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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.

1. 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 [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, [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

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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 (3D) 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 3D 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.

2. Methods

2.1. 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

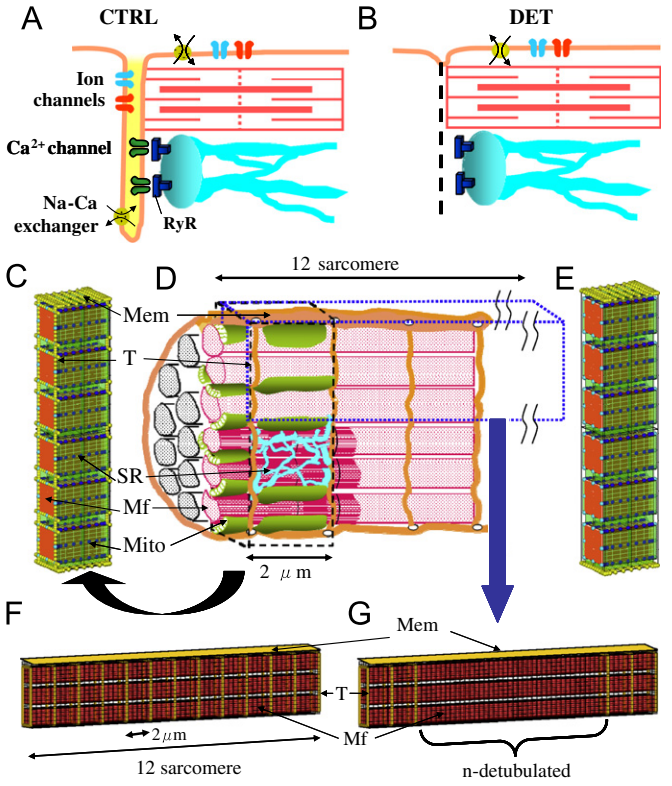

Fig. 1. (A) Control model (CTRL) with normal t-tubule structure. (B) Detubulated model (DET) with orphan ryanodine receptor. (C–E) Schematic diagram of the 3D cardiomyocyte model. (D) Six myofibrils of one sarcomere length were modeled in finite element mesh with subcellular structures (C, CTRL; E, DET). 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.

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:

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

where $\mathbf{D}$ describes the diffusivity of Ca^{2+}, $[Ca^{2+}]_i$ is the concentration of Ca^{2+} at position i, and $f_i([Ca^{2+}]_i)$ 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