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SYMPOSIUM PAPER

Optical microscopy versus scanning electron microscopy in urolithiasis

Y. M. Fazil Marickar · P. R. Lekshmi · Luxmi Varma · Peter Koshy

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Abstract Stone analysis is incompletely done in many clinical centers. Identification of the stone component is essential for deciding future prophylaxis. X-ray diffraction, Fourier transform infrared spectroscopy, and scanning electron microscopy (SEM) still remains a distant dream for routine hospital work. It is in this context that optical microscopy is suggested as an alternate procedure. The objective of this article was to assess the utility of an optical microscope which gives magnification of up to 40× and gives clear picture of the surface of the stones. In order to authenticate the morphological analysis of urinary stones, SEM and elemental distribution analysis were performed. A total of 250 urinary stones of different compositions were collected from stone clinic, photographed, observed under an optical microscope, and optical photographs were taken at different angles. Twenty-five representative samples among these were gold sputtered to make them conductive and were fed into the SEM machine. Photographs of the samples were taken at different angles at magnifications up to 4,000. Elemental distribution analysis (EDAX) was done to confirm the composition. The observations of the two studies were compared. The different appearances of the stones under optical illuminated microscopy were mostly standardized appearances, namely bosselations of pure whewellite, spiculations of weddellite, bright yellow colored appearance of uric acid, and dirty white amorphous appearance of phosphates. SEM and EDAX gave clearer pictures and gave added confirmation of the stone composition. From the references thus obtained, it was possible to confirm the composition by studying the optical microscopic pictures. Higher magnification capacity of the SEM and the EDAX patterns are useful to give reference support for performing optical microscopy work. After standardization, routine analysis can be performed with optical microscopy. The advantage of the optical microscope is that, it is easy to use and samples can be analyzed in natural color.

Keywords Urinary stone · Optical microscopy · Electron microscopy · Whewellite · Weddellite · Uric acid · Apatite

Introduction

In most of the industrialized countries, around 20% of population is suffering from urolithiasis [1]. Urinary stones are of different composition, shape, and color, and some among them may be mixed layered [2]. Identification of the stone component is essential for deciding future prophylaxis. Stone analysis is incompletely done in many clinical centers. A combination of refined morphological and structural examinations of stone with optical microscopy complemented by compositional analysis using Fourier transform infrared (FTIR) spectroscopy [3] provides a precise and reliable method for identifying the structure and crystalline composition, and permits quantification of stone components. If the stone appears to be heterogeneous in origin, analysis is carried out for core, cross-section, and surface of calculi [4]. Pathogenic origin, identification, and quantification

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Y. M. F. Marickar (✉)
Department of Surgery, Zensa Hospital,
Trivandrum 695-009, India
e-mail: fazilmarickar@hotmail.com

P. R. Lekshmi · L. Varma · P. Koshy
Department of SEM, National Institute for Interdisciplinary
Science and Technology (NIIST), Trivandrum 695019, India

of different parts of the stones can be studied through the combination of FTIR techniques with optical microscopy [5]. X-ray diffraction [6], FTIR spectroscopy [7], Raman spectroscopy [8] and scanning electron microscopy along with elemental distribution analysis X-ray (SEM-EDAX) [9] still remain a distant dream for routine hospital work.

The scanning electron microscope (SEM) images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern [10]. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition, and other properties such as electrical conductivity. In order to authenticate the morphological analysis of urinary stones, and to study whether they are layered in structure, and to study more about the crystalline structure, SEM analysis is being used. EDAX [11] along with SEM makes it possible to get an idea about the percentage composition of each element present in a particular sample.

It is in this context that optical microscopy is suggested as an alternate procedure. The human eye cannot distinguish very small degrees of unsharpness. Optical microscopy can be used to take the images of the sample in their original color. Further, it can analyze samples, even if they are in air or water. Hence it can be applied in a variety of disciplines [12]. The optical microscope [13] uses visible light and a system of lenses to magnify images of small samples. Optical microscope is the oldest and simplest of the microscopes.

The objective of this article was to assess the utility of an optical microscope that gives clear picture of the surface of the stones. This was done by comparing the optical microscopy findings with those of the SEM and EDAX.

Materials and methods

A total of 250 urinary stones of different types were collected from the urinary stone clinic. Samples were washed and photographed, and wet chemical analysis was carried out. They were then observed under optical microscope and the photographs of the surface were taken at different angles using Olympus zoom stereo microscope up to magnification of 40 and recorded with Olympus 8 mega pixel camera.

Twenty-five representative samples, which were predominantly single component stones were taken for SEM analysis. Before inserting the sample into SEM machine, they were gold sputtered using a sputter coater [14] to coat non-metallic samples with a thin layer of gold to make them conductive and ready to be viewed by SEM. Representative areas were studied using X-ray probe and compositional analysis of the elements was performed. The findings of the optical microscopy were compared with the observations of SEM and EDAX.

Results

In the 250 samples analyzed by optical microscopy and wet chemical methods, the stone composition was predominantly identified to be COM (Whewellite)—158 (63%), COD (Weddellite)—73 (29%), uric acid—18 (7%), and phosphates 13 (5%). Of the representative 25 samples for which SEM and EDAX were also done, 11 (44%) were predominantly COM, 6 (24%) were predominantly COD, 5 (20%) were uric acid, and 3 (12%) were phosphates. The findings of OM and SEM-EDAX tallied well in all the samples studied. Four representative samples are depicted in the figures shown below.

The COM stone showed a dark brown, bosselated, mulberry appearance on the surface (Fig 1a). The bosselations were more clearly delineated in the optical micrograph (Fig. 1b). SEM picture at 1,000 magnification (Fig. 1c) did not show the bosselations, but showed dumbbell like shapes on the surface indicating the stone as whewellite. EDAX (Fig 1d) shows prominent peaks of Ca, C, and O which confirms the composition as calcium oxalate, thereby confirming the optical micrograph result.

The weddellite (COD) stone showed the typical spiculated appearance on the surface on ordinary photographs (Fig. 2a). The light brown, spiculated surface of dipyramidal crystals (sugar candy appearance) was well shown in optical micrograph (Fig. 2b) indicating the stone as weddellite. SEM of the surface (Fig. 2c) showed the broken layers of the dipyramidal crystals indicating the composition as COD. EDAX (Fig. 2d) shows prominent peaks of Ca, C, and O which confirms the optical micrograph report of calcium oxalate.

The uric acid stone showed the reddish yellow, smooth surface in the ordinary photograph (Fig. 3a) as also in the optical micrograph (Fig. 3b) indicating the stone as uric acid. SEM of the surface of the stone (Fig. 3c) shows the cotton candy appearance of uric acid surface. EDAX (Fig 3d) shows prominent peaks of C, N, and O confirming the optical micrograph result of uric acid.

The phosphate stone showed the white amorphous surface in the ordinary photograph (Fig. 4a) as also shown in optical micrograph (Fig. 4b) indicating the stone as phosphate. SEM of the surface of the sample (Fig. 4c) showed the usual surface of phosphate appearance. EDAX (Fig 4d) shows prominent peaks of P, Ca, C, and O confirming the optical micrograph result that the stone is phosphate. The SEM appearances of the surface of the stone were tallying with the optical microscopic findings.

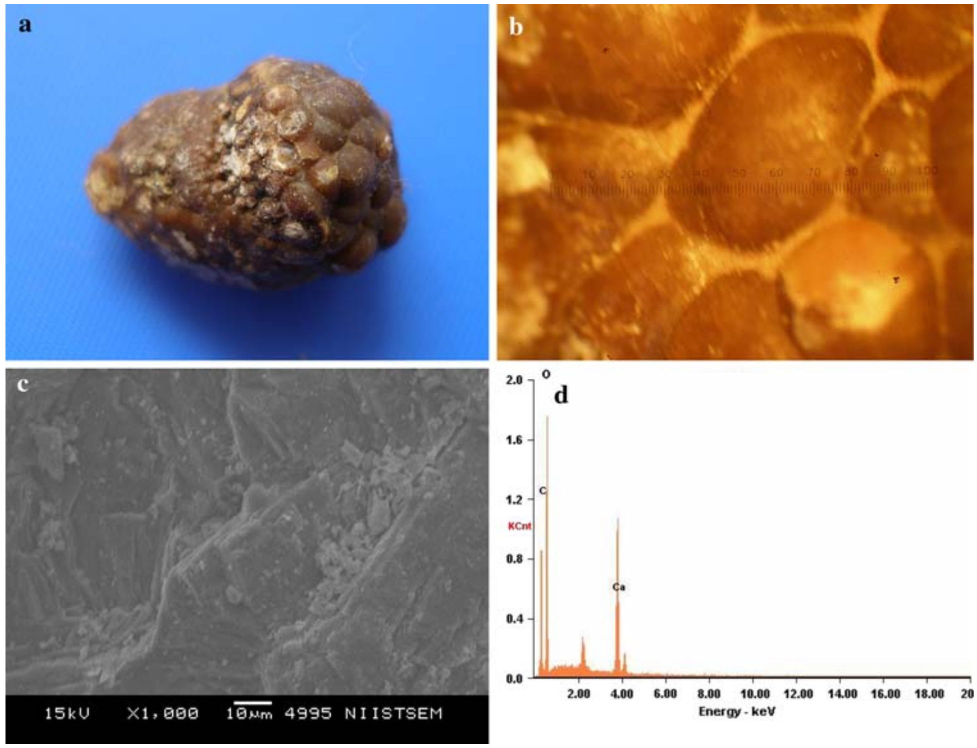

Fig. 1 Whewellite calculus: **a** Ordinary photograph; **b** Optical micrograph; **c** Scanning electron micrograph; and **d** EDAX

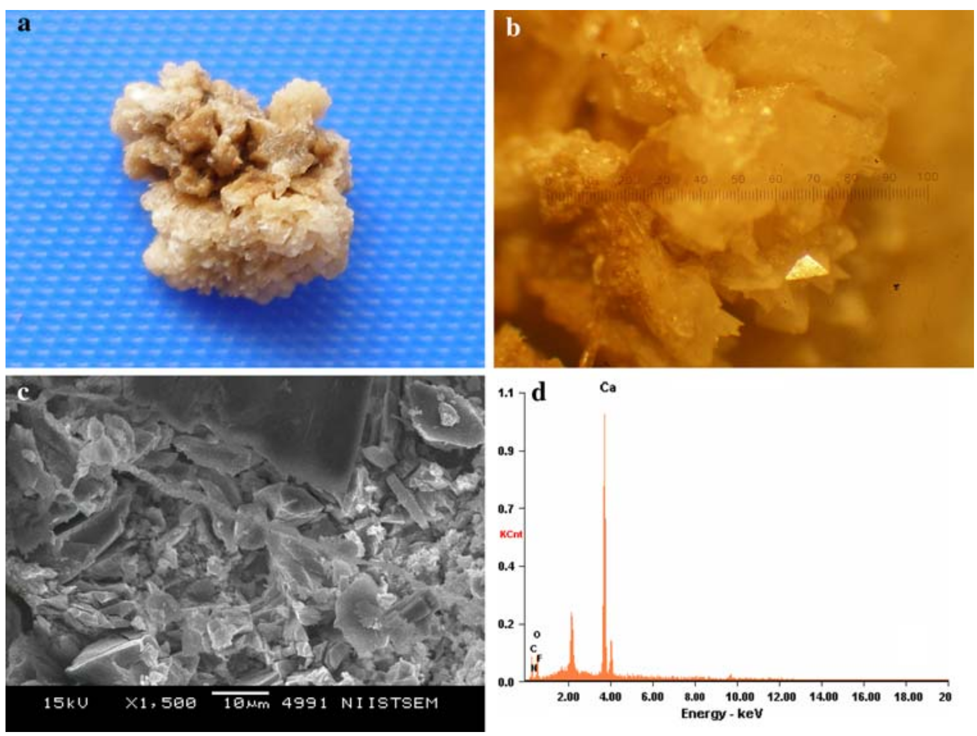

Fig. 2 Weddellite calculus: **a** Ordinary photograph; **b** Optical micrograph; **c** Scanning electron micrograph; and **d** EDAX

Discussion

The reason for the lack of interest in performing stone analysis among clinicians is the lack of proper feasible investigative set up in most hospitals.

SEM can give magnification up to 50,000. High resolution of SEM gives the evidence of very minute crystalline particles in a single stone. The combination of higher magnification, larger depth of focus, greater resolution [15] and ease of sample observation makes the SEM one of the most

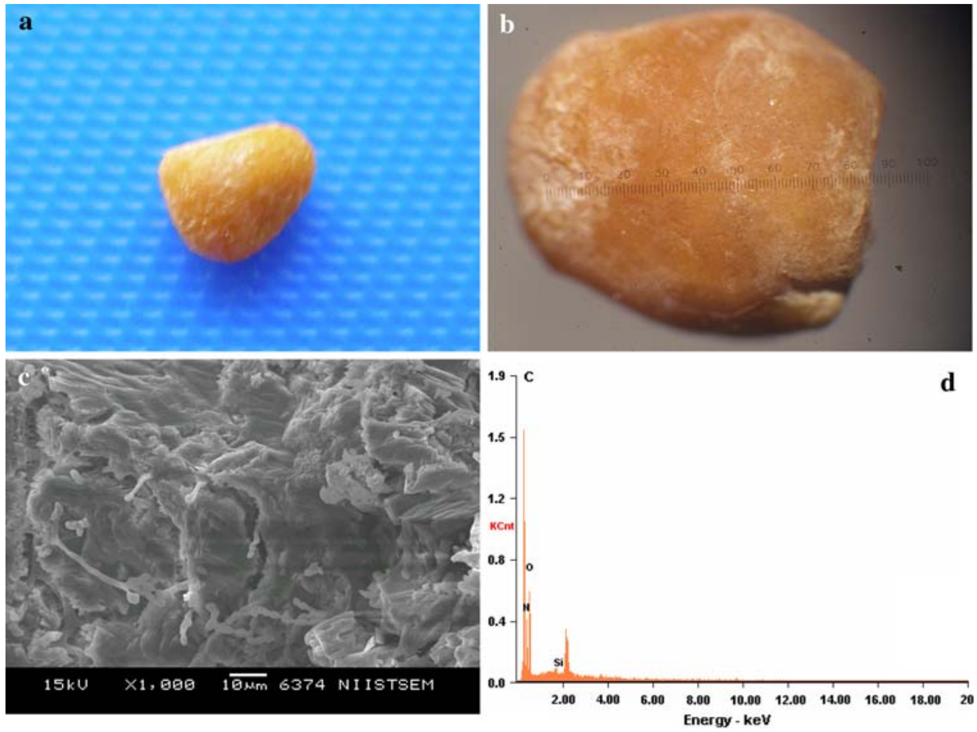

Fig. 3 Uric acid calculus: **a** Ordinary photograph; **b** Optical micrograph; **c** Scanning electron micrograph; and **d** EDAX

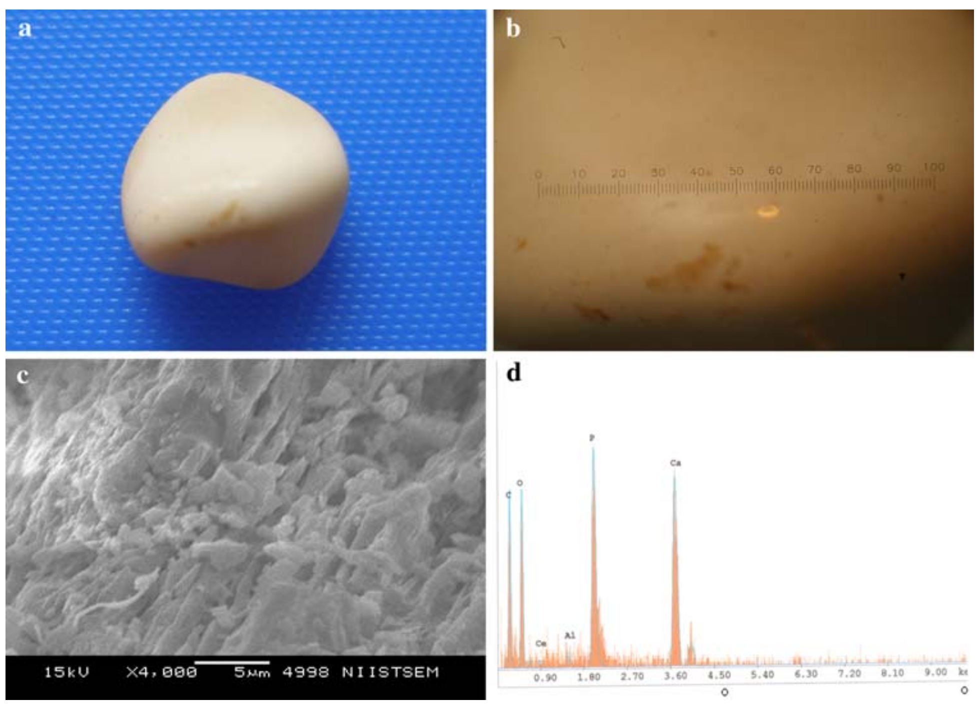

Fig. 4 Apatite calculus: **a** Ordinary photograph; **b** Optical micrograph; **c** Scanning electron micrograph; and **d** EDAX

heavily used instruments in research areas today. In the optical photograph taken at high resolution, only a section of the radiolarian is in sharp focus. The attractive nature of optical microscopy is that it is so easy to perform. The samples can be analyzed in air or water. The images are in natural color with magnification of 100–1,000 times. Even though OM does not give magnifications to the extent given by SEM, the available magnifications are sufficient to identify the actual composition of the stones. The equipment itself is not very costly and can be afforded by most hospital settings. Modern semiconductor electronics with charge coupled devices allow image processing also. Occasional standardization of the OM results may be performed using SEM and EDAX in appropriate research centers. In the lower SEM image, the whole specimen is in focus. SEM has better depth of field because the electrons used are focused through the use of an electro magnetic field. Stones with layered structure and crystal growth in them could be well analyzed using SEM methods. Higher magnification capacity of the scanning electron microscope and EDAX patterns are useful to give reference support for performing optical microscopy work. After standardization, routine analysis can be performed with optical microscopy. But SEM is of choice because of its depth of focus and resolving capacity. SEM will also show lamellar structures up to 10 micrometer in size and provide evidence that these structures are composed of smaller particles. But it is costly and so optical microscopy is preferred in much clinical analysis.

Conclusion

It is concluded from the study that optical illuminated microscopy can be added as a routine investigative modality in usual clinical laboratories handling stone analysis work. The patterns, however, have to be standardized using SEM and EDAX as research tools for authentication of the optical microscopic findings.

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