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

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Introduction

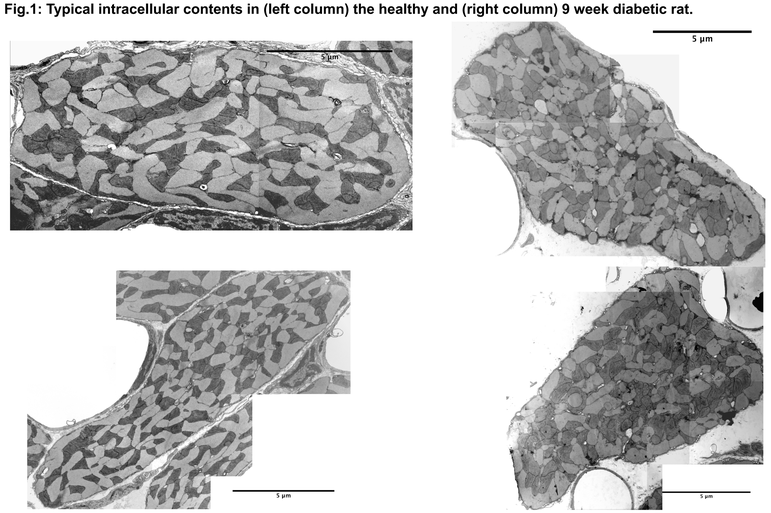
Diabetic cardiomyopathy is accompanied by reduced cardiac output and contractility and is known to exhibit alterations in both structure and function at the sub-cellular scale [1-3]. Previous studies have reported changes in quantity of calcium handling proteins, calcium handling as well as mitochondrial 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 the role this may play 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 column) the healthy and (right column) 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 virtually absent in healthy cells.

Morphological Differences in Structure in Healthy and 9-Week STZ-Induced Diabetics

Fig. 2 shows comparisons of key morphological parameters of mitochondria and myofibrils.

The average size of the diabetic cells (118 square micron) was twice that of the healthy cells (65 microns). Myofibril diameters remained consistent, thus a drop in density (no. per square micron) is expected. However, it is evident from the area fraction that there are indeed fewer contractile filaments in diabetic cells.

Mitochondrial density also decreases in disease, but area fraction remains similar due to increased size (from swelling) of these organelles, consistent with [2].

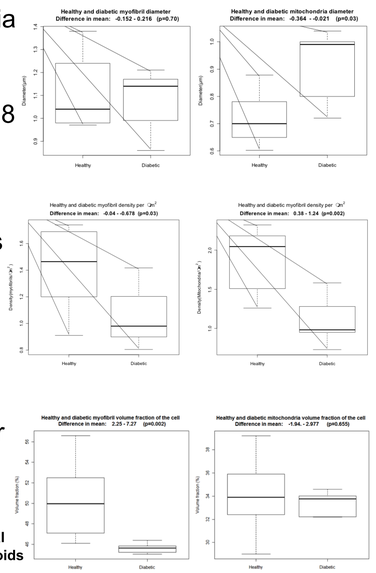
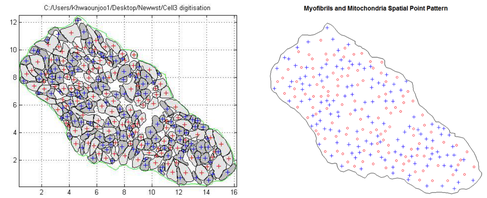


Fig 2: Statistical comparisons of myofibrillar (left) and mitochondrial (right) morphologies (organelle diameter, density - number of centroids per square micron, %area of whole cell

Modeling Spatial Organisation of Organelles

The centroids of the organelles were calculated and described as a spatial point patterns for further analysis (Fig. 4).



A "multi-strauss hardcore" model was chosen to describe the statistical variation in spatial characteristics between the organelles. The "hardcore" represents a hard limit on the minimum distance between two points (e.g. between two organelle centroids) and the "strauss" refers to a loo====--- ser constraint on distances between organelles. "Multi" simply refers to the existence of multiple "point types".

In fitting this model to the 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) were fitted to match the statistical variations in these distances observed in the healthy and diabetic groups. Comparison of these parameters in the two groups

Differences in Spatial Organisation

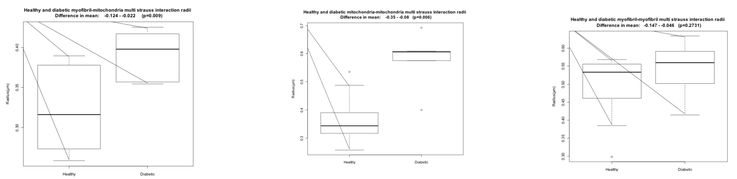


Fig. 4: Statistical comparison of fitted values of the three key parameters that measure spatial organisation - (Left) myofibril to mitochondrion, (Middle) mitochondrion to mitochondrion and (Right) myofibril to myofibril distances.

Fig. 4 shows that distances between mitochondria are increased 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.

Effects on Calcium Dynamics

We have developed methods to statistically fuse LM protein data to TEM data to generate integrated models of cell ultrastructure [5].

Conclusions and Future Work

We have developed methods to model 3D cardiac cell anatomy and function in a realistic fashion.

The next step is to incorporate Hinch model of calcium dynamics.

References

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