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The cardiac muscle duplex as a method to study myocardial heterogeneity

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ABSTRACT

This paper reviews the development and application of paired muscle preparations, called duplex, for the investigation of mechanisms and consequences of intra-myocardial electro-mechanical heterogeneity. We illustrate the utility of the underlying combined experimental and computational approach for conceptual development and integration of basic science insight with clinically relevant settings, using previously published and new data. Directions for further study are identified.

1. Introduction

Cardiac pump function builds on, and requires, the interaction of regional spatio-temporal heterogeneities, from differences in electrical activation timing and gradients in load-dependent local stress-strain behaviour, to variation in passive and active cellular electro-mechanical properties (Katz and Katz, 1989). Indeed, cells isolated from various parts of the left ventricular (LV) wall have different ion handling protein densities (Antzelevitch and Fish, 2001; Wolk et al., 1999) and mechanical properties (Bollensdorff et al., 2011; Cazorla and Lacampagne, 2011; Cordeiro et al., 2004; Wan et al., 2003). Contractile protein isoforms differ (Litten et al., 1985; Stelzer et al., 2008), with V3 myosin dominating in the sub-endocardial layers, while in sub-epicardial myocytes the V1 isoform is more common in many species. These myosin isoforms differ in the velocity of cross-bridge cycling, where V1 allows for greater velocity of cardiomyocyte shortening compared to V3 (Litten et al., 1985; VanBuren et al., 1995). Also, owing to differences in the expression levels of several ionic mechanisms, cells from sub-endocardial and sub-epicardial layers further differ in action potential (AP) shape and duration (Bryant et al., 1997; Stones et al., 2008), as well as in the kinetics of intracellular Ca^{2+} handling (Cordeiro et al., 2004; Laurita et al., 2003). As a result, together with faster contraction dynamics, sub-epicardial cardiomyocytes also demonstrate shorter AP and swifter Ca^{2+} transients than the (physiologically earlier-activated) sub-endocardial cells.

At the tissue level, the structure and function of the LV have been shown to be highly heterogeneous (Ashikaga et al., 2007, 2009; Bogaert and Rademakers, 2001; Sengupta et al., 2006b). Regional mechanical function in the LV varies longitudinally from basal to apical segments, in transmural regions, and between the free wall and the septum (Ashikaga et al., 2009; Sengupta et al., 2006a). The timing of electrical excitation in the LV wall is also known to be heterogeneous, with excitation generally spreading from apex to base and from sub-endocardial to sub-epicardial layers (Sengupta et al., 2006b).

List of abbreviations: 1D, One-dimensional; AP, Action potential; APD, Action potential duration; APD80, AP duration at 80% repolarization; ΔAPD, Difference in APD; BM, Biological muscle; CRT, Cardiac resynchronization therapy; DR, Dispersion of repolarisation; ENDO, Sub-endocardial; EO model, Ekaterinburg-Oxford model; EPI, Sub-epicardial; F–V, Force–velocity relationships; LV, Left ventricular; L_{MAX}, Muscle length at which maximum isometric peak force is developed; ML, An initial muscle length; MP, Membrane potential; N cell, Normal cell; SC cell, Sub-critical cell; SR, Sarcoplasmic reticulum; SFR_{IM}, Intra-myocardial slow force response; VM, Virtual muscle.

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Despite a large body of research characterising cardiac regional heterogeneity, its role in the mechanical and electrical function of the myocardium, and the bilateral relationships between electrical and mechanical activity, remain under-appreciated.

Significant progress in understanding the role of heterogeneity phenomena has been achieved using simple models of heterogeneous myocardium, called "muscle duplexes", first developed in the 1960s by Tyberg and co-workers (Tyberg et al., 1969), and later expanded by Bing and colleagues (Shimizu et al., 1996; Wiegner et al., 1978). Tyberg's team studied mechanical consequences of interactions between end-to-end ('*in series*') connected normal and ischaemic muscles. Bing's group added a computer interface to record muscle contraction in normal conditions, and then to apply this as a command signal to the same muscle after exposure to hypoxia. The authors interpreted this kind of signal exchange as *in series* interactions between the muscles, although the signal applied by the setup was in fact uni-directionally applied to the biological sample.

The cardiac muscle duplex approach was subsequently developed further, and specifically applied to investigations into cardiac regional heterogeneity, by our group (Markhasin et al., 1999; Rutkevich et al., 1997). A muscle duplex represents the simplest physiological model of mechanically interacting segments of the myocardium. It comprises two isolated and mechanically coupled muscle elements, exposed to *bilateral* mechanical interactions during their contractions. As this system is simple, it allows one to unravel basic properties of heterogeneous myocardium, as they emerge from element interactions. We have refined the duplex method by implementing a number of principally different configurations, including *in series* and *in parallel* coupling of biological and virtual (computer-modelled) muscles (Markhasin et al., 2003), and by extending its capability by simultaneous registration of cellular electrical activity or Ca^{2+} dynamics (Markhasin et al., 2012). This allowed us to reveal and explain a number of basic effects characteristic of heterogeneous myocardium in norm and pathology.

Here, we review the duplex techniques and illustrate essential results obtained with our approach. Most of these results have been published in previous papers, and the relevant sources are identified in the text.

2. Muscle duplex approach

To address the effects of mechanical interactions between spatially distinct but mechanically coupled segments of native myocardial tissue we use the simplest case model – the muscle duplex (Markhasin et al., 2003). Muscle segments are mechanically connected either *in series* or *in parallel* and different sequences of muscle stimulation with varying time lags (from 0 to ±100 ms) are applied to simulate time delays between regional excitation throughout the myocardial tissue. The physiological relevance of the duplex model stems from the fact that mechanical signal transduction in cardiac tissue is more far-reaching and two to three orders of magnitude faster than electrical excitation propagation: mechanical stimuli travel near the velocity of sound in liquids, i.e. about 3×10^2 m/s, compared to electrical excitation in the order of 10^{-1} to 10^0 m/s. Mechanical effects (stress or strain) from earlier activated myocardial segments are therefore almost immediately transmitted even to distant surrounding tissue, potentially affecting its subsequent activity via acute mechano-mechanical (Shiels and White, 2008), mechano-electrical (Kohl et al., 1999), mechano-chemical (Ennis et al., 2013) and mechano-structural feedback (Kohl et al., 2003).

We developed and explored six principal duplex configurations (Markhasin et al., 2003; Protsenko et al., 2005), using either *in series* or *in parallel* mechanical connection between coupled muscles, implemented for the three sets of element combinations: (1) a biological duplex comprising two isolated multicellular myocardial preparations (biological muscles [BM]; i.e. thin papillary muscles or trabeculae); (2) a virtual duplex comprising two computational models of the electromechanical activity of cardiac muscle (virtual muscles [VM]; see below for details); or (3) a hybrid duplex comprising one BM and one VM. A schematic illustration of all the duplex settings is presented in the electronic supplemental data (see Fig. S1).

2.1. Main features of mechanical interactions between in series and in parallel coupled muscles

In the *in parallel* duplexes, dynamic interactions of elements occur at identical lengths, for example during shortening-lengthening phases of isotonic or auxotonic contractions of the pair, working from (against) a defined and externally applied mechanical pre- (after-)afterload. Here, element forces add up to total duplex force, while element deformations are equal at any given time (see Fig. 1 and Fig. 2, left panel). This kind of dynamic behaviour of coupled muscle segments mirrors interactions between *in parallel* ventricular layers (e.g. sub-endocardial and sub-epicardial regions), where individual regional forces are in balance with the external mechanical load during overall chamber deformations (Ashikaga et al., 2007; Sengupta et al., 2006a).

The *in series* duplexes can be used to investigate dynamic interactions between 'end-to-end' coupled muscles, as they occur during externally isometric contractions. When imposing externally isometric conditions on the whole duplex, the outer ends of muscles are kept iso-positional, while internal changes in element lengths are allowed and registered (see Fig. 2, right panel, and Fig. 3 showing muscle interactions). Mechanical activity of these *in series* duplex elements is governed by coinciding yet opposite length changes, as they 'pull' at each other, while forces in the elements are equal at any given time. Therefore, each duplex element contracts *auxotonically*, as is the case in real tissue. The ensuing dynamic behaviour of coupled muscle elements is conceptually similar to isovolumetric contraction (or relaxation) of the ventricles, where internal regional deformations occur at constant ventricular volume (Ashikaga et al., 2009; Sengupta et al., 2006a).

2.2. Main features of the biological, virtual and hybrid duplex settings

2.2.1. Biological duplexes

These consist of two mechanically linked BM that are kept in separate chambers with independent stimulation, perfusion and temperature control systems. Mechanical interaction is implemented using a computer-controlled system to interrelate input/output signals of muscle force and length changes between both BM, with an internal time step of 100 μs, while prescribing the relevant duplex constraints (i.e. in externally isometric *in series* duplexes – opposite length changes and equal force, or for contracting *in parallel* duplexes – opposite force changes and equal shortening). The principal features of the experimental duplex method and a schematic illustration of the computer-based control algorithm used to imitate real-time mechanical interactions are available elsewhere (Markhasin et al., 2003; Protsenko et al., 2005). In addition to the mechanical activity of both BM, the duplex setting allows us to measure action potential (AP) time-courses using floating microelectrodes (see an example of AP recordings in Section 2.3, and (Markhasin et al., 2012)). Unfortunately, due to the dynamic nature of the preparation, it has been difficult to measure AP in both BM simultaneously and for prolonged periods, even with

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