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Mechanical stimulation in the engineering of heart muscle☆

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ABSTRACT

Recreating the beating heart in the laboratory continues to be a formidable bioengineering challenge. The fundamental feature of the heart is its pumping action, requiring considerable mechanical forces to compress a blood filled chamber with a defined in- and outlet. Ventricular output crucially depends on venous loading of the ventricles (preload) and on the force generated by the preloaded ventricles to overcome arterial blood pressure (afterload). The rate of contraction is controlled by the spontaneously active sinus node and transmission of its electrical impulses into the ventricles. The underlying principles for these physiological processes are described by the Frank–Starling mechanism and Bowditch phenomenon. It is essential to consider these principles in the design and evaluation of tissue engineered myocardium. This review focuses on current strategies to evoke mechanical loading in hydrogel-based heart muscle engineering.

Contents

1. Introduction

Recapitulating disease mechanisms underlying heart failure development and progression in tissue engineered myocardium, alongside designing surrogate heart muscle for repair applications, promises to advance heart failure diagnostics and therapy beyond the current state-of-the-art. The development of human pluripotent stem cells from either blastocyst stage embryos [1] or somatic cell reprogramming [2], concomitant with robust protocols for directed cardiomyocyte differentiation ([3–5]; reviewed by Burridge et al. [6]) provides the basis for reconstructing human cardiac tissue for disease modelling and therapeutics. Early studies in chick [7] and rodent [8–13] models provided substantial insight into the conditions required for heart muscle reconstitution in the laboratory. Subsequent investigations defined mechanical [14,15] and electrical stimulation as key requirements for biomimetic culture [16]. Furthermore, the need to formulate a "natural" mix of cardiomyocytes and stroma cells was essential to advance rodent [17,18] and human ([19], own unpublished results) models of tissue engineered myocardium. Despite these palpable advances, stem cell-based myocardial tissue engineering approaches fail to attain adult stage maturation and should be, for the most part, considered as models akin to the embryonic heart. Whether this is a fundamental limitation

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for anticipated *in vitro* and *in vivo* applications remains to be investigated. It nevertheless illustrates the lack of insight into the interplay between specific growth-promoting biological and biophysical factors during human heart development. This review will discuss considerations pertaining to mechanical conditioning and maturation in hydrogel-based (collagen/fibrin) myocardial tissue engineering.

2. Cardiomyocyte maturation

The term "maturation" is commonly used to compare morphological and/or functional properties of cultured cardiomyocytes to those residing natively in the adult heart. In most instances, it is over-ambitiously used to describe a specific aspect of development rather than the complex state of the maturing heart. Human embryonic development encompasses the first eight weeks post-fertilisation, culminating in the formation of the four chambered heart [20–22]. Studies on tissue engineered myocardium rarely recapitulate this developmental window. In light of the difficulties to classify maturation as a whole, we prefer to stratify maturation according to the following categories (Fig. 1): (A) *structural* maturation (i.e., sarcomere assembly, myofiber orientation, mitochondrial distribution, t-tubule formation), (B) *functional* maturation (i.e., force of contraction, conduction velocity, calcium handling, electrophysiology, responsiveness to pharmacological interventions), (C) *metabolic* maturation (i.e., shift from anaerobic glycolysis to β-oxidation of fatty acids), and (D) *molecular* maturation (i.e., the shift from a so-called fetal to adult gene expression profile). Note that all of these categories are affected in heart failure, suggesting a re-introduction of fetal or even embryonic "maturation" in response to overload. To eventually gain a more comprehensive overview of cardiac maturation as a whole, we must take advantage of a systems biology approach and integrate phenotypic data collected from these four categories. All aspects of maturation will be influenced by the application of developmental stage adapted biophysical stimuli during the tissue engineering process. Making use of next generation sequencing, state-of-

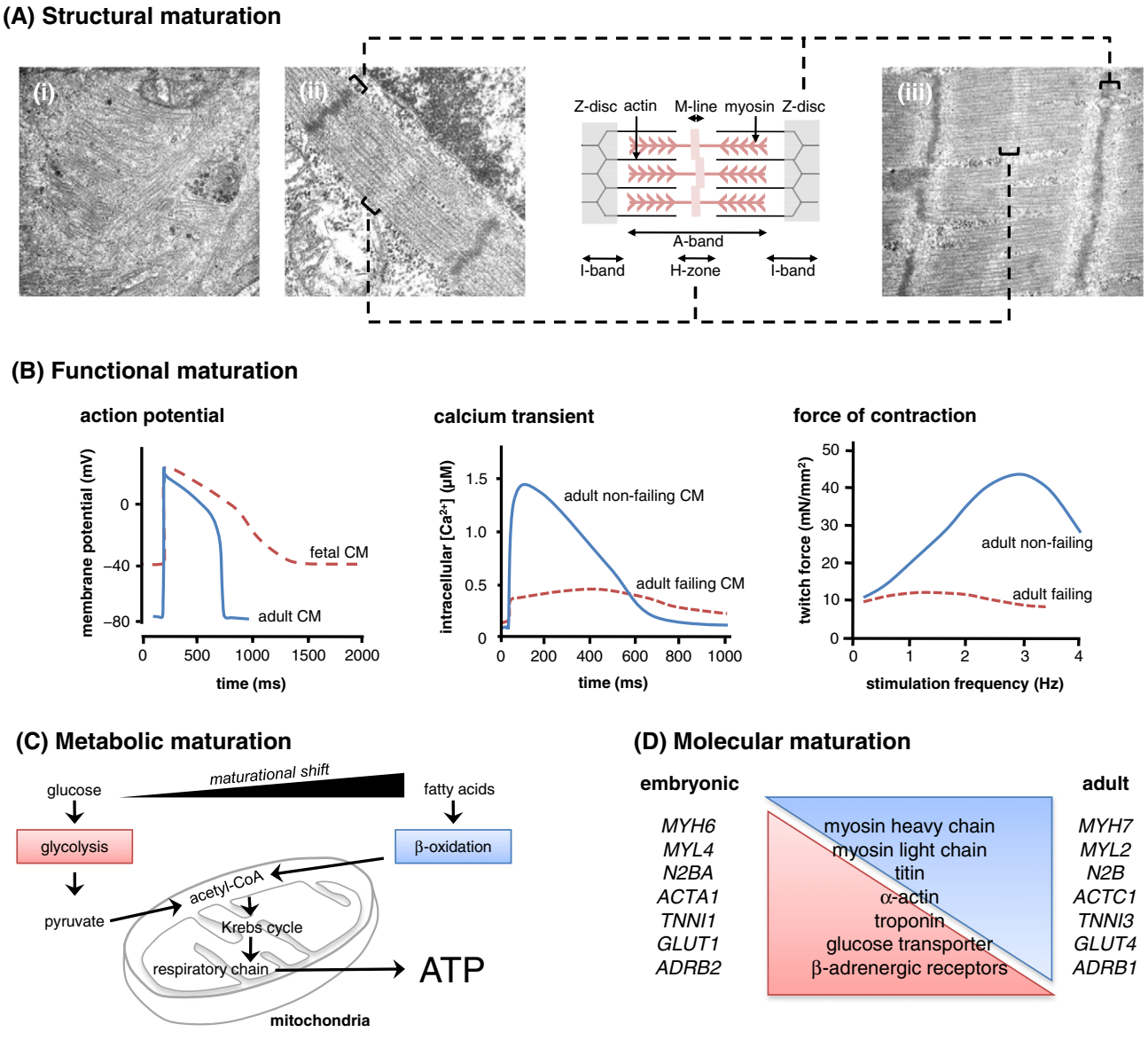

Fig. 1. Parameters to evaluate cardiomyocyte maturation. (A) *Structural maturation*: electron micrographs of cardiomyocytes from different developmental stages demonstrating myofiber disarray (i: from embryonic body culture) or a more developed sarcomere assembly with distinguishable Z-, I-, A-, H-, and M-bands (ii: from a neonatal rat cardiomyocyte; iii: from a rat EHM). (B) *Functional maturation*: schematic of human fetal and adult isolated cardiomyocyte action potentials [63], adult non-failing and failing cardiomyocyte calcium transients [64], and force of contraction from adult non-failing and failing isolated left ventricular myocardium at increasing stimulation frequencies [52]. (C) *Metabolic maturation*: embryonic/fetal stage cardiomyocytes produce ATP mainly via anaerobic glycolysis whereas β-oxidation of fatty acids predominates postnatally [65]. (D) *Molecular maturation*: examples for canonical changes in human gene transcription underlying functional, structural, and metabolic maturation are summarised [65].