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Review

Mechano-electric interactions in heterogeneous myocardium: development of fundamental experimental and theoretical models

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Abstract

The heart is structurally and functionally a highly non-homogenous organ, yet its main function as a pump can only be achieved by the co-ordinated contraction of millions of ventricular cells. This apparent contradiction gives rise to the hypothesis that ‘well-organised’ inhomogeneity may be a pre-requisite for normal cardiac function. Here, we present a set of novel experimental and theoretical tools for the study of this concept. Heterogeneity, in its most condensed form, can be simulated using two individually controlled, mechanically interacting elements (duplex). We have developed and characterised three different types of duplexes: (i) *biological duplex*, consisting of two individually perfused biological samples (like thin papillary muscles or a trabeculae), (ii) *virtual duplex*, made-up of two interacting mathematical models of cardiac muscle, and (iii) *hybrid duplex*, containing a biological sample that interacts in real-time with a virtual muscle. In all three duplex types, *in-series* or *in-parallel* mechanical interaction of elements can be studied during externally isotonic, externally isometric, and auxotonic modes of contraction and relaxation. Duplex models, therefore, mimic (patho-)physiological mechano-electric interactions in heterogeneous myocardium at the multicellular level, and in an environment that allows one to control mechanical, electrical and pharmacological parameters. Results obtained using the duplex method show that: (i) contractile elements in heterogeneous myocardium are not ‘independent’ generators of tension/shortening, as their ino- and lusitropic characteristics change dynamically during mechanical interaction—potentially *matching* microscopic contractility to macroscopic demand, (ii) mechanical heterogeneity contributes differently to action potential duration (APD) changes, depending on whether mechanical coupling of elements is *in-parallel* or *in-series*, which may play a role in mechanical *tuning* of distant tissue regions, (iii) electro-mechanical activity of mechanically interacting contractile elements is affected by their activation sequence, which may optimise myocardial performance by *smoothing* intrinsic differences in APD. In conclusion, we present a novel set of tools for the experimental and theoretical investigation of

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cardiac mechano-electric interactions in healthy and/or diseased heterogeneous myocardium, which allows for the testing of previously inaccessible concepts.

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1. Introduction

The topic of cardiac heterogeneity has seen a surge of interest and awareness over the past decade or so.¹ The exceedingly large body of data on the (patho-)physiological relevance of cardiac heterogeneity has been addressed in previous reviews (Katz and Katz, 1989; Lew, 1991; Wolk et al., 1999; Antzelevitch and Fish, 2001). We will, therefore, only briefly address some of the findings that are of principal relevance to this study.

Mechanical heterogeneity of the heart is well established at the organ and tissue levels (Lew, 1991), but information on the underlying cellular manifestations of mechanical heterogeneity has only recently started to emerge. Thus, ventricular myocytes, isolated from sub-epicardial tissue layers of rat and ferret show lower diastolic stiffness than sub-endocardial myocytes (Cazorla et al., 2000). This corresponds well to the lower mechanical systolic tension in the sub-epicardium, compared to sub-endocardium.

Mechanical heterogeneity applies not only to passive properties of cardiomyocytes. Sarcomere length—active tension relationships are significantly greater and slightly steeper in sub-endocardial than sub-epicardial cells in rat and ferret (Cazorla et al., 2000; Natali et al., 2002).

¹ A simple search in PubMed for 'cardiac heterogeneity review' yields more than 2200 entries since 1990, yet only 260 in the two preceding decades.

Sub-epicardial myocytes from Guinea pig show higher velocities of unloaded contraction and relaxation than sub-endocardial ones (Bryant et al., 1997). This asynchronous activity could be explained by regional differences in expression of myosin isoforms V_1 and V_3 (Sartore et al., 1981; Litten et al., 1985). Isomyosin V_1 supporting faster cycling of cross-bridges and quicker cell shortening than the V_3 isoform (Carey et al., 1979), is prevalent in the sub-epicardium, while the sub-endocardium contains more V_3 (Litten et al., 1985).

Thus, cardiac mechanical heterogeneity may be observed from the molecular to the whole organ levels in norm and pathology. It is a dynamic property of the heart, and subject to changes during development and pathology.

Electrophysiological heterogeneity, too, may be observed at all levels of integration, from the sub-cellular (Liu et al., 1993) to the whole organ, where it forms the basis of our understanding of the ECG T-wave (regions of the ventricle that are excited last repolarise first, Cohen et al., 1976). The time-lag between activation of sub-endocardial and sub-epicardial cells in normal human myocardium is in the order of 10 ms (Taggart et al., 2000). One should expect, therefore, that action potential duration (APD) in sub-epicardial cells is (at least 10 ms) shorter than sub-endocardial APD. Indeed, cells isolated from different regions of the ventricular wall demonstrate pronounced APD gradients, with transmural differences in APD exceeding 30–40 ms (fast sub-epicardial cells have shorter APD, Bryant et al., 1997; Cheng et al., 1999).

Thus, mechanical and electrical heterogeneity co-exist in ventricular myocardium. While the significance of electrophysiological gradients for normal and disturbed cardiac function is generally appreciated (Katz and Katz, 1989), surprisingly little is known about the role of mechanical heterogeneity. Also, the interplay between physiological and patho-physiological gradients, and the cross talk between cardiac mechanical and electrical activity in heterogeneous myocardium are little understood. This is, at least in part, caused by a lack in suitable experimental and theoretical models, as native tissue preparations miss access and control of local parameters, while lower-order cellular or multi-cellular preparations do not usually implement heterogeneity. In this review, we discuss a novel platform of experimental and theoretical tools for the investigation of cardiac mechano-electric interactions in controlled cardiac heterogeneity, using two individual cardiac muscles (either biological or virtual) that are mechanically linked *in-parallel* or *in-series*—the duplex method (Solovyova et al., 2002).

2. Methods

2.1. Brief history of duplex model development

The duplex model represents myocardial heterogeneity in its most reduced form: a structure, consisting of two distinct myocardial elements that are mechanically interconnected.

Elements can be either biological samples (B), such as thin papillary muscles or trabeculae, or virtual muscles (V) represented by mathematical models. Elements can be teamed-up in three principal combinations: biological duplex (B–B), virtual duplex (V–V), or hybrid duplex (B–V). In each case, connection of elements can be either *in-parallel* or *in-series* (see Fig. 1), yielding *six principal configurations* of the duplex model.

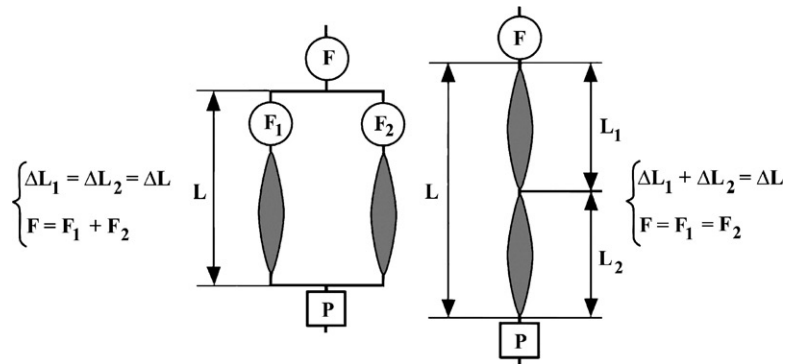


Fig. 1. Schematic representation of duplex models with individual elements (here biological samples) connected either *in-parallel* (left) or *in-series* (right). P is the total load applied to the duplex, F is duplex tension, and L is duplex length. F_1, F_2 are instantaneous tension and L_1, L_2 instantaneous length of individual elements (see equations for the relation of individual and total F and L in each configuration).

One of these configurations—the *in-series* biological duplex—was implemented in 1969, when Tyberg and colleagues investigated the effects of asynchrony and local metabolic disturbances on pairs of cat papillary muscle (Tyberg et al., 1969). This study showed (i) that mechanical characteristics (like length-force and force-velocity relationships) of individual and mechanically interconnected muscles differ, (ii) that asynchronous stimulation of elements leads to a redistribution of element contractility without necessarily causing a change in total duplex force, and (iii) that hypoxic muscle preparations could be distended by their normoxic counterpart during systole. This was seen to support the idea of a mechanically induced readjustment in fiber lengths during the so-called ‘entrant’ phase of ventricular contraction (Wiggers, 1925), and to explain paradoxical segment lengthening in ischaemic foci of myocardium (Tanant and Wiggers, 1935).

A second configuration—an *in-series* hybrid duplex—was partially implemented in 1978 (Wiegner et al., 1978), when physiological force patterns of a rat papillary muscle were recorded, stored, and then re-applied (as an external mechanical command sequence) to the same muscle after rendering it hypoxic. A reversed protocol (recording hypoxic muscle mechanics and re-applying it to the same preparation in normoxic conditions) was implemented by the same team, about two decades later (Shimizu et al., 1996). This partial implementation of a hybrid duplex did not allow for interaction of biological and virtual muscle (virtual muscle mechanics were applied from a pre-determined look-up table, rather than computed as a function of real-time activity of the biological partner element). These studies reconfirmed that asynchronous motion of ischaemic and non-ischaemic regions, seen in regional myocardial disease, can be explained by the interaction of muscles with different contractility.

Thus, out of the six potentially valuable configurations of the duplex method, two have what amounts to a very restricted track record. Early implementations of the method largely preceded the recent raise in awareness of myocardial heterogeneity as a (patho-)physiologically relevant entity, which may explain their successive demise. To the best of our knowledge, the Ekaterinburg lab is the only one to have conducted a concerted effort into the development of the duplex

method as a platform technology for research into cardiac mechanical heterogeneity (Bliakhman et al., 1989; Landesberg et al., 1996; Markhasin et al., 1997, 1999, 2002; Solovyova et al., 2002).

2.2. The biological duplex

In order to extend the method beyond Tyberg's earlier work (Tyberg et al., 1969), we focused initially on developing an *in-parallel* biological duplex (Fig. 1, left). The duplex elements—individual cardiac muscle preparations, usually trabeculae or papillary muscles from rat or rabbit—are maintained using independent preparation survival systems, including separate perfusion chambers, stimulating electrodes, mechanical recording systems, and temperature regulation. This allows us to control 'mechanical heterogeneity', for example by drug application or by unilateral changes in pre-load and/or bath temperature. The system can also mimic various 'spatial interrelations' of elements by introducing a variable time delay in electrical activation of the mechanically coupled elements (such as that occurs as a result of the slow, compared to mechanical impulse transmission, propagation of electrical excitation in ventricular tissue).

Force registration is via two isometric force transducers (compliance $< 1 \mu\text{m g}^{-1}$) that are mounted to micrometer screws for individual preload adjustment. One end of each cardiac muscle preparation is connected to a force transducer, while the other end is attached to the common lever of a fast-acting, computer-controlled servomotor (response time $< 2 \text{ ms}$ for a 1 mm stepwise displacement).

Length registration is via a photodiode array, attached to the servomotor lever, allowing length registrations over a range of $\pm 2.5 \text{ mm}$ at 1 kHz, with a resolution of $2 \mu\text{m}$.

The control software supports definition of complex mechanical protocols, using pre-defined settings and/or feedback from force transducers to control the servomotor (for further detail on methods see, Rutkevich et al., 1997). This allows for the investigation of muscle properties in isometric, isotonic, and auxotonic modes of contraction. Dynamical adjustments can be performed to mimic the sequence of mechanical changes during a normal cardiac cycle, both for individual and coupled duplex elements. At the same time, the software provides on-line analysis of the main mechanical characteristics, such as the force—velocity relationship, length—force relationship, and end-systolic length—characteristic time of relaxation relationship (Markhasin et al., 1999).

2.3. The virtual duplex

The virtual duplex is a mathematical representation of biological duplexes. Initial virtual duplexes used the Ekaterinburg model of cardiac muscle (Katsnelson et al., 1990; Izakov et al., 1991), which has been validated against a wide range of experimental findings (Katsnelson and Markhasin, 1996; Katsnelson et al., 2000; Solovyova et al., 2002). The model is based on mathematical descriptions of active and passive mechanical properties of myocardium, calcium activation of contractile proteins, co-operativity, and feedback from cardiac mechanics to calcium handling.

More recently, we extended the model's utility (Solovyova et al., 2003) by merging the Ekaterinburg mechanics representation (Solovyova et al., 2002) with the cardiac electrophysiology model (Noble et al., 1998) from the OXSOF family. The combined model (even without stretch activated currents accounted for) simulates a wide range of experimental data on cardiac mechano-electric interactions by involving mechano-dependent modulation of Ca^{2+} handling as a mechanism of mechano-electric feedback (MEF). This model underlies our simulations of the effects of mechanical heterogeneity on mechanical and electrical processes, and their dynamic cross-talk.

Virtual duplexes form a unique tool for the design and analysis of experiments, including the theoretical identification of molecular and cellular candidate-mechanisms that may underlie macroscopic responses. They aid hypothesis formation and provide a test-bed for 'dry screening' of interventions that would be suitable for experimental hypothesis evaluation. It is this interaction with 'wet' research where the utility of 'pure' virtual duplexes culminates.

2.4. The hybrid duplex

In a hybrid duplex, a biological cardiac muscle preparation interacts mechanically, in real-time, with a virtual one. Computed mechanical activity of the virtual muscle is applied to the biological sample via D/A output to servomotor (controlled by virtual muscle tension if connected *in-series*, and by its length if *in-parallel*). In turn, the mechanical activity of the biological sample is recorded by a force transducer and/or a photodiode array and, via A/D input, applied to the model calculations (using either biological muscle length changes or its force depending on duplex configuration). The total time required for signal processing from A/D input to D/A output is 100 μs (including 50 μs computing time per cycle of interaction, Solovyova et al., 2002).

Hybrid duplexes can be subjected to either isometric or isotonic modes of contraction (note that *in-parallel* duplex elements behave as independent units in isometric mode, while *in-series* duplex elements act independently of each other during pure isotonic contractions). The modelling algorithms therefore support equal shortening of natural and virtual muscles *in-parallel*, and equal force of *in-series* natural and virtual muscles.

The following is an example of the principal approach to controlling isometric contraction of a hybrid *in-series* duplex (algorithm applied during each time step of the control cycle):

1. biological muscle length is measured and subtracted from the pre-set duplex length to determine virtual muscle length;
2. virtual muscle length is used as input for the mathematical model simulating the virtual muscle, and instant virtual muscle tension is calculated;
3. virtual muscle tension is used as an external mechanical command and applied to the biological muscle;
4. biological muscle contraction is subject to changed load during the subsequent time interval (100 μs), after which a new instantaneous length is recorded to return to step 1 of the control cycle.

Thus, biological and virtual elements of a hybrid duplex interact in *real-time* and affect *each other* in a highly dynamic setting. The main advantages of this approach are that (i) virtual muscle activity can be set in a pre-defined relation to biological sample parameters, (ii) virtual muscles

can be set to mimic normal and disturbed cardiac activity (including hypertrophied and hypoxic muscle), and a single biological muscle sample can be exposed to a variety of normal and/or patho-physiologically disturbed patterns of duplex partner activity, and (iii) virtual muscle activity can be ‘dissected’ to identify potential mechanisms and causal chains of events.

The critical parameter for implementation of the hybrid duplex is the time step that governs the control cycle (here 100 μ s). This requires optimisation of algorithms for numerical integration and computing speed, use of low-inertia servomotor and force transducers, and fine-tuning to reduce device interaction and noise.

2.5. *Interventions*

Experiments on duplexes that contain biological samples require thorough assessment of basic mechanical characteristics of muscle preparations prior to duplex formation, in order to team them up with an appropriate biological or virtual partner. After duplex formation, mechanical characteristics of elements and the duplex as a whole are re-assessed, before subjecting them to experimental interventions. A reduction in superfusate temperature of one biological element by 3–4°C, for example, may be applied to slow down contraction/relaxation in that element. This can be used to mimic differences in sub-epicardial (fast) and sub-endocardial (slow) speed of contraction/relaxation in normal myocardium (as referred to above). Similarly, there is a physiological time-lag in electrical activation of spatially distinct regions of myocardium, which can be addressed by introducing variable delays in electrical stimulation of elements. Finally, drug application and metabolic interventions are used to mimic the interaction of cardiac muscle elements in various (patho-)physiological states, including hypoxia or adrenergic states.

2.6. *Constraints*

Duplexes containing biological elements show significant variability in key mechanical characteristics (length, force, length–force and force–velocity relationships) of individual samples, which makes quantitative analysis difficult. The assembly of pure biological duplexes, therefore, requires extensive testing and element matching (a problem that can be circumvented by hybrid duplex formation). Furthermore, an ideal set of data would include simultaneously recorded mechanics, calcium transients and representative trans-membrane electrical recordings, which we are unable to do at present.

Duplexes containing virtual muscles, on the other hand, suffer from the known shortcomings of mathematical models: even if they would reproduce all known behaviour, they would not necessarily be representative of cardiac muscle, as future interventions may reveal relevant discrepancies in biological and model behaviour. In fact, such discrepancies are usually an important source of information regarding our understanding of biology and its implementation in the model. It is essential, therefore, to compare and validate work on virtual muscles with research on biological samples. Within this framework, modelling is set to provide major advances in our understanding of cardiac mechano-electrical behaviour in heterogeneous myocardium, and we view the hybrid duplex as a particularly promising tool as it combines ‘the best of both worlds’.

3. Data and discussion

3.1. Mechanical effects of heterogeneity

We have previously published a number of reports (Markhasin et al., 1997, 1999, 2002; Solovyova et al., 2002), which allow us to suggest that:

1. Cardiac myocytes in situ are not ‘independent’ generators of tension/shortening; their ino- and lusitropic characteristics depend on mechanical interaction with adjacent and distant cardiomyocytes, and they are subject to constant and dynamic change, potentially *matching* local contractility to global demand.
2. Cardiomyocyte electro-mechanical function is modulated by the sequence of ventricular activation, potentially providing a mechanical *tuning* effect on cells that are activated last.
3. Cardiac mechano-electrical heterogeneity is a required feature of normal cardiac function.

These suggestions are based on the observation that, firstly, in all heterogeneous duplex models tested, the mechanical characteristics of duplex elements were significantly changed by mechanical coupling (Solovyova et al., 2002). Secondly, differences between isolated and coupled behaviour were strongly affected by the electrical stimulation sequence, which could be used to enhance or reduce the effects of mechanical interaction. Thus, delaying stimulation of the faster duplex element (*in-parallel* setting) caused the force–velocity relationship of individual elements to approach each other in a non-linear dependence of stimulation delay (up to a perfect overlap). Delay in the activation of the slow element, in contrast, increased the discrepancy in force-velocity relations of elements (see, for example, Fig. 13 in Solovyova et al., 2002). This is in-line, thirdly, with the suggestion that electrical and mechanical heterogeneity are essential pre-requirements for normal biological function (Katz and Katz, 1989), where faster (sub-epicardial) elements are stimulated later (electrical conduction delay), which would reduce the inherent transmural discrepancy in mechanical and electrical activity.

An example is illustrated in Fig. 2, where *in-series* duplexes (hybrid and virtual) were subjected to various stimulation delays during externally isometric contraction. Fig. 2A shows isometric contraction of the three individual elements that were subsequently used to create hybrid and virtual duplexes (Fig. 2B and C). In heterogeneous duplexes, whether hybrid or virtual, peak force generation and characteristic time of relaxation (a measure of the speed of contraction/relaxation) showed a significant functional advantage when the faster duplex element was stimulated later than the slower one (positive delay times in Fig. 2B and C). At a delay in fast muscle stimulation of 30–40 ms (as is observed transmurally), force generation approached an optimum, while delayed activation of the slow element had negative effects of force and speed of contraction/relaxation (negative delay times in Fig. 2B and C).

Interestingly, homogeneous duplexes, created by pairing two fast muscles with similar force generation patterns, responded to stimulation delays with a decrease in force production (Fig. 2B, dotted line). This re-emphasises the view that ‘well-organised’ heterogeneity is a pre-requisite for normal cardiac activity, and it shows that electrical heterogeneity (such as transmural activation delay) *necessitates* mechanical heterogeneity for optimal mechanical performance.

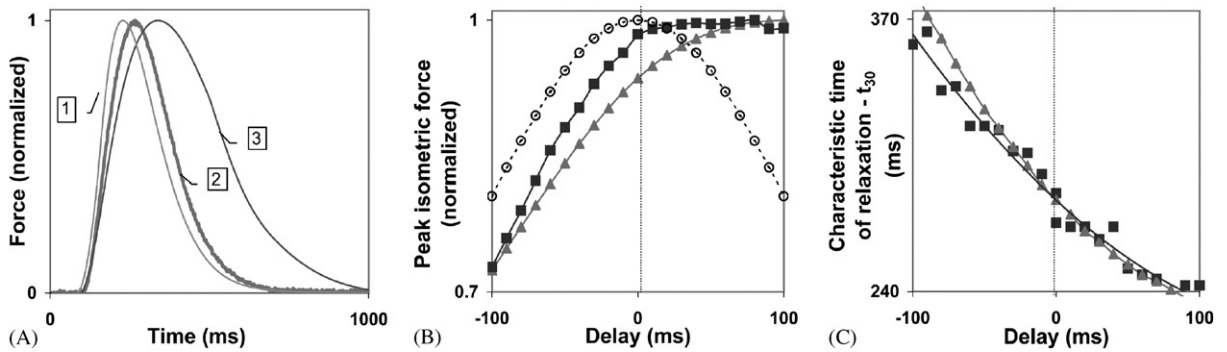


Fig. 2. Effect of stimulation delays on inotropic and lusitropic characteristics of *in-series* hybrid and virtual duplexes. A: time courses of isometric force development in the individual elements used to form duplexes, including a fast virtual muscle (1), a fast natural muscle (2), and a slow virtual one (3). B: summary of peak force generation in homo- (dotted line) and heterogeneous duplexes (solid lines) during a series of stimulation delays. Both heterogeneous duplexes ((■)—hybrid duplex of elements 2 and 3; (▲)—virtual duplex of elements 1 and 3) increased peak force when stimulation of the faster element was delayed (positive delay time) and decreased peak force when stimulation of the slower element was delayed (negative delay time). In contrast, any stimulation delay of elements in a homogeneous duplex (○—virtual duplex of two copies of element 1) reduced peak force generation. C: relaxation time in heterogeneous duplexes continuously decreased with an increasing stimulation delay of the faster element. The natural muscle was a trabeculum from rat right ventricle; time of relaxation was taken at 30% of the peak force during isometric relaxation.

3.2. Mechano-electric interactions

Cardiac electrical and mechanical activity are inseparably connected and affect each other, for example via excitation–contraction coupling (Bers, 2002) and MEF (Kohl et al., 1999). While excitation–contraction coupling has been extensively studied, there is only limited information on cardiac MEF, and virtually none on MEF in heterogeneous (i.e. realistic) myocardium. Circumstantial evidence suggests, that MEF may contribute to arrhythmogeneity, in particular in pathologically disturbed myocardium with enhanced mechanical heterogeneity (Eckardt et al., 2001), but underlying mechanisms are unknown.

In order to investigate MEF in heterogeneous myocardium, we replaced the Ekaterinburg model of cardiac mechanics with the Ekaterinburg–Oxford description of cardiac electro-mechanical activity (Solovyova et al., 2003). Virtual muscles with fast (Fig. 3, left-hand side) and slow (Fig. 3, right-hand side) mechanical characteristics were simulated and interconnected either *in-series* (Fig. 3, upper panels) or *in-parallel* (Fig. 3, lower panels; note that thin lines depict element activity *in isolation* and thick lines represent activity of the same element *in duplex*).

In the example, illustrated in Fig. 3A and B, contraction of the *in-series* duplex is externally isometric. After duplex formation, individual elements can shorten at the ‘expense’ of lengthening their *in-series* partner (compare thick lines to thin control curves). Initially, the faster muscle (Fig. 3A) distends its slower counterpart (Fig. 3B), followed by a more pronounced period of slow muscle shortening. This affects the time course of force development of each element, as well as Ca^{2+} transients ($[\text{Ca}^{2+}]_i$) and Ca^{2+} -troponin C binding ($[\text{Ca}^{2+}]_{\text{TnC}}$). The early shortening of the fast muscle decreases peak $[\text{Ca}^{2+}]_{\text{TnC}}$, increases early and reduces late $[\text{Ca}^{2+}]_i$, and causes an

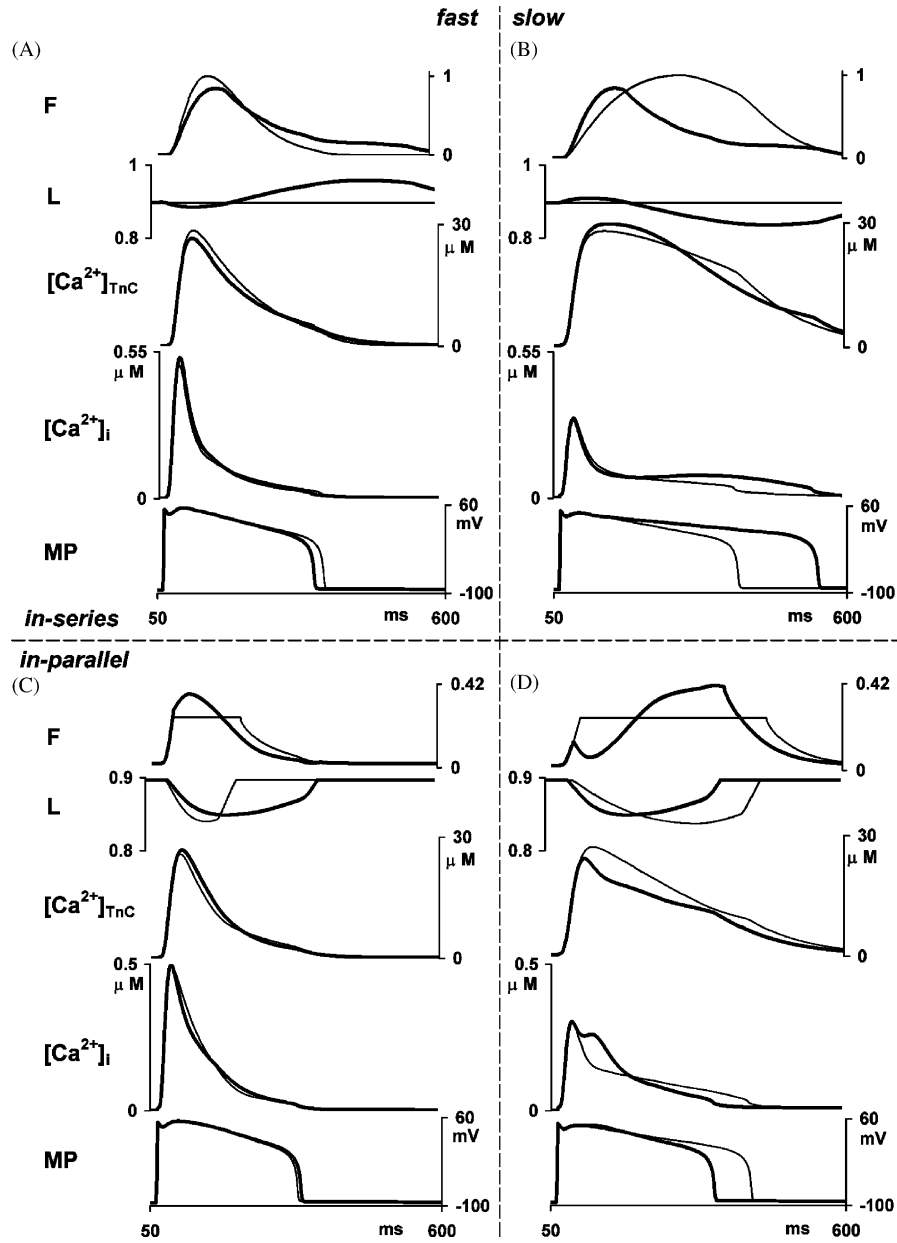


Fig. 3. Summary of the effects of mechanical coupling (top: *in-series*; bottom: *in-parallel*) of heterogeneous virtual muscles (left: fast muscle; right: slow muscle) on mechano-electric function of the individual elements (thin lines: element activity before coupling; thick lines: activity of the same element after duplex formation). In each panel, the following parameters are presented (from top to bottom): force (F), length (L), concentration of calcium bound to troponin C ($[Ca^{2+}]_{TnC}$), concentration of 'free' cytosolic calcium ($[Ca^{2+}]_i$), and trans-membrane potential (MP). Contraction of the *in-series* duplex is externally isometric, and that of the *in-parallel* duplex—isotonic. For detailed explanation see Chapter 3.2.

abbreviation in APD. The behaviour of the slow virtual muscle is principally different, as its early distension by the fast muscle increases peak $[Ca^{2+}]_{TnC}$. This, together with the enhanced release of calcium from troponin C during slow muscle shortening, contributes to a maintained elevation of $[Ca^{2+}]_i$ during the late calcium transient and prolongs the APD. Thus, MEF in heterogeneous myocardium may affect APD via changes in calcium handling (in particular by modulating $Na^+ - Ca^{2+}$ exchange currents during early phase of action potential plateau), even when stretch-activated currents are not accounted for in the model (see details of model analysis in Solovyova et al., 2003). The model predictions correspond well with experimental data on the role of mechano-dependent modulation of Ca^{2+} handling for cardiac MEF, as reviewed in this issue by Calaghan et al. (2003). The direction and amplitude of APD changes is affected by the timing of mechanical changes relative to the cycle of contraction/relaxation of individual muscle segments. This could, for example, contribute to action potential lengthening in ischaemic foci (which can be seen as being equivalent to an *in-series* slow muscle element).

The effects of formation of an *in-parallel* duplex on isotonic contraction of the above virtual muscles are illustrated in the bottom half of Fig. 3 (afterload is set to 25% of peak isometric force of the respective preparations). After duplex formation (thick lines) the contribution of individual elements to total duplex tension varies, with an initial peak in fast muscle force production (Fig. 3C), followed by a more pronounced contribution to total duplex tension by the slow muscle (Fig. 3D; note that the sum of forces is constant once it reaches the pre-determined afterload level). Again, as a consequence of altered mechanical activity $[Ca^{2+}]_{TnC}$ of both elements in the duplex (thick lines) differs significantly from control activity before duplex formation (thin lines), which produces corresponding changes in $[Ca^{2+}]_i$ that affect $Na^+ - Ca^{2+}$ exchange (not shown) and APD.

The relative changes in fast and slow virtual muscle behaviour, induced by duplex formation (*in-parallel* as well as *in-series*), are of opposite directionality. Interestingly, the overall effect of *in-parallel* duplex formation on APD is opposite to that caused by *in-series* connection of the same elements. Thus, mechanically induced changes in APD in heterogeneous myocardium depend not only on the timing of mechanical interventions relative to the cardiac cycle (as suggested before, Kohl et al., 1998), but also on the mode of contraction (isometric or isotonic) and on the prevalent type of mechanical interaction (*in-series* or *in-parallel*). This may be of relevance in interpreting the discrepancy of *in vitro* and *in situ* APD data of cells from different layers of the ventricular wall: isolated sub-epicardial (fast) cells tend to have shorter APD than, for example, isolated sub-endocardial (slow) ones (Antzelevitch and Fish, 2001), whereas such a gradient is not observed in the intact human heart (Taggart et al., 2000, 2003). While the latter will, at least in part, be attributed to electrotonic cancellation of APD differences, it is noteworthy that isolated cell studies are performed in mechanically unloaded cells, and that *in-parallel* mechanical loading (as occurs in native ventricle) may, via MEF, contribute to a pronounced reduction in transmural APD gradients.

Therefore, heterogeneous myocardium displays complex, dynamic interactions of mechanical and electrical events, which depend on factors such as mode of contraction, spatial arrangement of muscle elements, relative timing of mechanical and electrical events, and electrical stimulation sequence. These multiple interactions are of key importance for extrapolation of *in vitro* data to the *in situ* setting, and the duplex method provides a novel handle on investigating them.

4. Conclusions

We have developed and characterised a novel platform for experimental and theoretical studies into cardiac mechano-electric function that takes into account (patho-)physiological heterogeneity of myocardium. Our studies support the notion that ‘well-organised’ inhomogeneity is a prerequisite for normal cardiac function, and suggest that electrical and mechanical heterogeneity are normally ‘synergistic’. Pathologically disturbed patterns of cardiac heterogeneity contribute to arrhythmogenesis, and mechanisms underlying this behaviour may now be assessed using the new duplex method.

5. Editor’s note

Please see also related communications in this volume by [Power et al. \(2003\)](#) and [Stevens and Hunter \(2003\)](#).

Acknowledgements

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