fetal CMs as opposed to an adult CMs. This is particularly true given the ever-increasing push to utilize iPS cell-derived CMs as a cell source in tissue engineering and regenerative medicine applications, as well as in the development of so-called organs or bodies on-a-chip systems. Moreover, the development of systems to mimic the biophysical environment present *in vivo* could be of significant use in drug discovery and development studies where CMs that mimic the functional phenotype present in adult tissue would be a valuable asset.

To achieve greater CM maturation and function *in vitro*, many researchers have sought to understand the role of mechanical and electrical stimulation in CM gene and protein expression. There has been a significant amount of effort in development of culture platforms that improve CM function compared to traditional 2D culture, where CMs do not align and remain relatively immature [25–27]. The addition of mechanical stimulation can increase maturation and contraction via hypertrophic pathways and the addition of electrical signaling leads to enhanced cell–cell coupling and improved calcium handling. Even with these improvements, current tissue engineering strategies still result in constructs that are functionally dissimilar to native myocardium. Engineered tissues are only able to achieve contractile stresses on the order 5 kPa [27–31], while native human heart tissue is on the order of 15–30 kPa [32]. As such, dual stimulation methods have been implemented which aim to combine both mechanical and electrical stimulation in a physiologically relevant way [33–36], but optimization of these platforms is still required.

In this review, we outline the efforts to improve CM function *in vitro* using mechanical and electrical stimulation, paying close attention to system design and the level of control of cell phenotype. First, the motivation for mechanical and electrical stimulation in the context of cardiac tissue development is discussed, followed by the cell culture and bioreactor systems that have been developed to promote functional CM phenotypes, where mechanical, or electrical stimulation are used individually. Herein, 2-dimensional (2D) and 3-dimensional (3D) culture systems are both reviewed, where the definition of a 2D system is one where CMs are grown on top of a substrate as compared to within (3D) the substrate or biomaterial. We close with a review of strategies for combining electrical and mechanical stimulation in physiologically relevant ways before concluding with a discussion of areas that remain to be addressed by those in the field.

2. Biological basis for mechanical stimulation

Beating, or the generation of contractile force, is a key component of both cardiac development and general cardiac function [37–40]. In humans, the heartbeat of a fetus is measured during pregnancy and the rate of the heartbeat is often used as a marker of fetal health and development. In adults, changes in heart rate or beat frequency can indicate disease, especially noticeable during a heart attack or in patients with arrhythmias. In order to better understand the biological foundation for these basic observations, investigators have utilized animal models, demonstrating that the course murine heart development is similar to that of the human, thus enabling the utilization of mice in the study of genetic and developmental abnormalities, specifically those related to changes in mechanical forces and cardiac specific gene expression [39–42]. Research has shown that bulk mechanical properties of the ventricular tissue change as the animal ages, suggesting that local structural changes, such as ECM crosslinking density, tissue composition, and cell–extracellular matrix (ECM) interactions play key roles during development [42–44] and changes in the aging heart or in disease models suggest that structural changes post-development are signs of cardiovascular disease [45].

These differences observed at the tissue level are also detectable at the cellular level. Changes in basic cellular processes, such as gene expression, protein expression, and cellular communication are influenced by changes in intracellular tension and/or extracellular stress. Specifically, changes in stiffness are transmitted via integrin binding, receptor tyrosine kinase activation, and GTPase activation at the cell membrane, in turn affecting signaling pathways involving important proteins such as Rho/ROCK (Rho-associated protein kinase) (95–97),