Critical role of cardiac t-tubule system for the maintenance of contractile function revealed by a 3D integrated model of cardiomyocytes

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Abstract

T-tubules in mammalian ventricular myocytes constitute an elaborate system for coupling membrane depolarization with intracellular Ca^{2+} signaling to control cardiac contraction. Deletion of t-tubules (detubulation) has been reported in heart diseases, although the complex nature of the cardiac excitation-contraction (E-C) coupling process makes it difficult to experimentally establish causal relationships between detubulation and cardiac dysfunction. Alternatively, numerical simulations incorporating the t-tubule system have been proposed to elucidate its functional role. However, the majority of models treat the subcellular spaces as lumped compartments, and are thus unable to dissect the impact of morphological changes in t-tubules. We developed a 3D finite element model of cardiomyocytes in which subcellular components including t-tubules, myofibrils, sarcoplasmic reticulum, and mitochondria were modeled and realistically arranged. Based on this framework, physiological E-C coupling was simulated by simultaneously solving the reaction-diffusion equation and the mechanical equilibrium for the mathematical models of electrophysiology and contraction distributed among these subcellular components. We then examined the effect of detubulation in this model by comparing with and without the t-tubule system. This model reproduced the Ca^{2+} transients and contraction observed in experimental studies, including the response to beta-adrenergic stimulation, and provided detailed information beyond the limits of experimental approaches. In particular, the analysis of sarcomere dynamics revealed that the asynchronous contraction caused by a large detubulated region can lead to impairment of myocyte contractile efficiency. These data clearly demonstrate the importance of the t-tubule system for the maintenance of contractile function.

Introduction

In mammalian ventricular myocytes, the cell membrane (sarcolemma) invaginates to the cell interior at a regular interval to form the well-organized transverse (t-) tubule system. As voltage-operated L-type Ca^{2+} -channels are clustered in the t-tubules where they face the junctional part of the sarcoplasmic reticulum (SR) in close proximity, the t-tubule system is believed to play a crucial role in coupling the depolarization signal with Ca^{2+} -release from the SR (Brette and Orchard, 2003). In fact, atrial myocytes with a poorly-developed t-tubule system exhibit slow propagation of the Ca^{2+} signal from the periphery to the center of the cell (Kirk et al., 2003). Further, experimental deletion of t-tubules (detubulation) in ventricular myocytes by osmotic expansion (Brette et al., 2002; Kawai et al., 1999) or long term culture (Louch et al., 2004) results in an asynchronous rise in intracellular Ca^{2+} concentration $[\text{Ca}^{2+}]$ upon electrical stimulation as in pathological deletion (Dibb et al., 2009; Heinzel et al., 2008). Nevertheless, the causal relationship between t-tubule structure, $[\text{Ca}^{2+}]$ dyssynchrony, and contractile dysfunction of the myocytes often observed in diseased hearts remains unclear. For instance, structural remodeling of the t-tubule system is not only observed in the advanced stage of heart failure (Song et al., 2006), but it can occasionally start early in the compensated stage of hypertrophied hearts without contractile dysfunction (Wei et al., 2010). Remodeling includes the t-tubular disarray observed in mechanically unloaded myocytes, in which abnormal Ca^{2+} release is observed without appreciable changes in action potential and density of t-tubules as estimated from membrane fluorescence (Ibrahim et al., 2010). Furthermore, t-tubule alteration is not always observed in heart failure, while the localized drop out in excitation-contraction coupling (ECC) efficacy apparently caused by t-tubule remodeling can be explained by the altered action potential waveform characterized by the reduced transient outward current (Bers, 2006; Harris et al., 2005).

An alternative approach to study the functional impact of t-tubules is numerical simulation, with which we can perform *in silico* experiments under totally controlled conditions.

The crucial part of the modeling of cardiac myocytes is the representation of the calcium-induced calcium release (CICR) mechanism. Until recently, the majority of studies used the ‘common pool model’ (Stern, 1992) in which all Ca^{2+} currents are mediated by the L-type Ca^{2+} channel (LCC) and the release flux from the SR flow into a common compartment, and the Ca^{2+} concentration in this compartment controls the activity of the SR Ca^{2+} release channel (ryanodine receptor: RyR). However, as this modeling strategy cannot reproduce the graded Ca^{2+} release, alternative formulations have been devised. For example, Greenstein et al. (2006) proposed a local control model in which simulated the gating of individual LCCs and RyRs in dyadic space to successfully reproduce the graded Ca^{2+} release. Nevertheless, both modeling approaches treat the subcellular spaces as a lumped compartment and ignore geometrical factors, and as such are unsuitable for examining the effect of structural changes in t-tubules. Three-dimensional (3-D) models of cardiac myocytes studying the impact of 3D t-tubules on electrophysiology and 3D Ca^{2+} dynamics have also been reported (Cheng et al., 2010; Lukyanenko et al., 2009; Yu et al., 2011). However, although these models reproduce either a simple or realistic shape of t-tubule structure, other subcellular components are not explicitly modeled and the diffusion of Ca^{2+} is calculated in the homogeneous cytosolic space assuming a uniform distribution of troponin C throughout the cytosol.

Thus, as yet there are no simulation models that integrate the electrophysiology and contraction in a detailed 3-D structure to examine the functional impact of t-tubule structure in health and diseased states. In the present study, we extended our previous system (Okada et al., 2005) to develop a finite element model of cardiomyocyte integrating electrophysiology, Ca^{2+} dynamics, and contractile process with detailed subcellular structures to evaluate the impact of the spatial arrangement of t-tubules in the signal transduction involved in ECC. The simulation results reproduced both the propagation of Ca^{2+} signals and the contractile behavior for both normal and detubulated myocytes previously reported in experimental studies. Furthermore, the detailed information provided on Ca^{2+} concentration, stress, and sarcomere strain beyond the limit of

experimental approach demonstrated not only the importance of t-tubules in contractile function but also the utility of this model for studying cardiac mechanics.

Methods

3D finite element model of the myocyte

The structure of the 3D finite element model of myocytes is shown in Fig. 1C-E. Ion channels, pumps, and exchangers are distributed in surface sarcolemma and t-tubules with specific densities, as previously reported for guinea pig ventricular myocyte (Pasek et al., 2008). Mitochondria were modeled as a Ca^{2+} sink, although energy metabolism was not incorporated in this study. To reduce computational costs the segment containing three myofibrils of one sarcomere length was modeled together with the adjacent cell membrane and organelle (2816 nodes and 2107 elements). The rationale behind this modeling strategy was the longitudinal periodicity and axial symmetry of the myocyte. Subcellular components including the mitochondria, myofibril (A-zone, I-zone, and M-line), junctional and network sarcoplasmic reticulum (JSR and NSR respectively), cell membrane, and t-tubules were located at the appropriate nodes to reproduce the anatomical structure and occupy the relative volume (Aliev et al., 2002; Bers, 2001; Chen-Izu et al., 2006; Cortassa et al., 2006). The function of each subcellular component was primarily based on the previously proposed mathematical formulation for guinea pig ventricular cardiomyocyte (Cortassa et al., 2006). Additionally, to investigate the effect of regional disorganization of the t-tubule system, we created larger models consisting of three myofibrils of 12-sarcomere length (Fig. 1F and G). In these cases, to save the computational cost, coarse meshes were used and half of the model (6 sarcomere length) was calculated considering its symmetry. The model consisted of 4340 nodes and 3420 elements. The reaction-diffusion fields for Ca^{2+} were defined in the cytosolic space, as follows:

$$\frac{d[\text{Ca}^{2+}]_i}{dt} = \nabla \cdot (\mathbf{D}_i \nabla [\text{Ca}^{2+}]_i) + f_i([\text{Ca}^{2+}]_i) \quad \text{Eq. 1,}$$

where $\mathbf{D}$ describes the diffusivity of Ca^{2+} , $[\text{Ca}^{2+}]_i$ is the concentration of Ca^{2+} at position i , and $f_i([\text{Ca}^{2+}]_i)$ is the function describing the reaction of Ca^{2+} which corresponds to elementary processes of each subcellular components including mitochondria, JSR and so on, and which

was defined appropriately for each node to reproduce the anatomical structure.

The diffusion coefficient for the longitudinal direction was estimated from an *in vitro* experiment (de Graaf et al., 2000) and set at $D^{Ca} = 0.3$, ($\mu m^2/ms$). With regard to the coefficient in the transverse direction, we multiplied this value by 0.6265 based on *in vivo* cardiac myofibril results (Vendelin and Birkedal, 2008). Every node on the membrane and t-tubule permeates ions according to the local ion concentration and shares a membrane potential. As experimental data suggested that the whole cell membrane is electrically well coupled (Orchard and Brette, 2008), the membrane potential was treated as uniform throughout the model. Ion channel distributions on peripheral and t-tubule membrane were defined as previously reported (Pasek et al., 2008) (see Supplemental Table 2). At the Ca^{2+} release site the channel distribution is different from other regions of the t-tubule membrane, and only the L-type Ca^{2+} channel (LCC) is clustered without other ion channels. Here, the LCC, JSR, subspace, and the local cytosolic space share a common node, but possess their own Ca^{2+} concentrations for finite element analysis. Upon depolarization of the membrane potential triggered by stimulation current, LCC opens and the Ca^{2+} current through the LCC (I_{Ca}) flows into the subspace and activates ryanodine receptors (RyR) located on the JSR. This triggers Ca^{2+} release from the JSR (J_{rel}) into the subspace, and the released Ca^{2+} moves to the cytosolic node to diffuse throughout the entire cytosol. Within the cytosolic space, Ca^{2+} is either taken up by the Ca^{2+} uniporter of the mitochondrial node, bound to troponin C on the thin filament, or is sequestered by Ca^{2+} pump (SERCA2) on the NSR, before eventually being transported back to the JSR. Further details outlining the model can be found in Supplementary Material.

The contraction model developed by Negroni et al. (Negroni and Lascano, 1996) was located at each point of the A-zone and generated force depending on the local Ca^{2+} concentrations and sarcomere length. Using these force values as boundary conditions, the following mechanical equilibrium and compressibility control condition on variables ($\mathbf{u}$, λ) were solved using the finite element method:

$$\begin{aligned} \int_V \left(2 \frac{\partial W}{\partial \mathbf{C}} + \lambda \mathbf{J} \mathbf{C}^{-1} \right) : \delta \mathbf{E} dV + \int_{S_t} \tilde{\mathbf{t}}_b \cdot \delta \mathbf{u} dS &= 0 \\ \int \delta \lambda \left((\mathbf{J} - I) - \frac{2\lambda}{\kappa} \right) dV &= 0 \end{aligned} \quad \text{Eq. 2,}$$

where $\mathbf{u}$ is displacement, W is strain energy potential, $\mathbf{C}$ is right Cauchy-Green deformation tensor, $\mathbf{E}$ is Green-Lagrange strain tensor, J is Jacobian of $\mathbf{C}$, V is analysis volume, λ is a Lagrange multiplier corresponding to -1/2 of the pressure, κ is bulk modulus, $\tilde{\mathbf{t}}_b$ is the stress boundary condition calculated from A-zone force generation, and S_t is the surface where stress boundary condition is defined.

The cell was assumed as nearly incompressible hyperelastic material, with anisotropic constitutive equations for myofibril and isotropic constitutive equations of Mooney-Rivlin material for the other spaces previously reported (Okada et al., 2005). Full details of the mathematical modeling can be found online in Supplementary Material.

Detubulated myocyte models

The detubulated model (DET) was created by removing the t-tubules from the control model (CTRL) (Fig. 1A) along with the Ca^{2+} channels, $\text{Na}^+ - \text{Ca}^{2+}$ exchangers, and other channels distributed in it. As a result, the Ca^{2+} release channels (RyR) on the SR were left as orphan RyR (Fig. 1B). To analyze the effect of regional disorganization of the t-tubule system, we created two regionally detubulated models with either a large (8 sarcomere-length: DET8) or small (4 sarcomere-length: DET4) detubulated region, and compared them with the non-detubulated control myocyte model (Fig. 1F and G). We also compared the models in which half of the t-tubules were removed homogeneously (every other transverse tubule was deleted: homogenous model) or by deletion of the middle six t-tubules as a cluster (clustered model).

Simulating the adrenergic stimulation

In CTRL and DET models, the influences of adrenergic stimulation were simulated by up-regulation of L-type Ca^{2+} channel current by 50%, lowering the threshold $[\text{Ca}^{2+}]$ level for

Ca^{2+} release from RyR by 20%, and tripling the maximum velocity of SR Ca^{2+} pump, as previously reported (Brette et al., 2005; Brette et al., 2004). We also simulated a case in which only the L-type Ca^{2+} current was up-regulated (Harris et al., 2005).

Computation

All the program codes (including solver and finite element integration) were written in-house using Fortran language, and the computation was performed using an Intel Xeon CPU (3.2 GHz).

Results

Ca²⁺ propagation and contraction in detubulated myocyte

The Ca²⁺ propagation and unloaded contraction of control and detubulated myocyte models in time-lapse images are shown in Figure 2 (see also Supplemental Movie 1). In CTRL, [Ca²⁺] increased simultaneously at the surface membrane and Ca²⁺ release sites distributed along the t-tubules, and rapidly propagated towards the A-line region. Consequently, all the sarcomeres in the model contracted synchronously. By contrast, [Ca²⁺] in DET was initiated only at the surface membrane and propagated slowly to the center of the cell, resulting in slow and asynchronous contraction.

Next, for comparison with the experimentally observed transverse line scan using confocal microscopy, we simulated isometric contraction, and sampled and plotted the [Ca²⁺] on the line running transversely along the t-tubules after the onset of excitation, as well as the local Ca²⁺ transients sampled at the sub-sarcolemma (SS) and core (CC) regions (Fig. 3A and B). The regularly spaced bright spots in these figures correspond to the Ca²⁺ release sites. There was a significant delay in local Ca²⁺ transients in the core region of DET compared with CTRL, and both the time to peak and the end of the whole-cell Ca²⁺ transient, averaged over the entire region, was significantly delayed compared with CTRL (black lines in the bottom panels). The propagation velocity of Ca²⁺ transients was 31 $\mu\text{m/s}$, comparable to those observed experimentally ($48.4 \pm 5.2 \mu\text{m/s}$; Yang et al. (2002)). Upon adrenergic stimulation, the amplitude of the Ca²⁺ transient increased and its duration decreased in CTRL (Fig. 3C). In the detubulated myocyte the lag between the periphery and the core was reduced (Fig. 3D), such that the whole-cell Ca²⁺ transient resembled that of the control (blue lines in the bottom panels). For validation, we compared these results with the experimental observations of Brett et al. (2004) (Fig. 3G). Our simulation model reproduced the effect of detubulation under both baseline and adrenergic stimulation. On the other hand, when only the I_{Ca} was up-regulated, the re-synchronization effect was minimal and a significant delay in the local Ca²⁺ transient in the

cell center remained. Propagations in the DET model under baseline (without adrenergic stimulation), up-regulation of I_{Ca} , and β -adrenergic stimulation are shown in Figure 4. For baseline, I_{Ca} up-regulation, and β -adrenergic stimulation, propagation times were 150, 115, and 35 ms, respectively, and propagation velocities were 31, 40, and 133 $\mu\text{m}/\text{sec}$, respectively.

We also examined the role of $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) current distributed in t-tubules (Pasek et al., 2008) by comparing the averaged Ca^{2+} transients (Fig. 5A), L-type Ca^{2+} channel (Fig. 5B), and NCX (Fig. 5C) currents between the CTRL and the DET models. The total amounts flowing in and out of the model were compared for the currents. While the Ca^{2+} transient of the CTRL model exhibited a sharp rise triggered by the L-type Ca^{2+} channel current, the transient of the DET model triggered by diffuse Ca^{2+} increased slowly to its peak. After the peak, the Ca^{2+} transient of the DET model remained elevated. In the DET model, both the L-type Ca^{2+} channel current and the NCX current were significantly decreased compared with the control model, reflecting the localization of these currents in the t-tubules. These differences in currents contributed to the slow decay in the DET model, although the decay phase of the Ca^{2+} transient was dominated by the SR Ca^{2+} pump for both the CTRL and DET models.

Propagation of Ca^{2+} is influenced by the contraction mode

Next, we compared transverse line scans of the Ca^{2+} signals under different loading conditions (Fig. 6). For the isotonic contraction, afterload was set at 25% of the maximum isometric force. As the afterload was reduced (A: isometric $\rightarrow$ B: isotonic $\rightarrow$ C: unloaded), shortening of the myocyte increased the cell width. Although the initial propagation speed was similar ($\sim 30 \mu\text{m}/\text{s}$), the resultant widening of the space between the adjacent Ca^{2+} release sites caused a progressive delay in Ca^{2+} propagation ($\sim 20 \mu\text{m}/\text{s}$), and the propagation was finally terminated under the unloaded condition (Fig. 6C). A similar phenomenon was reported in the experimental study by Yang et al. (2002) (Fig. 6D).

Regional loss of t-tubules

The effects of regional detubulation on Ca^{2+} transient and contraction were examined using the

12-sarcomere model under isometric conditions (Fig. 7A). In contrast to the control myocyte showing synchronized Ca^{2+} transient and contraction, slow propagation of Ca^{2+} in the detubulated region progressively delayed the completion of contraction as the detubulated region became increased (Fig. 7A and Supplemental Movie 2). The amplitude and timing of the peak isometric force were also decreased and delayed, respectively, depending on the size of the detubulated region (Fig. 7B). To further analyze the contractile dysfunction of the detubulated myocyte, we examined the Ca^{2+} transient, sarcomere length, and developed force at each sarcomere (Fig. 8). The presentation mimicking the longitudinal line scan mode clearly demonstrated a progressive delay of Ca^{2+} propagation toward the center of the detubulated region (Fig. 8 top panels). Although the total muscle length was made isometric, asynchrony of the local Ca^{2+} transient in the detubulated models induced the internal shortening of the early-activated sarcomere and lengthening of the resting sarcomere (Fig. 8, middle panels). In particular, the large internal shortening observed in the DET8 model with the large detubulated region is the likely cause of the greater depression in initial rise of isometric force (Fig. 7B), via inhibition of development of force locally (Fig. 8 bottom panels). To investigate the effect of the size of individual detubulation, we also compared the homogeneous model and the clustered model that both had the same number of t-tubules. The homogeneous model showed no variation in sarcomere length, and the force developed smoothly, similar to the control myocyte without detubulation (Fig. 9). Internal shortening in the clustered model caused the depression of force development in the initial phase (Fig. 7C), and may also have induced a deleterious effect. In addition, although energy metabolism was not considered in this simulation, internal shortening is expected to reduce the contractile efficiency by wasting energy according to the Fenn effect.

Discussion

3D model of cardiomyocyte

In the present study, we extended our previous 3D cardiomyocyte model to incorporate ion currents distributed heterogeneously in the surface membrane and the t-tubule system to examine the impact of the t-tubule system on cardiac function. We adopted the sarcomere model of Negroni and Lascano (1996) for cardiac contraction. Although this simple model is not capable of reproducing the cooperative activation of actin-myosin interactions, we previously reported its robustness and compatibility with the FEM model (Okada et al., 2005; Watanabe et al., 2004). These complex models can reproduce various phenomena including cooperativity, quick-stretch, and release, but may require significant computational cost. The models of electrical activity by Cortassa et al. (2006) do not differ significantly from the model by Rudy (Livshitz and Rudy, 2007), but allow us to extend the model to include metabolic processes in future studies. However, as this model is a lumped parameter model, the distributions of ion channels in the t-tubule system and the volume fraction of the sub-compartments require adjustment for the current 3D model.

In the detubulated model, Ca^{2+} transients initiated at the peripheral membrane by depolarization propagate to the center of the cell slowly by a series of reaction diffusion processes (CICR). As a result, the Ca^{2+} transient averaged over the entire region showed a delay in time to peak and a slow decay. Slow decay was also observed in local Ca^{2+} transients due to the loss of t-tubular Na-Ca exchange current, as suggested experimentally (Kawai et al., 1999; Pasek et al., 2008; Yang et al., 2002). In addition, under adrenergic stimulation, the detubulated model showed faster Ca^{2+} propagation and the synchronization of the Ca^{2+} transient was restored. The lower threshold $[\text{Ca}^{2+}]$ level for Ca^{2+} release and the larger Ca^{2+} release flux through up-regulation of LCC and SR (up-regulation of SR Ca^{2+} pump resulted in higher content and larger release) accelerated the CICR and the reaction-diffusion processes. This synchronizing effect of beta adrenergic stimulation on detubulated cells was also observed in

experimental studies (Brette et al., 2004; Smyrniak et al., 2010). In contrast to our simulation results, Harris et al. (2005) was able to recover synchronous Ca^{2+} release in failing feline myocytes only by increasing I_{Ca} induced by the normalization of action potential. However, although the authors did not examine the t-tubule morphology in their failing myocytes, they show a spotty Ca^{2+} release pattern suggesting that a large area of couplon loss did not take place in their preparations. Considering our simulation result with the small detubulated region (DET4 or homogeneous model), we speculate that if the area of detubulation is small, only the increase in I_{Ca} could allow recovery of the normal cardiomyocyte E-C coupling function, while if the area of detubulated is large, the recovery may not be possible even with multiple compensatory mechanisms.

Impact of t-tubule disorganization on cardiac dysfunction of the diseased heart

In cardiomyocytes isolated from diseased hearts, t-tubules are lost heterogeneously and the area of disorganization increases progressively (Cannell et al., 2006; Heinzel et al., 2008; Louch et al., 2004; Louch et al., 2006). Our simulation revealed a clear dependence of cardiac dysfunction on the area of the detubulated region. When the gap (detubulated region) was small, force generation was not appreciably affected due to the rapid propagation of the Ca^{2+} transient by diffusion and the slow time course of force development. By contrast, a large gap introduced a significant delay in the Ca^{2+} transient and in contraction in the detubulated region. Such dyssynchrony in contraction impairs the force generation and reduces the efficiency of contraction as the early contracting segment elongates the resting segment (internal shortening), thus wasting mechanical energy. These two factors may synergistically deteriorate cardiac function. In addition, our results suggest that the size of the detubulated region, together with t-tubule density, are the important factors for cardiomyocyte function, since the size of detubulation determines the delay of Ca^{2+} transient and the degree of inhomogeneity. In human heart failure there are large areas of t-tubule disorganization comparable to our large gap model (Fig. 5 of (Cannell et al., 2006)). However, in the murine model of heart failure the majority of

the delayed Ca^{2+} release region had a width of less than 4 μm (Louch et al., 2006). Nevertheless, even with such small gaps in the t-tubule system, concomitant alterations in Ca^{2+} handling proteins can induce cardiac dysfunction. In this study, however, we only evaluated the effect of t-tubule disorganization.

Limitations of the model

For our model, we used both detailed single-sarcomere models and 12-sarcomere models with coarse mesh. However, even with the coarse mesh model, the analysis volume was insufficient to impose a three-dimensionally inhomogeneous deformation. A whole cell model with a more realistic sub-cellular structure is required for further analysis, although compromising the computational cost would be a prerequisite for such modeling. On the other hand, uncertainty in the subcellular distributions of functional components including ion channels also limits the analysis (Pasek et al., 2008). The modeling study can be used to estimate these unknown parameters, although further experimental clarification and verification are required. Finally, we assumed that the $[\text{Ca}^{2+}]$ of the t-tubular space was equal to that of bulk extracellular space. However, the $[\text{Ca}^{2+}]$ of t-tubular space was estimated to decrease by 13.8% at onset of Ca^{2+} release under pacing at 1 Hz (Pasek et al., 2008). The diffusion process in the extracellular space needs to be implemented in future model development.

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Figure Legends

Fig. 1. A) Control model (CTRL) with normal t-tubule structure. B) Detubulated model (DET) with orphan ryanodine receptor. C-E) Schematic diagram of the 3D cardiomyocyte model. D) Six myofibrils of one sarcomere length were modeled in finite element mesh with subcellular structures (C, CTRL; E, DET). Ion channels and exchangers are distributed over the t-tubules and sarcolemma. Mem: sarcolemma, Mf: myofibril, Mito: mitochondria, SR: sarcoplasmic reticulum, T: t-tubule. F) Twelve sarcomere mesh with normal t-tubule structure. G) Twelve sarcomere mesh with t-tubule deleted in the mid n-sarcomeres.

Fig. 2. Time lapse images of Ca^{2+} propagation in control (CTRL) and detubulated (DET) myocyte models under unloaded conditions. Ca^{2+} concentrations in the laterally arranged six sarcomeres are shown in color-coding indicated below. The numbers in the middle indicates the time (msec) after the stimulation.

Fig. 3. Simulated transverse line scans of control (CTRL) model (A and C) and detubulated (DET) model (B and D) under control conditions (A and B) and under beta adrenergic stimulation (C and D) of isometric contraction. Each panel shows a line scan image of Ca^{2+} concentration with time courses of the local Ca^{2+} concentration sampled at the CC and SS (attached below). The bottom panels compare the whole cell averaged Ca^{2+} transients of A vs. C (in E) and B vs. D (in F). G) Experimental data of line scans and traces of local and averaged Ca^{2+} transients from Brette et al. (2004) are provided for comparison by copyright permission of Elsevier.

Fig. 4. Simulated transverse line scans and averaged Ca^{2+} transients of the detubulated model during the isometric contraction under baseline (A and D, black line), I_{Ca} up-regulation (B and D,

blue line), and β -adrenergic (C and D, red line) conditions.

Fig. 5. Ca^{2+} transients and currents in CTRL (black line) and DET (red line) models under isometric condition. A) Ca^{2+} transients averaged over the sarcomere. B) L-type Ca^{2+} channel current. C) Na^+ - Ca^{2+} exchanger current.

Fig. 6 Transverse line-scan images of detubulated (DET) model under different contraction modes. A) Isometric contraction. B) Isotonic contraction. C) Unloaded contraction. While a single tangential line (white line) was well fit to the Ca^{2+} propagation in A, additional lines (green) were required in B and C due to the reduction of propagation velocity in the core region. Propagation velocity calculated from the slope of the tangential lines were similar in all cases at the initial part (white line: 31 $\mu\text{m/s}$), but decreased in B (21 $\mu\text{m/s}$) and C (18 $\mu\text{m/s}$), both calculated from the slope of the green lines. Blue arrows indicate the initiation of widening. D) Experimental transverse line-scan image of detubulated myocyte demonstrating incomplete propagation of Ca^{2+} . Two tangential lines were fit, similar to above (reproduced from Yang et al. (2002) by copyright permission of the American Heart Association).

Fig. 7. Effect of regional detubulation on the Ca^{2+} transient and contraction. A) Time-lapse images of $[\text{Ca}^{2+}]$ in color-coding during isometric contraction are shown for CTRL (left), DET4 (middle), and DET8 (right) myocytes. B) Isometric forces are compared among the CTRL (black), DET4 (red), and DET8 (blue) myocytes. C) Isometric forces are compared among the CTRL (black), homogeneous detubulation (red), and clustered detubulation (red). Forces are normalized for peak isometric value of CTRL in B and C.

Fig. 8. Local Ca^{2+} transient and developed force of DET4 (left column) and DET8 (right column) models. Top rows: Longitudinal line-scan showing the local $[\text{Ca}^{2+}]$ with longitudinal

position in horizontal axis and time after stimulus in vertical axis. Length change (middle rows) and developed force (bottom rows) of each sarcomere located in the position numbered in the longitudinal scan are shown.

Fig. 9. Local Ca^{2+} transient and developed force of homogeneous (left column) and aggregated (right column) models. Top rows: Longitudinal line-scan showing the local $[\text{Ca}^{2+}]$ with longitudinal position in the horizontal axis and time after stimulus in the vertical axis. Length change (middle rows) and developed force (bottom rows) of each sarcomere located in the position numbered in the longitudinal scan are shown. In the homogeneous model, force and local $[\text{Ca}^{2+}]$ traces of each sarcomeres are superimposed.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

Figure 8.

Figure 9.