JCMM Express
Images in Cellular/Molecular Medicine

J. Cell. Mol. Med. Vol 13, No 5, 2009 pp. 995-998

Three-dimensional electron microscopic reconstruction of intracellular organellar arrangements in vascular smooth muscle – further evidence of nanospaces and contacts

Wing Chiu Tong [a, #], Michele Sweeney [b, #], Carolyn J. P. Jones [c], Henggui Zhang [d], Stephen C. O'Neill [e], Ian Prior [f], Michael J. Taggart [b, *]

[a] *Institute of Membrane & Systems Biology, University of Leeds, Leeds, UK*
[b] *Institute of Cellular Medicine, Newcastle University, Newcastle Upon Tyne, UK*
[c] *Maternal & Fetal Health Research Group, University of Manchester, Manchester*
[d] *School of Physics & Astronomy University of Manchester, Manchester, &*
[e] *Cardiovascular Research Group, University of Manchester, Manchester, UK and*
[f] *School of Biomedical Sciences, University of Liverpool, Liverpool, UK*

Received: March 31, 2009; Accepted: April 14, 2009

Abstract

The sarcoplasmic reticulum (SR) of smooth muscle is crucial for appropriate regulation of Ca^{2+} signalling. In visceral and vascular smooth muscles the SR is known to periodically lie in close register, within a few nanometres, to the plasma membrane. Recent work has focussed on reconstructions of the ultrastructural arrangement of this so-called peripheral SR that may be important for the genesis of phenomena such as Ca^{2+} sparks. Here, we turn our attention to vascular smooth muscle and explore the 3-dimensional (3D) ultrastructural positioning of SR found deeper in the cell that is involved in the propagation of Ca^{2+} waves. We use digital reconstruction and volume rendering of serial electron microscopic sections from isolated resistance arteries, pressurized *in vitro* to mimic cellular geometric conformations anticipated *in vivo*, to map SR positioning. We confirm that these central portions of SR are in close register with mitochondria and the nucleus with all three organelles tightly enveloped by a myofilament/cytoskeletal lattice. Nanospacings between the SR and individual mitochondria are visible and in three dimensions as the SR contorts to accommodate these organelles. Direct connection of the SR and nuclear membranes is confirmed. Such 3D positioning of centrally located SR further informs us of its likely role in the manifestation of spatiotemporal Ca^{2+} dynamics: signal encoding may be facilitated by spatially directed release of Ca^{2+} to influence several processes crucial to vascular smooth muscle and resistance artery function including myofilament activation by Ca^{2+} waves, mitochondrial respiration and gene transcription.

Keywords: smooth muscle • electron microscopy • sarcoplasmic reticulum • digital 3-dimensional reconstruction

The extent of tone development in small arteries is a key determinant of peripheral vascular resistance. The level of tone is controlled by the contractile state of smooth muscle cells whose orientation in arterial walls is predominantly circular, thereby encircling the lumen. In turn, vascular smooth muscle cell contractility is controlled by the regulation of the spatiotemporal dynamics of intracellular Ca^{2+}. These can take many forms. For example, those that are restricted temporally (milliseconds duration) and spatially (a few microns) such as Ca^{2+} sparklets and Ca^{2+} sparks. In response to excitatory agonists, however, Ca^{2+} fluctuations with broader spatial (tens of microns) and temporal (seconds duration) distributions are seen. These are often demonstrated as waves of Ca^{2+} which originate in one or more discrete points of the cell and then propagate throughout the rest of the cell [1–3]. Propagation of Ca^{2+} waves is now known to be dependent upon release of Ca^{2+} from the sarcoplasmic reticulum (SR). In order to better understand the spatiotemporal nature of these Ca^{2+} dynamics, and the functional changes for the smooth

[#]These authors contributed equally.
*Correspondence to: Professor Michael J. TAGGART,
Institute of Cellular Medicine, 3rd Floor Leech Building,
Newcastle University, Newcastle Upon Tyne NE2 4HH, UK.
Tel.: +44 (0)191 222 6988; Fax: +44 (0) 191 222 5066
E-mail: michael.taggart@ncl.ac.uk

doi:10.1111/j.1582-4934.2009.00770.x

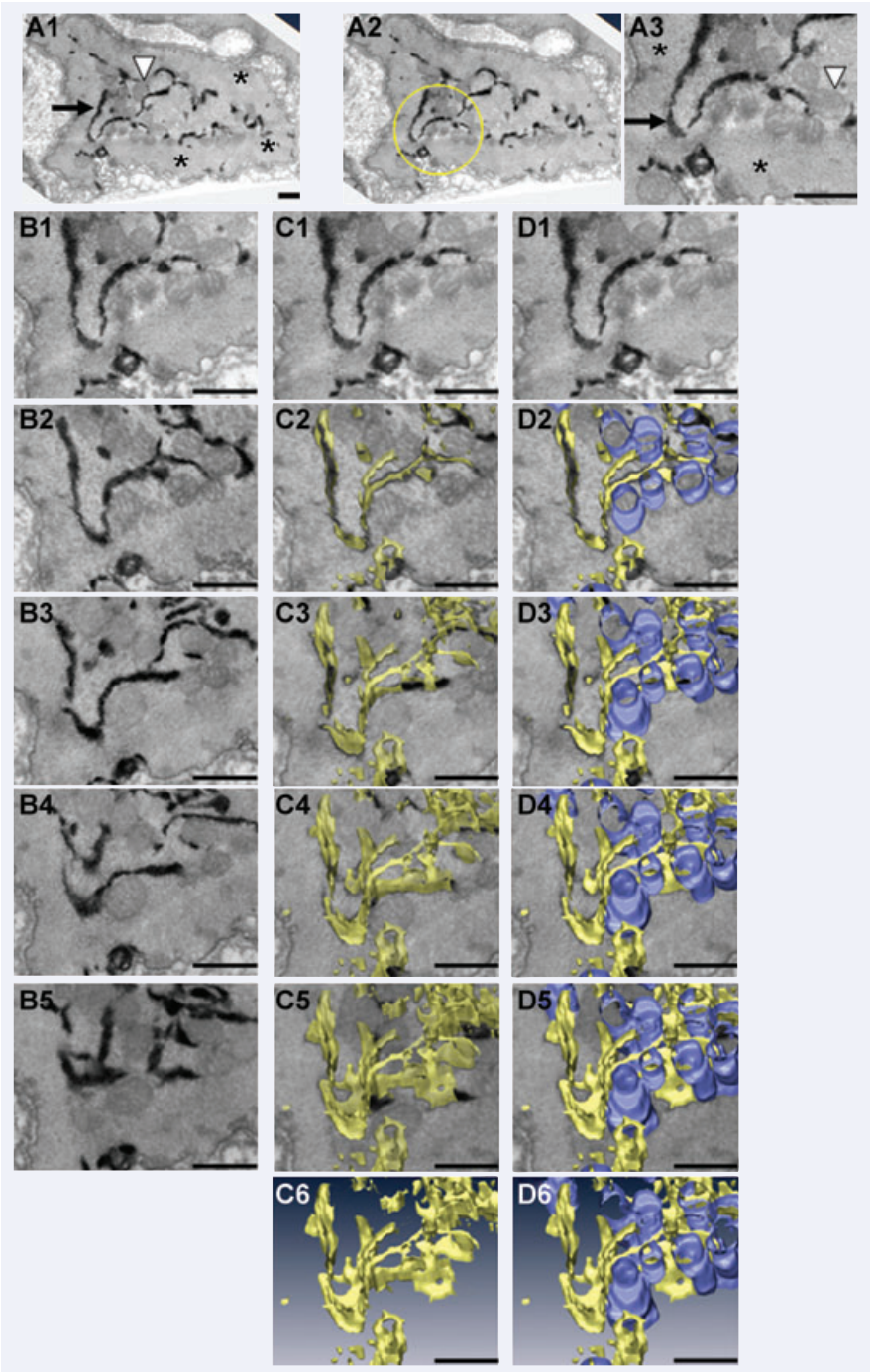

Fig. 1 Three-dimensional reconstruction of resistance artery smooth muscle SR relationship with mitochondria. **A1**, longitudinal cross section of resistance artery indicating centrally located SR (stained black, indicated by black arrow) and mitochondria (white arrowhead indicates a mitochondrion) enveloped by myofilament lattice (black asterisks). **A2**, same section with yellow circle denoting the enlarged portion viewed in **A3**. **B1**–**B5**, consecutive 70-nm-thick sections showing contortions of SR around mitochondria. **C1**–**C5**, digitized tracings of SR location from each section appearing cumulatively. **D1**–**D5**, cumulative digitized tracings of mitochondrial positioning, in addition to SR, from each section. **C6**, digitized rendering of SR 3D reconstruction. **D6**, digitized rendering of mitochondria and SR 3D reconstruction. Scale bars: **A1**–**A2**, 2 μm; **B1**–**D6**, 300 nm.

muscle cell that such changes may impart, it is necessary to understand the spatial arrangement of SR in vascular smooth muscle cells. Although a considerable amount is known about the SR arrangement in two dimensions (*x*–*y*) [see 1, 4 and references therein], less is known about the 3-dimensional (3D) arrangement. It is important to consider this as the dissipation/propagation of Ca^{2+} elevations away from any specific site of origin will effect a functional response dependent upon the location of likely Ca^{2+}-sensitive targets. Recent electron microscopy and digital reconstruction approaches have attempted to address this by examining the relationship between peripherally located SR and elements of the adjacent plasmalemma [5–7]. Such studies and techniques,

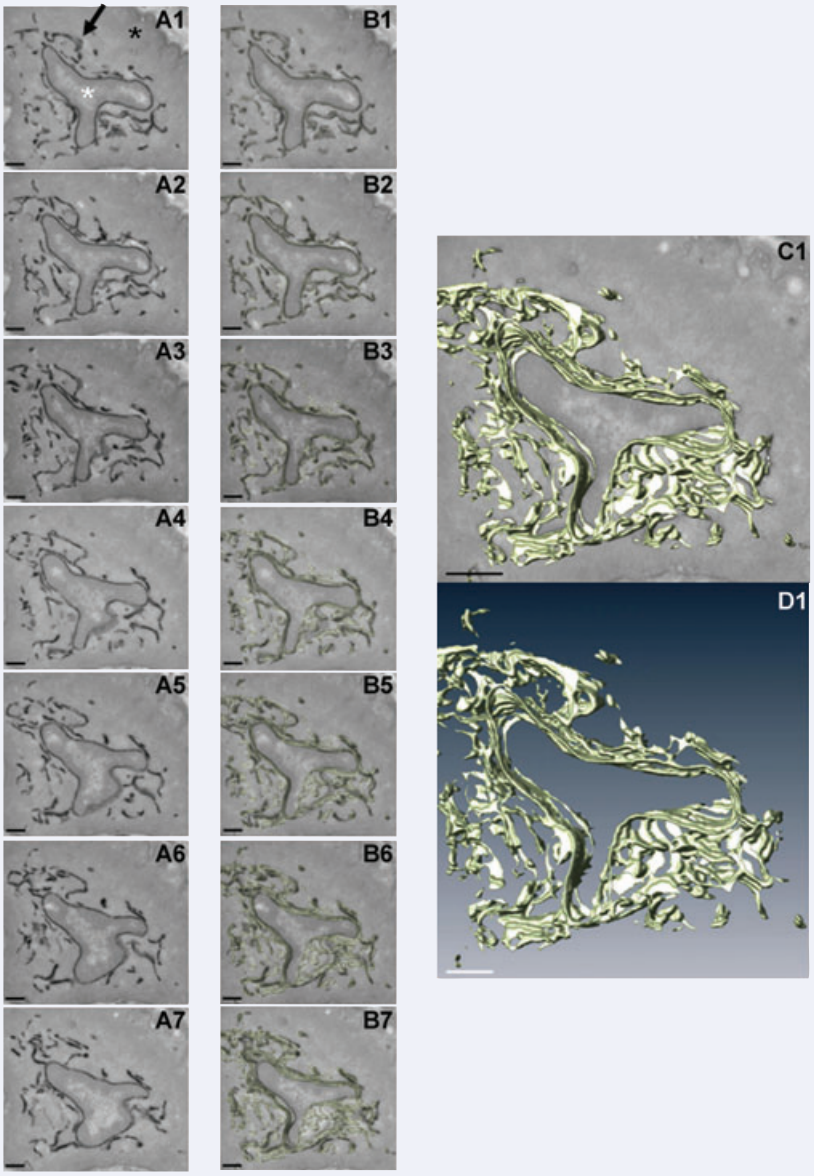

Fig. 2 Three-dimensional reconstruction of resistance artery smooth muscle SR relationship with the nucleus. **A1**, longitudinal cross section of resistance artery indicating centrally located SR (stained black, indicated by black arrow) and nucleus (white asterisk, nuclear membrane also stained black) enveloped by myofilament lattice (black asterisk). **A1**–**A7**, consecutive 70-nm-thick sections showing contortions of SR in relation to nuclear membrane. **B1**–**B7**, digitized tracings of SR location and nuclear membrane from each section appearing cumulatively. **C1**, merged picture of the digitized accumulations of **B1**–**B7**. **D1**, digitized rendering of the cumulative tracings of SR and nuclear membrane locations. Scale bars: **A1**–**B7**, 1 μm; **C1** and **D1**, 400 nm.

including the use of titling electron microscopy, have shown the prevalence of 'nanospaces' between such peripheral SR and the plasmalemma and caveolae [8–10]. Here we present further evidence of 3D SR spatial arrangements in smooth muscle cells but with some differences: First, we have concentrated on the relationship of centrally located SR with other intracellular organelles. Second, we have utilized a method for increasing SR contrast in electron microocopy (EM) capture that aids in the digitized volume rendering of the organelle [1, 4]. Third, we have investigated the SR structure in smooth muscle cells of isolated resistance arteries that were maintained at physiological pressures throughout the fixation procedure so as to retain a conformation close to that anticipated *in vivo*.

Adult male Wistar rats were killed according to UK Home Office guidelines. Third-order mesenteric arteries were isolated and mounted in an arteriograph chamber under isobaric conditions (60 mmHg intralumenal pressure), equilibrated in physiological salt saline (PSS [mM]: NaCl 119, KCl 4.7, $MgSO_4.7H_20$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.17, K_2EDTA 0.03, glucose 5.5, $CaCl_2.2H_20$ 1.6 at pH 7.4 at 37°C), constricted with high K^+-PSS to ensure tissue viability and relaxed in PSS. While remaining pressurized, vessels were then placed in EM fixative (2.5% gluteraldehyde in 0.1 M sodium cacodylate buffer pH 7.3), post-fixed for SR staining (2% osmium tetroxide, 100 mM sodium cacodylate buffer, 0.8% [w/v] potassium ferricyanide) and embedded in TAAB (TAAB Laboratories Equipment

Ltd, Aldermaston, Berks, UK) epoxy resin as previously described [1]. The central portion of the fixed vessel was identified and transverse ultrathin serial sections (70 nm) were cut and laid consecutively on formvar/carbon-coated copper grids with a 2 × 1 mm slot (Agar Scientific, Stansted, UK). Samples were viewed without further contrasting in a Philips CM10 electron microscope (Philips Electronics UK Ltd, Guildford, Surrey, UK) at an accelerating voltage of 80 kV and digital images were captured using a Deben camera and stored as 4 MB TIFF files. These images were aligned, organelles volume rendered and images accumulated and visualized using Amira software. Three-dimensional stereo movies of the assembled data utilized 500–1000 frame tilts per video.

In smooth muscle cells the centrally located SR, mitochondria and nucleus are enshrouded by a myofilament/cytoskeletal filament lattice. Examination of serial sections where the centrally located mitochondria were visible showed that the black stained, electron dense SR is in close proximity to individual mitochondria (Fig. 1). Digital 3D reconstruction of these serial sections followed by volume rendering results in the visualization of SR contortions between and around the mitochondria (Fig. 1 and Supporting Information Files S1 and S2 ['render_SR' and 'render_SR_mito']).

On other occasions it can be seen that the SR makes direct contact with the nuclear membrane (Fig. 2). In 3D reconstructed files (Fig. 2 and Supporting Information File S3 ['render_SR_nuclear membrane']) the SR is indicated to emanate from the nuclear membrane at several points, supporting earlier suggestions [4] that the SR and nuclear membranes form a contiguous structure.

In conjunction with the previous reports mentioned above of assembling 3D EM information on the peripheral SR, this work enhances the notion that the SR may contribute to the complexity and variety of temporally and spatially distinct release of Ca^{2+} in arterial smooth muscle. This, one assumes, imparts benefit by allowing a multitude of signal processing possibilities utilizing Ca^{2+} as a second messenger that contributes to varied vascular functions. These may include energetically conservative tone maintenance and transcriptional-dependent phenotypic plasticity. Future reconstruction efforts covering a whole cell/tissue depth by EM is required, possibly in conjunction with fluorescent microscopy to integrate specific molecular identifications with structural definition [11], to fully uncover the morphological basis of spatiotemporal Ca^{2+} dynamics in smooth muscle.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

File S1. Videofile of rendered reconstruction data from Fig. 1 (C6) 'render_SR' with the SR coloured yellow.
File S2. Videofile of rendered reconstruction data fom Fig. 1 (D6) 'render_SR_mito' with the SR coloured yellow and mitochondria blue.
File S3. Videofile of rendered reconstruction data from Fig. 2 (D1) 'render_SR_nuclear membrane' with the SR and nuclear membranes coloured yellow.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1582-4934.2009.00770.x
(This link will take you to the article abstract).

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

References

1. **Shaw L, Sweeney MA, O'Neill SC, Jones CJ, Austin C, Taggart MJ.** Caveolae and sarcoplasmic reticular coupling in smooth muscle cells of pressurised arteries: the relevance for Ca^{2+} oscillations and tone. *Cardiovasc Res.* 2006; 69: 825–35.
2. **Lamont C, Weir G.** Different roles of ryanodine receptors and inositol (1,4,5)-triphosphate receptors in adrenergically stimulated contractions of small arteries. *Am J Physiol.* 2004; 287: H617–25.
3. **Zacharia J, Zhang J, Wier WG.** Ca^{2+} signalling in mouse mesenteric small arteries: myogenic tone and adrenergic vasoconstriction. *Am J Physiol Heart Circ Physiol.* 2007; 292: H1523–32.
4. **Nixon GF, Mignery GA, Somlyo AV.** Immunogold localization of inositol 1,4, 5-trisphosphate receptors and characterization of ultrastructural features of the sarcoplasmic reticulum in phasic and tonic smooth muscle. *J. Muscle Res Cell Motil.* 1994; 15: 682–700.
5. **Moore ED,Voigt T, Kobayashi YM, Isenberg G, Fay FS, Gallitelli MF, Franzini-Armstrong C.** Organization of Ca^{2+} release units in excitable smooth muscle of the guinea-pig urinary bladder. *Biophys J.* 2004; 87: 1836–47.
6. **Lee C-H, Kuo K-H, Dai J, Leo JM, Seow CY, Van Breeman, C.** Calyculin-A disrupts subplasmalemmal junction and recurring Ca^{2+} waves in vascular smooth muscle. *Cell Calcium.* 2004; 37: 9–16.
7. **Fameli N, Van Breemen C, Kuo K-H.** A quantitative model for linking Na^{+}/Ca^{2+} exchanger to SERCA during refilling of the sarcoplasmic reticulum to sustain $[Ca^{2+}]$ oscillations in vascular smooth muscle. *Cell Calcium.* 2007; 42: 565–75.
8. **Popescu LM, Gherghiceanu M, Mandache E, Cretoiu D.** Caveolae in smooth muscles: nanocontacts. *J Cell Mol Med.* 2006; 10: 960–90.
9. **Gherghiceanu M, Popescu LM.** Caveolar nanospaces in smooth muscle cells. *J Cell Mol Med.* 2006; 10: 519–28.
10. **Gherghiceanu M, Popescu LM.** Electron microscope tomography: further demonstration of nanocontacts between caveolae and smooth muscle sarcoplasmic reticulum. *J Cell Mol Med.* 2007; 11: 1416–8.
11. **Agronskaia AV, Valentijn JA, van Driel LF, Schneijdenberg CTWM, Humbel BM, van Bergen en Henegouwen PMP, Verkleij AJ, Koster AJ, Gerritsen HC.** Integrated fluorescence and transmission electron microscopy. *J Struc Biol.* 2008; 164: 183–9.