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Research Article

Calcium mineralization analysis in human aortic valve using SEM, XRD and EDX

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Abstract

Calcific aortic stenosis is a slowly progressing disease with a large accumulation of calcium minerals. Several studies have been reported in the literature to investigate structural defects in the aort valve. However, to date, little is known about the morphological structure of calcification formation in the tissue of the aortic valve. In this study, morphology and structural analysis of calcification in the aorta valve were performed. In addition, the effects of other artifacts were investigated. Scanning electron microscopy (SEM) was used to determine the morphology of calcification structures deposited in aortic valve tissue. Energy Dispersive X-ray Analysis (EDX) and X-ray Diffractometer (XRD) were used to characterize the morphological structures observed by SEM. The results revealed that trace elements play a role in nucleation for the formation of calcification.

Keywords: Aortic valve, Calcification, EDX, SEM, XRD

SEM, XRD ve EDX yöntemleriyle insan aort kapaklarında kalsiyum mineralizasyon analizi

Özet

Kalsifik aort darlığı, kalsiyum mineral birikimi ile yavaş ilerleyen bir hastalıktır. Literatürde aort kapağındaki yapısal kusurları araştırmak için çeşitli çalışmalar bildirilmiştir. Bununla birlikte, bugüne kadar, aort kapağının dokusundaki kireçlenme oluşumunun morfolojik yapısı hakkında çok az şey bilinmektedir. Bu çalışmada, aort kapağında morfoloji ve kalsifikasyon yapısal analizi yapılmıştır. Aort kapak dokusunda biriken kalsifikasyon yapılarının morfolojisini belirlemek için taramalı elektron mikroskopisi (SEM) kullanılmıştır. SEM tarafından gözlemlenen morfolojik yapıları karakterize etmek için Enerji Dağılımlı X-ışını Analizi (EDX) ve X-ışını Difraktometresi (XRD) kullanılmıştır. Sonuçlar, eser elementlerin kalsifikasyon oluşum sırasında çekirdeklenmede rol oynayabileceği ortaya koyulmuştur.

Anahtar Kelimeler: Aort kapağı, Kalsinasyon, EDX, SEM, XRD

1. Introduction

Scanning electron microscopy (SEM) is a well-known, powerful surface morphology imaging tool. SEM can easily identify structures on a surface in a large range from nanostructures to macrostructures. It is possible to obtain information about the formation mechanisms of images obtained by image analysis techniques. Especially in order to investigate the morphological structures of biological samples, significant advances have been made in SEM technology in recent years. It is quite difficult

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for SEM devices that require vacuum to view and analyze the natural states of biological samples. Environmental SEM devices developed for this purpose are preferred (Donald, 2003; Stokes, 2008; Jiao, 2019). It is also possible to obtain SEM images by drying biological samples (Delogne et al., 2007; Shimshoni and Sagi, 2019; Schatten, 2011; Chang et al., 2014).

Energy dispersive X-ray (EDX) spectroscopy is an analytical technique used to obtain elemental analysis or chemical combination of a sample and it is usually integrated into SEM. It is also used to obtain quantitative elemental analysis and 2-dimensional elemental mappings of different compositions (Scotuzzi et al., 2017; Goldstein et al., 2017). In the last decade, EDX-integrated SEM has been used to rapidly and accurately demonstrate the morphology of biological samples and the chemical composition of structures in this morphology (Delogne et al., 2007; Jung et al., 2015; Li et al., 2017; Scimeca et al., 2018; Perrotta and Davoli, 2014). SEM-EDX system has several advantages such as; (a) direct measurement of the elemental concentration of the samples (> 0.1% by weight); (b) measuring atoms and their of cations and anions in a wide range from Be to U; (c) determination of minerals; and (d) an efficient system that saves time with rapid sample change (Timofeeff, 2013). The SEM-EDX also informs the system of a certain depth of the sample not only from the surface of the sample depending on energy of the electrons to ionize the sample.

X-ray powder diffraction (XRD) is used to determine crystal structure of the compounds. It also allows a quantitative and qualitative analysis of compounds that cannot be measured by other means. Besides structural analysis with XRD, the content of the crystal structures and the determination of the grain size can be obtained. The aim of the study is to investigate the composition of the material, especially the crystal structure, the size of the mineral by using XRD with the properties determined by SEM-EDX analytically. Thus, the degree of correlation with the results obtained with other techniques will be revealed.

Cardiac valve calcification is a common disease especially in the elderly and may affect valve function and may lead to heart failure and sudden death (Adler et al., 2002). On the other hand, aortic valve calcification is also associated with arteriosclerosis and coronary heart disease (Rashedi and Otto, 2015). However, the origin of aortic valve calcification is still unclear. This study aims to demonstrate the development of the mechanism of the disease by characterizing samples with different calcification levels.

2. Material and Methods

After aort valve replacement operations, 3 calcined aort valves were obtained. The collected aort valves were completely dried at room temperature for a long time in the oven. The main purpose of the aort valves by keeping them at room temperature is to determine the calcification structures in the aort valve without causing any structural changes and to take images of morphological structures by scanning electron microscopy without causing any change in the morphology of the calcification structures.

After the drying process, the X-ray powder diffractometer (PANalytical Empyrean, Cu-Kα, λ = 1.54060 Å) XRD peaks of each of these valves were taken and the crystal structures of the calcined structures formed in these aort valves were determined (Figure 1).

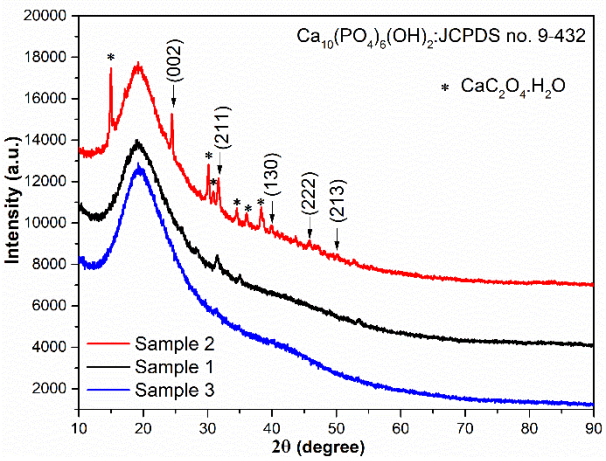

Figure 1. XRD peaks of calcined aort valves (Mi et al., 2014; Hlaing et al., 2015).

X-ray results revealed hydroxyapatite and calcium oxalate structures in the aort valves. In order to obtain the morphological structures, images were taken at different magnifications with the SEM device to obtain morphology of the calcined structures (Figure 2).

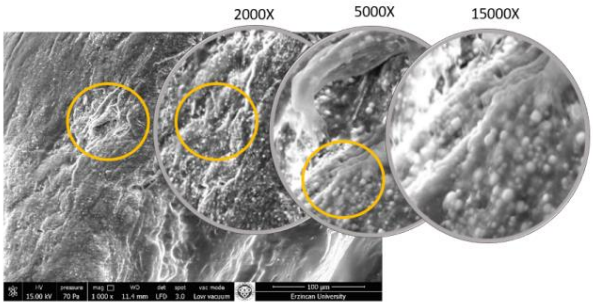

Figure 2. SEM images of calcined structures formed in the aort valves.

As can be seen from the SEM images, the calcined structures were formed in a spherical form from nano to micro structures. The formation of calcined structures in this way shows that they tend to grow from a nucleation.

Elemental analysis was performed with EDX device which is integrated to SEM device because of the fact that nucleation may cause other elements. The elemental analysis was performed both on these calcined islands and on the areas outside these islands (Figure 3). The elemental distribution obtained from these graphs is given in Table 1.

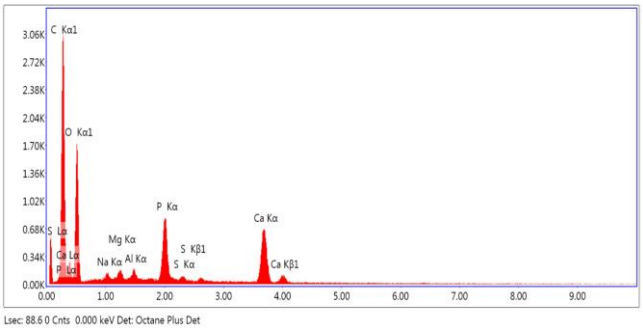

Figure 3. EDS graphs from calcined islands.

Table 1. Atomic ratios obtained from EDS graphs from calcined islands.

Element	Weight %	Atomic %
C K	48.67	60.86
O K	33.26	31.22
NaK	0.80	0.52
MgK	0.74	0.46
AlK	0.66	0.37
P K	5.37	2.60
S K	0.45	0.21
CaK	10.05	3.76

Element	Weight %	Atomic %
C K	49.38	59.67
O K	37.98	34.45
NaK	1.30	0.82
MgK	0.57	0.34
AlK	3.08	1.66
P K	2.49	1.17
S K	0.19	0.09
CaK	5.01	1.81

As can be seen from the EDS results and table, there are elements such as Na, Mg, S in calcined structures.

3. Conclusion

One of the most important cardiac diseases is the fact that the heart valves do not become

calcified and cannot perform their functions. In this study, it was observed that calcined structures were grown in heart valve tissue as spherical islands in the heart valves obtained. The fact that they are different in size from nano-sized to micro-sized aort valves shows that these calcined structures tend to grow from the nucleation stage. In addition, the elemental analysis in calcined structures, it was observed that Na, Mg, S elements except for calcium and phosphate on this calcined structure. It is thought that these structures may cause an effect to start nucleation of calcined structures. However, this effect should be examined with further investigations.

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