Project Report MD-984

Project Title: Arrhythmic substrate visualization to study the structural remodelling underlying atrial fibrillation progression from in-vivo models and human donors for computer simulations

Main Authors: Jazmín Aguado-Sierra, Constantine Butakoff, José Jalife, José Manuel Alfonso Almazán.

Summary:
The aim of this proposal is to characterise and generate a 3-dimensional model of the substrate that creates and maintains Atrial Fibrillation (AF) using tissue samples from human donor hearts. The tissue images will be segmented into a finite element model to create detailed multi-scale computational simulations of the arrhythmic tissue. The main objective of the project is to characterise the dynamics of wave propagation, structural and functional changes that enable AF to persist at different progression stages, by putting together computational, experimental and clinical tools for analysis and interpretation. The project has a multidisciplinary, multicenter and translational design that enable us both to generate both, an in vivo pig model, based on rapid atrial pacing that resembles clinical progression of AF, and to study isolated Langendorff-perfused human hearts from donors to correlate the propagation dynamics identified in the animal model with those present in the human heart.

Preliminary Results:

In February 2017 we obtained 48 hours of synchrotron time at the ID16A beamline (from February 4 at 8.00 am until 6 February at 8.00 am). For these experiments we had 25 vitrified samples (frozen at high pressure in liquid nitrogen): 10 control samples, 5 human samples, 5 animal protocol samples in the reentry zone and 5 other animal protocol samples in a remote area. Vitrification was carried out at the National Biotechnology Center of the Autonomous University of Madrid. The sample generation protocol consisted of:

1. Fixation of the tissue so that it is free of pathogens or toxic substances with paraformaldehyde and glutaraldehyde for at least 8 hours.
2. The tissue is cut using a manual slicing machine. The cut tissue was then rinsed thoroughly on a solution of phosphate buffered saline (PBS). The tissue was soaked in BSA (Bovine Serum Albumin) right before vitrification as a cryo-protectant.
3. The cut tissue was then placed in the leica carriers for samples of size 0.4 mm thick by 1.2 mm in diameter to be frozen in liquid nitrogen under high pressure in a Leica EM PACT2 equipment at the National Biotechnology Center in Madrid. (See picture 1)
4. Once vitrified, they were stored in liquid nitrogen for shipment to Grenoble. (See image 2)
5. A specialised company was hired to transport the samples at liquid nitrogen temperature (-196º Celsius) However, the shipping company completely failed and all samples were completely destroyed. They arrived at room temperature at the ESRF.
6. The only option was to transfer fresh tissue from Madrid, on plane, to repeat the procedure in Grenoble the afternoon before the start of the beam time.
7. Vitrification of 8 samples was achieved at the Grenoble Institut des Neurosciences with help of staff of the beamline ID16A.
8. By the start of this step, our beam time had just started. The next challenge was to remove the tissue from the carrier. The BSA created a sticky environment, so extracting the tissue from the carrier was not trivial. We tried pinching the tissue out, cutting the carrier, and in the end, in order to remove the tissue from the carrier, the carrier had to be briefly taken out of the liquid

nitrogen so that the surrounding tissue would defrost slightly, allowing us to scoop out the sample.

9. The tissue was then placed in a pin that would hold it steadily inside the chamber of the beam. (See Figures 3 and 4). We achieved the placement of the first sample at 10 pm of the 4th of February. By this time, only 3 more samples remained viable from the 8 we managed to freeze.

Image 1.

Image 2.

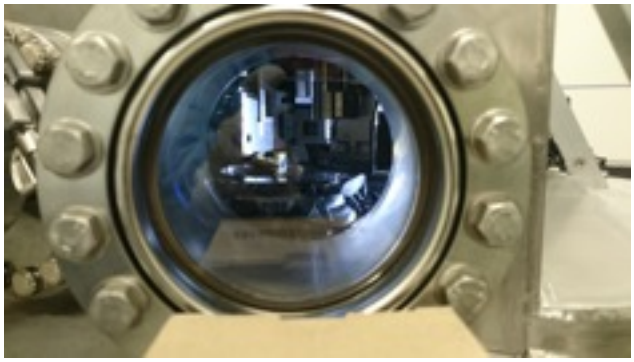

Image 3.

Image 4.

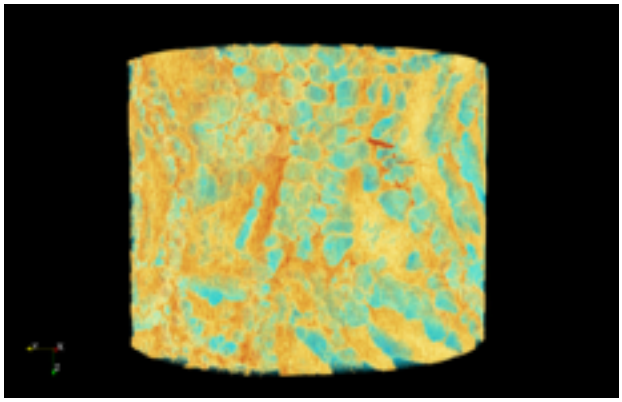

Image 5.

Image 6.

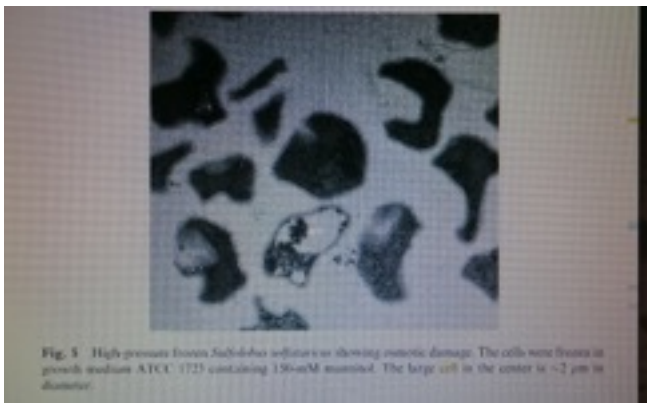

Image 7.

10. We managed to scoop out the 3 remaining samples to be then put in a pin for imaging next day. On the next day we put in a human sample. We imaged for the day, and while putting in the third sample in the evening of the 5th of February, the transferring system failed and it was too late to try to fix it and put in a new sample.
11. One of the reconstructed volumes can be seen in Figure 5. Figure 5 shows the sample of the experimental atrial fibrillation tissue. The resolution achieved was 50nm. There are a couple of features that we should observe: The tissue looks disrupted, but no crystals were observed. In the bibliography, we observed similar situations where osmotic damage occurred (Methods in Celular Biology. Volume 79. Cellular Electron Microscopy). We found that cryo-protectants like ficoll or dextran can minimise the disruption of the tissue. Pre-segmentation of the volumes showed no distinctive myocyte cell membrane. See image 8.

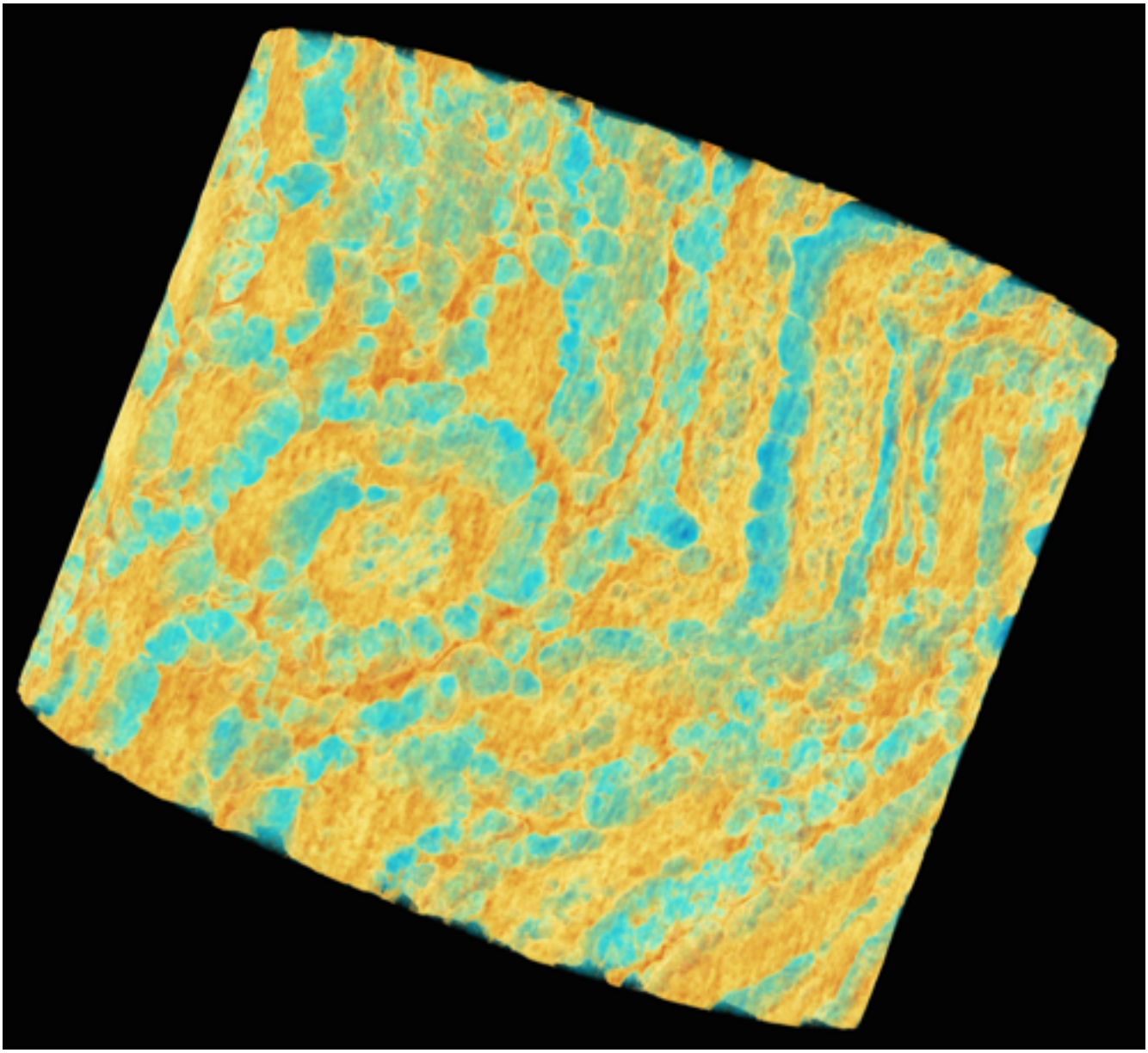

Image 8.

The obtained results have verified that the methodology that we need to follow for this project only requires a couple of improvements:

A. Try different cryo-protective substances (Ficoll or Dextran)

B. Acquire the Leica carrier cutter to extract the sample without thawing it.
C. Process the samples with the cryo-substitution method to embed the samples in resin to avoid transport in liquid nitrogen. The samples obtained would be similar to the one shown in figure 6.

We are working in fixing the problems faced during our experiments.

Expected Results

The expected result is to visualize, segment, characterize and quantify the structural remodeling of intact transmural atrial tissue in different stages of atrial fibrillation in various regions of interest. Importance: Three-dimensional quantitative evaluation of tissue structure, including the number and distribution of myofibroblasts, myocytes, collagen, blood vessels and gap junctions, will provide evidence of structural, regional and heterogeneous remodeling that will resolve some of the mechanisms of disease progression. FA in the animal model, which will also be compared with the clinical case for the validation of the experimental scenario.

Removal of tissue structure to create detailed models of finite tissue elements in health and at various stages of AF is a unique opportunity. A supercomputer center in close collaboration with a world-renowned cardiovascular research institute can process high resolution images and perform the most detailed tissue simulations of a highly prevalent disease such as AF. The impact for basic science and clinical translation of computational modeling is unprecedented. Attempts to model fibrosis have never taken into account the actual tissue structure.

The final objective of the project is to use computer models to evaluate, diagnose and treat patients with AF in a personalized way to avoid recurrence of AF after ablation and to predict outcomes in the daily clinic. The only way to generate reliable models is to understand and perform a complete characterization of the multi-scale nature of the problem.

Synchrotron measurements of atrial tissue under FA within this project will provide the most complete study, from tissue structure to the manifestation of the entire organ of disease and its progression in a large animal model, the closest to the human phenotype of AF.