ESEM–EDX Investigation of the Cross-sectional Microstructure and Elemental Chemical Composition of a Giant Human Cardiac Calculus

Hsiao-Huang Chang¹, Ching-Li Cheng², Pei-Jung Huang¹ and Shan-Yang Lin*³

¹Division of Cardiovascular Surgery, Department of Surgery, Taipei Veterans General Hospital, Taipei, ROC
²Department of Nursing, National Tainan Institute of Nursing, Tainan, Taiwan, ROC
³Department of Biotechnology and Pharmaceutical Technology, Yuanpei University, Hsin Chu, Taiwan, ROC

We used environmental scanning electron microscopy (ESEM) and energy-dispersive X-ray (EDX) microanalysis to study the microstructure topography and elemental composition of a cross section of a giant human cardiac calculus after cutting it in half. The giant cardiac calculus was isolated from an 80-year-old male patient after invasive open heart surgery. After treatment with formalin, the yellow-brown fist-shaped calcareous wet mass, 50 mm × 45 mm × 30 mm in size, became a brown-yellow hard calculus and was surrounded by a thick white tissue layer. The dissection of the whole cardiac calculus into two halves clearly revealed a dense layer on the encrusted surface and a bread-like texture of the internal structure of the calculus. The cross-sectional ESEM micrograph of the cardiac calculus illustrated that different agglomerates were deposited on the uneven petrous surface. The EDX spectra and elemental chemical mapping revealed that C and O were the major elements on the surface of the different irregular, near-spherical agglomerates with trace amounts of N, Na, P, and Ca, whereas the petrous surface showed higher amounts of C, O, P, and Ca, as well as trace amounts of Na. These elemental compositions were consistent with the surface microstructure analysis results, which showed that the agglomerates were mainly composed of cholesterol, and the petrous surface was mainly composed of calcium hydroxyapatite, as confirmed by our previous Raman spectroscopic detection. This study indicates that ESEM–EDX microanalysis may directly provide useful information about microstructural characteristics and the spatial distribution of elements on a cross-sectional surface of a halved cardiac calculus.

Keywords: cardiac calculus; ESEM; EDX; microanalysis; morphology; element; mapping; cholesterol; hydroxyapatite

1. Introduction

Scanning electron microscopy (SEM) is a well-known, powerful imaging research tool and has superior performance for visualizing the surface topography and composition of a sample. As conventional SEM requires samples to be imaged under high-vacuum conditions, one of the limitations of SEM is that it cannot be applied to wet materials and biological samples. To examine the natural state of wet samples via imaging, Danilatos first introduced the environmental scanning electron microscope (ESEM), which is capable of imaging non-conductive materials in their natural state under low-vacuum conditions [1-3]. ESEM allows the direct examination of hydrated or dried specimens without a conductive metallic coating. The application of ESEM could have advantages in the study of biological and biomedical specimens due to a reduction in sample preparation time [4-6]. Donald has introduced and discussed the ESEM technique and its key advantages [7].

Energy-dispersive X-ray (EDX) spectroscopy is an analytical technique used for elemental analysis or chemical characterization of a sample. It is also used for chemical composition microanalysis in conjunction with advanced SEM to provide high-resolution imaging, semi-quantitative elemental analysis, and qualitative X-ray elemental maps for investigating the architecture and composition of different samples [8-9]. Over the past decade, ESEM equipped with EDX has been extensively used to rapidly and accurately examine the morphology and elemental chemical composition of wet materials [10-12]. ESEM–EDX has several advantages over other sample analysis methods, including (a) direct measurement of the concentration of major elements (>0.1 wt.%) in samples in situ; (b) measurement of any available cations and anions above the atomic weight of Be; (c) determination of opaque minerals; and (d) rapid sample change over and the fast acquisition of analytical spectra [13]. ESEM–EDX provides a potentially useful complement to ESEM and not only gives a useful microscopic picture of the sample surface but also combines this information with an overview of the elemental composition of the sample surface.

Calcification in biological systems is a complex pathological phenomenon in which insoluble compounds or salts are deposited in different soft tissues [14-15]. These insoluble salt deposits gradually accumulate and aggregate into clusters, which cause a loss of flexibility and pliability in soft tissues, resulting in sclerosis, thickening, and/or deterioration [16-17]. Abnormal or inappropriate biomineralization in soft tissues is a vital structural or functional factor that causes many chronic diseases due to inflammation caused by mineral crystals [18-19]. In fact, almost all the tissues, including the brain, eye, teeth, salivary gland, heart, breast, liver, gall bladder, vessels, urinary tract, skin, muscle, joints, and other parts of the body, are easily calcified [14-15]. It has been reported that the prevalence and incidence of tissue calcification have been increasing worldwide in recent years [20-21]. Lithiasis is a very important medical issue because many adult populations suffer from different stone diseases that may be asymptomatic or symptomatic [20-22]. Cardiovascular calcification commonly occurs in the aortic valve of the heart or in the coronary arteries [23-25]. However, there is rare evidence for calculus formation in the human cardiac...
Recently, we reported a giant cardiac calculus that was isolated from an 80-year-old male patient after invasive open heart surgery. This calculus had occupied the left atrium of his heart and had possibly been present for a long duration. Analysis with a portable fibre-optic Raman analyzer revealed that localized deposits of cholesterol, calcium hydroxyapatite, and protein were heterogeneously distributed on the surface of the whole cardiac calculus and the cross-sectional surface of the halved cardiac calculus [26-27]. In this study, the ultrastructural topography and elemental composition of the cross-sectional surface of the halved cardiac calculus were investigated by ESEM–EDX microanalysis to thoroughly visualize and examine its microstructural features.

2. Materials and Methods

2.1 Cardiac calculus isolated from a patient

A giant cardiac calculus was isolated from an 80-year-old male patient, suffering from hypertension for more than 30 years, after invasive open heart surgery [26-27]. Informed consent for specimen analysis was obtained from the patient. The study was also approved by the Institutional Review Board at the Taipei Veterans General Hospital, and all procedures adhered to the guidelines in the Declaration of Helsinki. The formalized and dried cardiac calculus was cut into two halves and sent for further examination. The patient recovered smoothly after the operation and was discharged a week later.

2.2 ESEM–EDX microanalysis

The cross-sectional imaging of the microstructure topography of the halved cardiac calculus was performed using an environmental scanning electron microscope (FEI Quanta 250 FEG, Hillsboro, OR, USA) operating in low-vacuum mode. The microscope was operated at 5 kV using a secondary electron detector. Furthermore, the microscope equipped with an EDX (XFlash 5010, Bruker AXS Microanalysis, Berlin, Germany) operating in low-vacuum mode at 21°C and 55–60% relative humidity was also used to analyze surface topography images and elemental localization on the cross section of the halved cardiac calculus. EDX can generate images known as digital element distribution maps, which are compositional maps of the sample. The EDX maps were recorded using a silicon drift detector, which indicated the location of each element analyzed. The brightness in the maps is related to the intensity of the pixels of the elements in the sample. EDX can identify element concentrations of <0.1%. The EDX spectra of two selected locations on the cross section of the halved cardiac calculus were analyzed in more detail. The distribution mapping of the elements C and Ca was also performed.

3. Results

3.1 Morphological features

This giant cardiac calculus was isolated after invasive open heart surgery. A hard, heavy, yellow-brown, fist-shaped mass measuring 50 mm × 45 mm × 30 mm, with focal calcification and surrounded by fibrinous material over the surface, was obtained. However, this yellow-brown, calcified wet solid mass became a brown-yellow hard calculus covered by a thick white tissue layer after treatment with formalin (Fig. 1A). When the whole cardiac calculus was cut into two halves, the cross section clearly revealed a dense layer on the encrusted surface and a bread-like texture of the internal structure (Fig. 1B).
3.2 ESEM imaging observations

The surface topography of the cross section of the halved calculus assessed by ESEM is shown in Fig. 2A. It clearly shows the observed topographic shape and surface roughness of the calculus cross section. After the magnification of the ESEM image, different irregular, near-spherical agglomerates (Fig. 2B) and an uneven petrous surface structure were clearly seen (Fig. 2C). These irregular, near-spherical agglomerates were mainly composed of cholesterol, and the petrous surface was mainly composed of calcium hydroxyapatite, as confirmed by our previous Raman spectroscopic detection [28].
3.3 ESEM–EDX investigations

Figure 3A and B again shows the surface topography of the cross section of the halved calculus determined by ESEM. The element distribution maps of the elements C and Ca and the EDX spectra of the two selected locations are shown. From the ESEM image, the numerous irregular, near-spherical agglomerates that appear on the petrous surface of the halved cardiac calculus can clearly be observed. The superimposed images of the ESEM image and the X-ray dot distribution map of the elements C and Ca are shown in Figs. 3C and D. The whole cross section of the halved calculus was almost completely covered by the element C (shown in green), suggesting that the sample was composed of organic compounds (Fig. 3C). However, the distribution map of the element Ca (shown in red) was markedly lacking in the irregular, near-spherical agglomerates, indicating a lower concentration of Ca in this location (Fig. 3D). The two selected locations (*) for the irregular, near-spherical agglomerate location and ** for the petrous surface location) were examined by EDX microanalysis; the two locations contained different amounts of elements. The irregular, near-spherical agglomerate location contained significant amounts of the elements C and O with small amounts of N, P, and Ca. In contrast, higher amounts of Ca and P were markedly located on the petrous surface. These findings were consistent with our previous Raman spectral study [28], in which the irregular, near-spherical agglomerates mainly contained cholesterol and trace amounts of calcium hydroxyapatite, while the petrous surface predominantly consisted of calcium hydroxyapatite. The C, O, and N peaks indicated cholesterol and protein concentrations.

To further confirm the elemental distribution on the cross section of the cardiac calculus, another area of this halved calculus was chosen and magnified to evaluate content reproducibility. Figure 4 shows the ESEM microphotographs of the cholesterol- and calcium hydroxyapatite-rich areas, as well as the corresponding EDX spectra. The irregular, near-spherical agglomerates and uneven surface of the petrous portion were observed again. The EDX analysis of both areas revealed a greater concentration of C, a minor concentration of O, and trace amounts of N, Na, P, and Ca in the cholesterol-rich area. In contrast, the calcium hydroxyapatite-rich area exhibited higher amounts of the elements C, O, P, and Ca, as well as trace amounts of N and Na. This strongly suggests that ESEM–EDX is a useful technique for the major element chemical analysis of bio-samples.

4. Discussion

Pathological calcification is the abnormal tissue deposition of calcium salts, together with smaller amounts of different elements and other mineral salts in a wide variety of disease states, particularly in aging populations [14-17, 29]. In the
present study, the topography microstructure and elemental distribution on a cross section of a halved cardiac calculus were clearly determined by ESEM–EDX analysis.

ESEM is an attractive innovation and a fundamental advance in the field of electron microscopy. It has been proven to be an effective tool for non-metallic materials, because coating samples with carbon or gold is unnecessary. In particular, it allows the direct imaging of various hydrated biological samples in their natural state, which is not possible by conventional SEM [3-7]. ESEM combined with EDX is useful for the determination of the morphology of a material and the occurrence and distribution of compositional elements within it. The ESEM-EDX system is capable of providing fast and accurate results on the surface topographic features and elemental chemical composition of different samples, which provides a potentially useful complementary analysis for conventional electron microscopy procedures [8-12]. ESEM equipped with or without EDX may play a paramount role in ultrastructural investigations; it also permits the imaging of the overall appearance and/or specific features of biosamples formed in different environments.

Calcification occurs as a result of calcium build-up in body tissues. Over time, plaque can harden or disrupt normal bodily processes. Although cardiovascular calcification is well known, it is rare in the cardiac cavity. In these rare cases, calcium salts may be extracellularly deposited or precipitated within the cardiac tissue from the circulation or interstitial fluid, gradually enlarging within the atrium. However, the real origin and formation mechanism of this type of calculus in the heart are still unclear. Because of the localization of calcium deposits in the heart, these cases may be also classified as visceral calcification, but this dystrophic deposit is rarely detectable during a person’s lifetime [30]. In this study, the patient from which the cardiac calculus was isolated had hypercholesterolemia for a long time. Thus, we hypothesize that hypercholesterolemia might be associated with a higher incidence of cardiac calculus formation, as numerous studies have documented a correlation between lipids and calcification [31-32].

5. Conclusions

A giant, fist-shaped cardiac calculus was isolated from an older male patient after invasive open heart surgery. ESEM–EDX microanalysis was successfully used to examine the surface topographic features and elemental chemical composition of the cross section of the giant cardiac calculus. Numerous irregular, near-spherical agglomerates that appeared on the petrous surface of the halved cardiac calculus were clearly observed by ESEM. In addition, ESEM–EDX analysis showed that these agglomerates contained major amounts of the elements C and O, with trace amounts of N, P, and Ca, and that higher amounts of the elements C, O, Ca, and P were markedly present on the petrous surface.

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References