Towards Online Monitoring of Engineered Tissue Using Confocal Microscopy and Image Processing

Richard A Lasher, Frank B Sachse, Robert W Hitchcock
Department of Bioengineering and Cardiovascular Research & Training Institute, University of Utah, Salt Lake City, UT.

Statement of Purpose: Currently, evaluation of engineered tissue is limited to endpoint analyses, such as characterizing histology, gene expression and solutes [1]. Most of the applied analysis approaches are based on immunochemistry procedures, requiring excision, fixation and sectioning of the tissue as well as cell membrane perforation and labeling of proteins [2]. These analyses are time-consuming and do not allow for online monitoring. There is a need for online, high throughput monitoring techniques for evaluation of engineered tissue. In this work, we introduce an approach for microscopic imaging of living engineered tissue and its online monitoring. The approach is based on application of a non-toxic dye specific for the extracellular space and in vivo confocal microcopy. We hypothesized that the approach allows for online characterization of cell structure.

Methods: Our hypothesis was tested on living atrial and ventricular cardiac tissue excised from rabbits. Dextran-conjugated, lysine-fixable Texas Red with a molecular weight of 3 kDa and excitation/emission wavelengths of 595/615 nm (Molecular Probes, Eugene, OR) was applied locally to living tissue by placing 10 µL at a concentration of 12.5 mg/mL on the coverslip (Fig. 1). The tissue was placed onto the dye and the dye was allowed to diffuse into the tissue. The tissue was covered with approximately 1 mL of Tyrode's solution and the tissue was gently pressed down with a polycarbonate tube. Three-dimensional image stacks were acquired using a confocal microscope (BioRad MRC-1024, Hercules, CA).

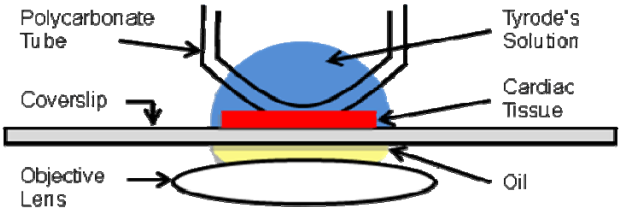

Figure 1. Experimental Setup.

We applied a framework for confocal image processing and analysis based on previously described methods [3,4]. Specifically, we focused on processing methods that allow for online analysis. The images were processed to remove background signal and correct for depth-dependent attenuation (Fig. 2).

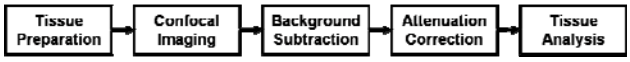

Figure 2. Experimental Methods.

Tissue analysis involved calculating volume fraction and average width of myocytes. Myocyte volume fraction was calculated by sampling regions of 300 x 300 x 25 voxels within the image stacks. Histograms of voxel intensity were plotted for each region and threshold values were chosen to distinguish intracellular space from extracellular space. Orientation of myocytes was determined by principal component analysis of the covariance matrix [5]. Average myocyte width was calculated using fast Fourier analysis [5] on lines perpendicular to the long axis of myocytes passing through the center of the image slice.

Results: We acquired 17 three-dimensional image stacks with dimensions of 1024 x 768 x 200 voxels and a spatial resolution of 200 x 200 x 200 nm (Fig. 3). We demonstrated that the dye readily diffused into the myocardium and was available in sufficient concentration for imaging and structural analysis.

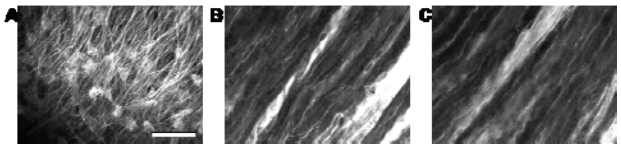

Figure 3. Raw image slices from ventricular tissue at epicardial surface *(A)* and moving into the myocardium 15 µm *(B)* and 30 µm *(C)*. Scale bar: 50 µm (A-C).

Five image stacks each of atrial and ventricular tissue were analyzed. Myocyte volume fraction was calculated by sampling at least 3 regions from each image stack. Histograms of voxel intensity indicated a normal distribution and threshold values were chosen to be the mode plus one standard deviation. Average myocyte width was calculated from multiple slices within each image stack. Myocyte width and volume fraction were larger for ventricular tissue than atrial tissue (Table I).

Table I. Quantitative Analysis (mean±stdev).

Tissue	Myocyte Width (µm)	Myocyte Volume Fraction
Atrial	12.4±2.2	75.0±4.4%
Ventricular	19.7±3.2	76.5±4.6%

Conclusions: Here we present an approach for quantitative online analysis of living tissue and cell morphology. The analysis yields data that are similar to published values for rabbit atrial and ventricular myocytes. We suggest that the approach will allow for online and high throughput monitoring of engineered tissue. In the future, we will apply this approach with dyes for functional labeling to study cardiac wave conduction and excitation-contraction coupling in cardiac tissue engineered constructs. We suggest that the method can be combined with catheter-based confocal imaging systems. This combination will allow for online analysis of tissue in standard in vivo bioreactors [6].

References:

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