SUBCELLULAR STRUCTURAL CHANGES IN DIABETIC CARDIOMYOPATHY AND ITS IMPACT ON CARDIAC CELL CALCIUM DYNAMICS

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Introduction

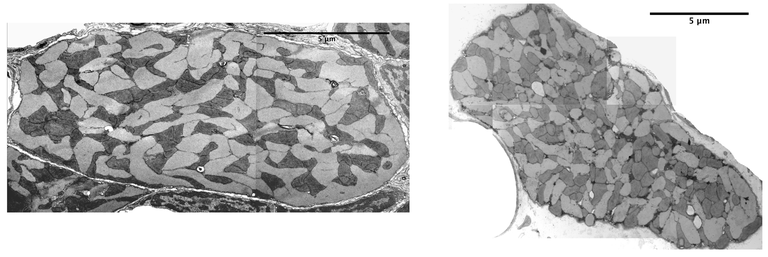
Diabetic cardiomyopathy is accompanied by reduced cardiac output and contractility and is known to exhibit alterations in both structure and function at the sub-cellular scale [1-3]. Previous studies have reported changes in quantity of calcium handling proteins, calcium handling kinetics as well as mitochondrial proteomics. However, limited attention has been given to contribution of sub-cellular structural alterations in cell function.

We present preliminary results of an analysis of the quantitative differences in structural organisation between healthy and diabetic cells and discuss its potential role in intracellular calcium dynamics.

Imaging Cardiac Cells in Health and Diabetes

Left ventricular mid-wall working myocytes from male adult wistar rats (200-250 g) were used in this study. Diabetic data were acquired from rats 9 weeks post injection with streptozotocin. Samples were prepared for electron microscopy similar to [4]. Thin sections of transversely oriented cells were acquired and imaged under a 120 kV FEI Tecnai transmission electron microscope.10 cells from the healthy tissue and 5 cells from the diabetic heart were randomly chosen.

Fig.1: Typical intracellular contents in (left) the healthy and (right) 9 week diabetic rat.



Visual inspection of the cell images consistently showed alterations in intracellular organisation in diabetes. Diabetic cell micrographs consistently showed the presenence of lipids and glycoproteins that were virtually absent in healthy cells.

Morphological Differences between Healthy and STZ-Induced Diabetics

Key differences from our study that were also reported in literature included:

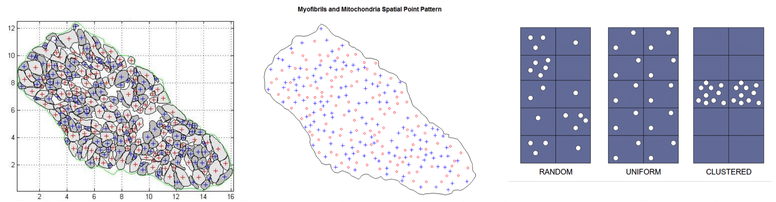
(1) Diabetic cells are roughly twice the size of healthy cells.

(2) Reduced area fraction and number per square micron of myofibrils in diabetes - potential contributor to reduced contractility.

(3) Increased diameter of mitochondria in diabetes, consistent with literature as well as reduced numbers per square micron.

Modeling Spatial Organisation of Organelles

The centroids of the organelles were calculated and described as spatial point patterns for further analysis (Fig. 2).



Fig, 2: The EM images are manually segmented (left) and reduced to centroid locations (middle) for spatial distribution analysis. Such analyses help measure spatial distributions as random, clustered or uniform in a quantitative manner (right).

A "multi-strauss hardcore" model was chosen to model the statistical variation in spatial relationships between the organelles. The "hardcore" represents a minimum distance between two points (e.g. between two organelle centroids) and the "strauss" refers to an extra repulsion to the hardcore that keep organelles apart. "Multi" simply refers to the existence of multiple "point types".

In fitting this model to data, specific parameters of interest were distances between pairs of myofibrils (Myo-Myo), pairs of mitochondria (Mito-Mito) and a mitochondrion and a myofibril (Myo-Mito). Comparison of fitted values of these parameters between the two groups are shown in Fig. 3.

Differences in Spatial Organisation

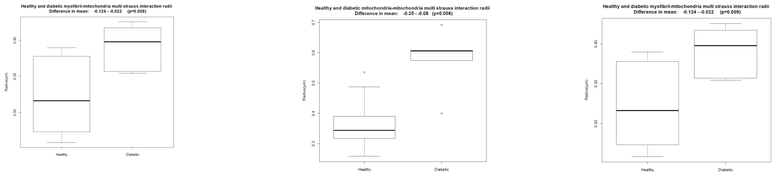


Fig. 3: Statistical comparison of fitted values of the three key parameters that measure spatial organisation - (left) myofibril to mitochondrion, (middle) mitochondrion to mitochondrion and (right) myofibril to myofibril distances.

Fig. 3 shows that distances between mitochondrial centroids increase in diabetic cells, consistent with increase in diameter of these organelles. Distances between a pair of myofibrils did not show significant differences between the two groups. However, distances between any pair of myofibril and mitochondrion is increased in diabetic cells. Thus, the reduced compactness of diabetic cell structure has been quantified.

The Strauss radii determine the area of organelle interaction beyond the physical boundaries of the organelles. We hypothesize that the Strauss radius is a reflection of the need for mitochondria and myofibrils to be juxtaposed due to energy requirements. Hence, integrating these observations in computational models can help determine the effects of these and other spatial parameters in cell function.

Integrating Structure and Function

We superimposed a statistical distribution of RyRs on the healthy and diabetic cells to acquire a realistic distribution of release sites for the particular cell geometries based on new techniques we have developed [5] to integrate LM with EM data.

Fig 4: Left: An EM tomogram segmented into myofibrils, mitochondria and sarcolemma. Middle: Confocal data set from [6] that was used to extract and simulate the statistical distribution of RyRs around z-discs. Right: Simulated RyR cluster pattern around myofibrils (red spheres), in the tomogram - orthographic views showing relationship between RyRs and z-disc and myofibrils.

Calcium release and diffusion from a select few of these simulated RyR clusters was modelled to visualise the spatio-temporal dynamics in the healthy and diabetic cells (Fig. 5). The diabetic cell simulation in Fig. 5 ignored the existence of inclusions and shows qualitatively similar characteristics to the release dynamics in the healthy cell.

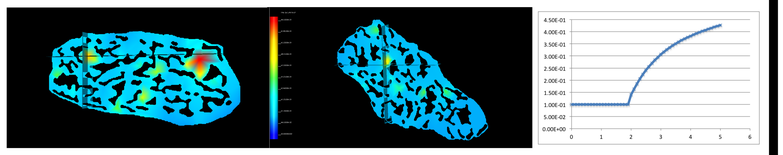


Fig. 5: Calcium release dynamics near selected RyR release sites. Left: Healthy; Middle: Diabetic without inclusions; Plot of time course of calcium release simulated; units in microM

Conclusions and Future Work

Quantitative differences in spatial organisation in cardiac cells in health and diabetes have been identified. A preliminary demonstration and analysis of the effects of excluding/including structural inclusions in diabetes has been conducted. A more thorough study is currently underway. These changes, and the reduced numbers of myofibrils and altered mitochondrial function may sum up to reduced contractility in diabetic cells. In future work we will quantify the critical factors using the presented integrated approach of structural and functional modeling.

References

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