SEM investigation of heart tissue samples

R Saunders and M Amoroso
Physics Department, University of the West Indies, St. Augustine, Trinidad and Tobago, West Indies.

Abstract. We used the scanning electron microscope to examine the cardiac tissue of a cow (Bos taurus), a pig (Sus scrofa), and a human (Homo sapiens). 1mm$^3$ blocks of left ventricular tissue were prepared for SEM scanning by fixing in 96% ethanol followed by critical point drying (cryofixation), then sputter-coating with gold. The typical ridged structure of the myofibrils was observed for all the species. In addition crystal like structures were found in one of the samples of the heart tissue of the pig. These structures were investigated further using an EDVAC x-ray analysis attachment to the SEM. Elemental x-ray analysis showed highest peaks occurred for gold, followed by carbon, oxygen, magnesium and potassium. As the samples were coated with gold for conductivity, this highest peak is expected. Much lower peaks at carbon, oxygen, magnesium and potassium suggest that a crystallized salt such as a carbonate was present in the tissue before sacrifice.

1. Introduction
The human heart generates electrical currents produced by the depolarisation-repolarisation processes of its muscle cells. These currents produce an associated time-varying magnetic field that can be measured and recorded non-invasively as the magnetocardiogram (MCG)[1]. Because of developments in the measurement of small magnetic fields the magnetocardiogram can be a very important diagnostic modality in medicine.

Cardiac muscle cells or myocytes show a single nucleus, have a length of approximately 100 µm and are approximately 25µm in diameter. The cardiac cells form a functional syncitium or branched network of individual myocytes connected through intercalated discs. The contraction of muscle fibres is normally explained by the sliding filament theory [2]. The close correlation between anatomical structure and physiological function may make it useful to address the Scanning Electron Microscopy (SEM) of the ventricular muscle of the mammalian heart, to better understand how the functions of the structures translate into the cardiac events and possible dysfunctions we may encounter in magnetocardiography. We therefore examined the cardiac muscle fibres of the cow (Bos Taurus), pig (Sus scrofa) and human (Homo sapiens).

2. Method
At least 3 samples of heart tissue were prepared and scanned from each species, using approximately 1 mm$^3$ blocks of tissue, from left ventricular heart muscle. For the cow and pig tissues, a larger tissue block was excised from the organ on site when the animal was slaughtered and immediately placed into a sterile specimen jar containing 96% ethanol. In the case of the human tissue, a block of tissue was excised from the organ by a pathologist before placing into 96% ethanol. Smaller 1 mm$^3$ blocks of tissue were cut from these larger blocks with a scalpel. The tissue blocks were then fixed in 96% ethanol followed by critical point drying (cryofixation) using a Tousimis Samdri 795 critical point dryer. They were placed on a
stub and viewed with a light microscope to check the orientation and position of the tissue blocks, sputter-coated with gold using a Denton Vacuum Desk II sputter-coating machine, then examined by electron microscope. Digital photos were taken at increasing magnifications. The samples were stored on stubs in a dark, dry cabinet at 26 ºC.

Fig.1: Human heart tissue 2400x  
Scalebar=5µm

Fig.2: Cow’s heart tissue 1200x  
Scalebar=10µm

Fig.3: Pig’s heart tissue 4580x  
Scalebar=2µm

Fig.4: Pig’s heart tissue 573x  
Scalebar=20µm

Fig.5: Pig’s heart tissue 2300x.  
Enlargement of area indicated in Fig. 4: A - elongated triangular prisms; B - irregular prisms and C – granules. Scalebar=5µm

Fig.6: Pig’s heart tissue 9150x  
Enlargement of the area indicated in Fig. 4 showing granules. Scalebar=1µm
3. Results

In each of the SEM images shown in the Fig.s 1 to 6, the scale bar (calibrated in µm) is superimposed on the lower left of the digital image for reference.

In the images of the tissues (Figures 1, 2 and 3), the expected ridged structure of the fibre bundles could clearly be seen; it was possible for structures of 0.5 micrometers to be differentiated visually in the images up to 19200x magnification. At greater magnifications it was difficult to further differentiate features of the specimen surface. In addition to this basic structure, small crystalline structures were found in the heart tissue of the pig (Figures 4, 5 and 6). These have been labelled as A (elongated triangular prisms), B (irregular prisms) and C (granules) in Figure 5. These structures were subsequently micro analysed using the EDVAC Genesis V5.2 x-ray analysis probe at two sites and spectral patterns were generated for each session of analysis. The following spectra (Figures 6 and 7) are representative of those obtained.

In all of the spectral patterns obtained the highest peak always occurred for Au (gold) at 2.00 and 9.6 keV ±0.4 keV. For the oblong solid (as indicated in Figure 5) C and O were the next highest peaks, where C occurred around 0.3keV and O occurred around 0.5keV. Peaks for K and Mg were next highest after that, K occurred at 3.5keV, Mg occurred at 1.2keV. For the rod-like structures (as indicated in Figure 5) C and K were the next highest peaks after Au. The third highest peaks appeared for Na and O, and the fourth highest peak occurred for N.

![Fig. 6: Spectrum obtained with microanalysis on oblong object](Image)

![Fig. 7: Spectrum obtained with microanalysis on rod-like object](Image)

| Table 1. Visual analysis: average dimensions of the solids |
|----------------------------------------------------------|
| Irregular prisms                                         | 7 x 1.75 x 2.75 µm ±0.125 µm                      |
| Elongated triangular prisms                              | 8.25 µm ±0.125 µm (length), 0.8±0.125 µm (side of face) |
| Granules                                                 | 0.66 - 0.45 µm ±0.125 µm diameter                  |

Figure 3b was visually similar in structure and relative dimensions to SEM images depicting collagen-associated calcific deposits in degenerative porcine bioprostheses [2].

4. Discussion

The ridged strands observed in SEM images of cardiac tissue are bundles of myofilaments [3]. The electron microscope examination confirms that the structure of ventricular cardiac tissue of the three large mammals studied is similar in appearance (at magnifications between 70x and 2400x). The presence of crystalline structures in the heart tissue of the pig (Figures 4 and 5) is of interest since it is possible that such structures may be present in the heart tissue of other mammals. This is atypical of healthy mammal heart tissue; during contraction of
cardiac myofibrils actin filaments slide inwards between myosin filaments[3] and the presence of crystals in cardiac tissue may impede motion and possibly sever the sliding fibres with resulting pain and loss of function. In the case of this particular sample of pig’s heart tissue, the star-shaped structure consists of regularly-shaped solids clustered in what appeared to be a break in a strand of cardiac tissue.

From microanalysis of solids A and B in Figure 5, in all the spectral patterns showed two dominant gold peaks. This was expected as the SEM samples were coated with gold for conductivity. The elemental analysis does not immediately identify the origin of the structure, but is consistent with the presence of crystallized salts. Pathological cardiovascular deposits tend to incorporate sodium, magnesium and carbonate, and are both morphologically and chemically heterogeneous [5].

The difficulty in placing any sort of marker to locate the original sites may have led to analysis of sites that were visually similar to the original sites but may actually have had different chemical compositions; isomorphic crystals are however likely to have the same elemental composition. Identification of the structure by visual comparison was inconclusive due to constraints on the accuracy of the image comparison as well as constraints on the available data used in the comparison. Measuring the approximate dimensions of the structures enabled us to rule out structures having similar shapes but differing in size.

5. Conclusion
The myofibrils in human, porcine and bovine cardiac tissue exhibit a similar ridged structure for cryofixed tissue scanned in the electron microscope. The presence of crystal-like objects found in one particular sample of pig’s heart tissue may be due to a pathological state of the animal before sacrifice. As the samples scanned in this study were not classified into groups corresponding to the state of health of the source, it would be interesting to further compare the appearance of tissue from sources with known diseases. The presence of such structures in the heart muscles will affect contraction and hence the associated magnetic field in the magnetocardiogram.

6. Acknowledgements
The authors wish to gratefully acknowledge Mr. David Hinds of the SEM laboratory, EWMSC for his expertise and assistance throughout this project.

7. References

1. G Baule and R McFee, 1963. Detection of the Magnetic Field of the Heart. American Heart Journal 66, 95-6.
2. Y S Lee, 1993. Morphogenesis of Calcification in Porcine Bioprosthesis: Insight from High Resolution Electron Microscopic investigation at Molecular and Atomic Resolution. Journal of Electron Microscopy 42, 156-165.
3. R E Klabunde, 2005. Cardiovascular Physiology Concepts. Lippincott Williams & Wilkins, 42-6.
4. D M K Cooper, 2003. Clinical Xenotransplantation – How close are we? The Lancet 362, 557-59.
5. B B Tomazic, L C Chow, C M Carey, A J Shapiro. 1997. An in Vitro Diffusion Model for the Study of Calcification of Bovine Pericardium Tissue. Journal of Pharmaceutical Sciences 86, No. 12, 1433 – 1438.
6. B B Tomazic, 2001. Physicochemical Principles of Cardiovascular Calcification. Zeitschrift fur Kardiologie 90: suppl 3, III68 – III80.