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Nanoscale three-dimensional imaging of the human myocyte

Matthew S. Sulkin [a,1], Fei Yang [b,1], Katherine M. Holzem [a], Brandon Van Leer [c], Cliff Bugge [c], Jacob I. Laughner [a], Karen Green [d], Igor R. Efimov [a,*]

[a] Department of Biomedical Engineering, Washington University in St. Louis, MO, USA
[b] Department of Radiation Oncology, School of Medicine, Washington University in St. Louis, MO, USA
[c] FEI Company, Hillsboro, OR, USA
[d] Department of Pathology and Immunology, School of Medicine, Washington University in St. Louis, MO, USA

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ABSTRACT

The ventricular human myocyte is spatially organized for optimal ATP and Ca^{2+} delivery to sarcomeric myosin and ionic pumps during every excitation–contraction cycle. Comprehension of three-dimensional geometry of the tightly packed ultrastructure has been derived from discontinuous two-dimensional images, but has never been precisely reconstructed or analyzed in human myocardium. Using a focused ion beam scanning electron microscope, we created nanoscale resolution serial images to quantify the three-dimensional ultrastructure of a human left ventricular myocyte. Transverse tubules (t-tubule), lipid droplets, A-bands, and mitochondria occupy 1.8, 1.9, 10.8, and 27.9% of the myocyte volume, respectively. The complex t-tubule system has a small tortuosity (1.04 ± 0.01), and is composed of long transverse segments with diameters of 317 ± 24 nm and short branches. Our data indicates that lipid droplets located well beneath the sarcolemma are proximal to t-tubules, where 59% (13 of 22) of lipid droplet centroids are within 0.50 μm of a t-tubule. This spatial association could have an important implication in the development and treatment of heart failure because it connects two independently known pathophysiological alterations, a substrate switch from fatty acids to glucose and t-tubular derangement.

1. Introduction

The ventricular human myocyte is uniquely optimized for hard labor without interruption for the entire lifespan of man. The densely packed ultrastructure consisting primarily of sarcomeres, mitochondria, transverse tubules (t-tubules), sarcoplasmic reticulum (SR), and lipid droplets is spatially organized to optimize ATP and Ca^{2+} delivery for contraction and relaxation. Mitochondria are proximal to both sarcomeres and SR Ca^{2+} pumps to reduce ATP diffusion length, while SR networks are adjacent to sarcomeres to accelerate delivery of Ca^{2+} to contractile proteins. T-tubules serve for three-dimensional spatiotemporal synchronization between electrical and calcium signaling, however, their role in metabolic synchronization in cardiac tissue is unknown.

Continuous progress has been made to improve the quality of electron microscope images of cardiac ultrastructure since the first attempts (Beams et al., 1949; Kisch, 1951; Kisch and Bardet, 1951; Kisch and Philpott, 1953; Kisch et al., 1948; Van Breemen, 1952, 1953), and recently three-dimensional visualization techniques have been utilized to further pursue structure–function relationships (Hayashi et al., 2009; Merchan-Perez et al., 2013; Yu et al., 2008). Quantitative analysis of cardiomyocyte ultrastructure has been extensively described in mouse (Herbener, 1976; Herbener et al., 1973; Kainulainen et al., 1979; Schaper et al., 1985; Tate and Herbener, 1976), rat (Craft-Cormney and Hansen, 1980; Guski et al., 1981; Laguens, 1971; Lund and Tomanek, 1978, 1980; Page et al., 1974; Reith and Fuchs, 1973; Schaper et al., 1985; Tomanek and Hovanec, 1981; Tomanek et al., 1979), and canine hearts (Becker et al., 1999; Gerdes and Kasten, 1980; Goldstein and Murphy, 1983; McCallister et al., 1978; Papadimitriou et al., 1974; Partin et al., 1972; Schaper et al., 1985), while fewer studies have investigated ultrastructure morphology of human cardiomyocytes (Fleischer et al., 1980; Laguens et al., 1979; Schaper et al., 1985).

Here, we utilize a dual-beam electron microscope for the first time in healthy human left ventricular (LV) tissue to create

Abbreviations: T-tubules, transverse tubules; SR, sarcoplasmic reticulum; LV, left ventricle; FIB, focused ion beam.

* Corresponding author. Address: Department of Biomedical Engineering, Washington University in Saint Louis, 390E Whitaker Hall, One Brookings Drive, St. Louis, MO 63130-4899, USA. Fax: +1 314 935 8377.
E-mail address: igor@wustl.edu (I.R. Efimov).

[1] These authors contributed equally to this work.

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high-resolution serial images at nanometer resolution for three-dimensional analysis. Our morphometric results, including ultrastructure volume and t-tubule geometry compare nicely to previous reports in mammalian ventricular myocytes. Further analyses indicate a novel finding—lipid droplets well beneath the sarcolemma are spatially located near t-tubules. The observed spatial association was compared against simulations that randomly placed lipid droplets within the volume and showed that the experimentally observed proximity could not be repeated.

2. Materials and methods

2.1. Tissue collection

The study was approved by the Washington University Institutional Review Board. A non-failing donor heart was provided by Mid-America Transplant Services (Saint Louis, MO). The donor was a 55 year old female with a history of hypertension and the cause of death was cerebral hypoxia. The heart was arrested with cardioplegic solution (110 NaCl, 1.2 $CaCl_2$, 16 KCl, 16 $MgCl_2$, 10 $NaHCO_3$ mmol/L) and explanted for experimentation. The donor heart was transported to the laboratory in cold (~4 °C) cardioplegic solution in 15 min. A transverse sample (~150 μm thick) of apical-posterior left ventricle was collected (Anchor, Soft Tissue Biopsy Device, Addison, IL) and both epicardial fat and papillary muscle were discarded.

2.2. Tissue fixation and embedding

Excised tissue was immediately immersion fixed in a modified Karnovsky's fixative (3% glutaraldehyde, 1% paraformaldehyde in 0.1 M sodium cacodylate buffer), post-fixed in cacodylate buffered

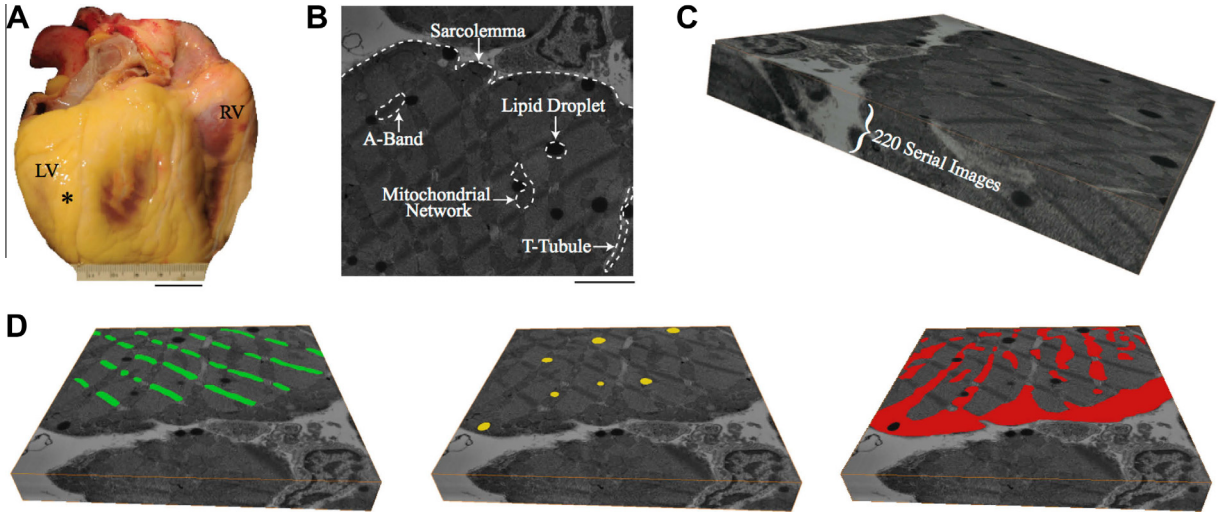

Fig.1. Ultrastructure segmentation. (A) Donated human heart with location of tissue sample marked by black asterisk. (B) Example two-dimensional scanning electron microscopy image with ultrastructure highlighted. (C) Serial section volume of 220 slices each 10 nm thick. (D) Example digital segmentation of A-bands (green), lipid droplets (yellow), and mitochondria (red) on first serial section. Scale Bars: 2 cm (A), 3 μm (B). Serial Section Volumes: 14.7 μm × 14.8 μm × 2.2 μm (C and D). RV: right ventricle; LV: left ventricle; T-tubule: transverse tubule. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

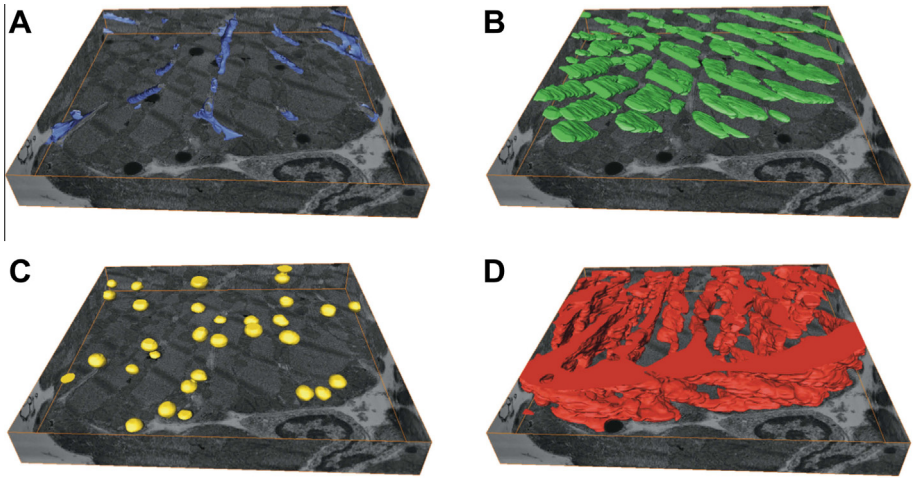

Fig.2. Three-dimensional reconstruction of ultrastructure with bottom serial section image and edges serving as a bounding box. (A) Transverse-tubules (purple). (B) A-band (green). (C) Lipid droplets (yellow). (D) Mitochondria (red). Serial Section Volumes: 14.7 μm × 14.8 μm × 2.2 μm (A–D). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)