Renal calculi composition studies with the use of microtomography

Grzegorz Tatoń[1], Eugeniusz Rokita[1,2], Andrzej Wróbel[2], Felix Beckmann[3], Piotr Thor[4]

[1]Jagiellonian University Medical College, Department of Biophysics, Cracow, Poland
[2]Jagiellonian University, Institute of Physics, Cracow Poland
[3]GKSS-Research Centre, Geesthacht, Germany
[4]Jagiellonian University Medical College, Chair of Pathophysiology, Cracow, Poland

KEY WORDS

image processing ◗ kidney stones composition ◗ three-dimensional imaging ◗ X-ray microtomography

ABSTRACT

Introduction. The usefulness of a three-dimensional microtomographic image histogram analysis in renal calculi composition studies was investigated. The results were compared to the X-ray crystallography (XRD) method.

Materials and methods. Renal calculi samples were studied *in vitro* with synchrotron radiation-based microtomography. Histograms of the three-dimensional stone images were analyzed. It was assumed that every stone compound results as a maximum in the histogram; therefore the identification of some compounds was possible. The compounds usually present in renal stones were considered. X-ray crystallography of the same stones pieces was performed to determine the samples composition.

Results. The XRD results correlate well with histogram findings. Compounds identified by crystallography were also identified by the histogram analysis. It was possible to identify other substances, which were not detected in XRD because of their smaller amount in the sample. Different calcium oxalates resulted in separated and slightly shifted image histogram peaks. Except from the maxima, connected to the considered pattern compounds, additional peaks in the stone histograms were also present.

Conclusions. The advantage of the proposed method relies on the possibility of calcium oxalates differentiation, differentiation of more compounds than XRD without additional effort, and also gives a perspective on the quantitative analysis of the compounds magnitude in the studied samples. Performed analysis was done with the resolution of a few micrometers, which is considerably better than the resolution of clinically used procedures at this time. The constant technological progress gives hope that in the future such a method could possibly be used for the clinical and noninvasive recognition of renal calculi types.

INTRODUCTION

The susceptibility of renal stones for different therapeutic methods used in practice [1] depends on their composition and structure [2, 3]. E.g. homogenous and compact calculi are less susceptible to lithotripsy than heterogeneous and structurally less compact stones [2]. The structure and composition of stones should be taken into account when choosing a therapeutic method while considering its' effectiveness and side effects [1, 4]. The optimization of urolithiasis treatment demands the development of reliable, precise, and preferably a noninvasive diagnostic method allowing for the determination of stone composition and structure.

In addition, modeling stone comminution, requires knowledge of the treated stone's composition and structure [2, 3, 5].

Computed tomography (CT) is one of the methods used for urolithiasis diagnosis [1, 2, 6]. Clinical CT investigations usually show evidence of stone presence, but their ability to study stone structure and composition is limited at the moment [3]. This is due to the resolving power limitations of CT scanners. The image resolutions currently achieved are in an order of magnitude of about 200-300 micrometers. On the other hand, constant technological advancement will allow higher CT abilities in the future. Therefore it is of value to also consider CT applications in this field. The methods of applying clinical CT scanners to the structural investigation of renal calculi *in vivo* were undertaken [2, 6].

From the practical point of view, at the moment, only the application of microtomography (μCT) gives more promising results [3, 7]. It can be used only *in vitro,* which is a considerable disadvantage if considering potential clinical applications, but the use of the X-ray microbeam allows achievement of much better resolutions than clinical scanners. A resolution on the level of a few micrometers can easily be achieved, allowing precise stone structure imaging and analysis.

The synchrotron radiation microbeam was used in this work. It allowed for the investigation of stone structure with a resolution of a few micrometers. The three-dimensional (3D) image histogram analysis was proposed as the solution allowing for the differentiation of minerals present in the stone. Because the dimensions of the monocrystals in renal stones could be in the order of magnitude of the achieved resolution, it is better to identify calculi minerals in the image histogram instead of on the 3D image. Every mineral type present in the sample in considerable amount reflects as a maximum in the histogram. The maximum position depends on the X-Ray's linear attenuation coefficient, which is unique for different materials.

MATERIALS AND METHODS

Eleven renal stones were investigated with X-ray diffraction (XRD) and μCT. Samples were obtained during percutaneous neph-

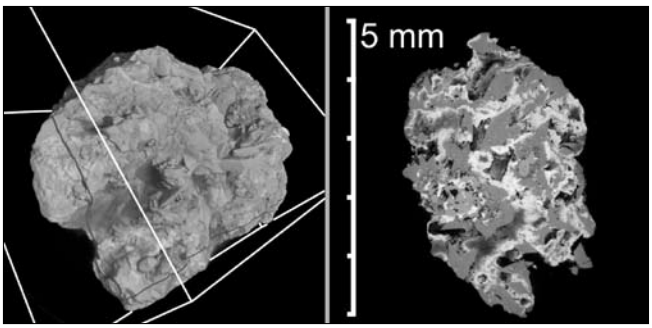

Fig. 1. Example of microtomographic reconstruction. 3D reconstruction of sample 1 (left) and its axial cross-section (right).

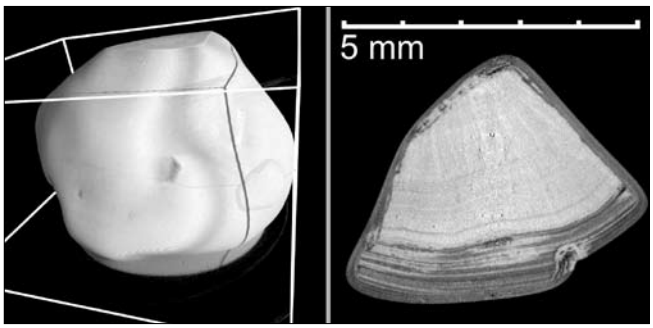

Fig. 2. Example of microtomographic reconstruction. 3D reconstruction of sample 6 (left) and its axial cross-section (right).

rolithotomy from patients with diagnosed nephrolithiasis. The same stone pieces, with a diameter of about 5 mm, were scanned with μCT and then investigated with XRD.

The XRD is reported as the best method with regard to accuracy in the field of renal calculi analysis [8]. Therefore XRD was performed in order to provide a reference for the determination of the crystal structures of all chosen samples. The XRD measurements were carried out with the use of PW1710 (Philips, Holland) powder diffractometer. It uses graphite monochromatized $Cu_{K\alpha}$ X-rays and allows measurements in the angular range of 12°-40°.

The μCT investigations were performed in DESY (German Electron Synchrotron, Hamburg, Germany). Samples were scanned with the BW2 beamline at HASYLAB equipped with μCT scanning system operated by the GKSS Research Center (Geesthacht, Germany). The applied system and procedures are described in detail in elsewhere [9]. There is no additional sample preparation required. The sample is mounted in a specially designed mechanical system, allowing precise movements and rotations. The sample irradiated by a wide monochromatic synchrotron beam is rotated and flat two-dimensional projections are acquired every 0.25° in the range of 0-180°. The detecting system consists of a CCD camera and a phosphor screen. The μCT system scans up to 1,530 image layers and up to 2.5 mm of the sample height at once. If the sample is higher than 2.5 mm it is shifted and scanned as many times as necessary. A single 2.5 mm high sample scan takes about 2.5 hours. The applied photon energy was 21 keV, while the achieved image voxel size was about 4 μm. The reconstructed 3D images represented the distribution of the X-rays linear attenuation coefficient normalized by the voxel size. Finally, the X-ray linear attenuation coefficients were recalculated linearly into 256-levels of grayscale (GS) and the 3D images were exported as a stack of TIFF (Tagged Image File Format) files. The recalculated GS will be used as the relative unit of the X-ray linear attenuation coefficient in this work.

Seven compounds, most frequently observed in renal calculi [6,7], were considered: hydroxyapatite (HAP), calcium oxalate monohydrate – whewellite (COM), calcium oxalate dihydrate - weddellite (COD), brushite (BRU), struvite (STR), uric acid (UA) and cystine (CYS). Because the presence of calcium oxalate with a hydration level between two and three ($(Ca_2C_2O_4*2.25H_2O)$ (COD/T)) was reported in XRD results, we also considered this form as well as calcium oxalate trihydrate – caoxite (COT). The X-ray linear attenuation coefficients for the energy of 21 keV were calculated and expressed in GS for all mentioned compounds and for air.

It was assumed that every component of the renal stone is reflected in the histogram as a separate Gaussian shaped peak [5, 8] since different minerals are characterized by different X-ray linear attenuation coefficients. The 3D image histograms were calculated and fitted with the set of Gaussian functions. It was possible to determine if a particular compound constitutes the stone by comparing the fitted Gaussian positions on the attenuation scale and the attenuations for the "pattern" compounds mentioned above. The findings of XRD were compared to the results of the 3D image histogram analysis.

Table 1. Results of X-ray diffraction (XRD) measurements and image histogram analysis. HAP – hydroxyapatite; COM, COD, and COT – appropriately calcium oxalate mono-, di-, tri-hydrate; COD/T – $CaC_2O_4*2.25\ H_2O$; BRU – brushite; STR – struvite; UA – uric acid; UA1, UA2 – different uric acid phases.

Sample	XRD	Histogram
1	BRU, COD/T	UA, BRU, COD, COT
2	COD/T	UA, COM, COD, COT
3	COD/T	UA, COD, COT
4	UA	UA1, UA2
5	STR, HAP	STR, CYS, COD
6	COM	UA, COD, COT
7	COD/T	UA, COD, COT
8	COM	UA, COD, COT
9	COD/T	COD, COT
10	COM, COD/T	UA, COM, COD, COT
11	COM, COD/T	UA, COD, COT

RESULTS

The results of the XRD investigations are presented in the second column of Table 1. The usual components of crystals in studied samples are different phases of calcium oxalates. Even if the sample composition seems to be more complicated on the basis of the 3D image reconstruction, no more than two crystal components were identified in the XRD.

On the basis of μCT investigations the 3D reconstructions of investigated stones were performed. Examples of the images obtained are presented in Figures 1, 2, and 4. As comparing Figures 1 and 2, different types of stones were studied. Clearly, besides the 3D views, the cross-sectional imaging of any plane is also possible. The cross-sections are even more helpful than the 3D rendered images because they can be used for the assessment of the renal calculi structure complexity. It is possible to see how many different compounds can be expected from the histogram analysis.

The 3D image histogram allowed for the identification of compounds present in the investigated stones. The results of identifying the compounds on the basis of histogram analysis are placed in the last column of Table 1. The examples of histogram fitting results are shown in Figure 3.

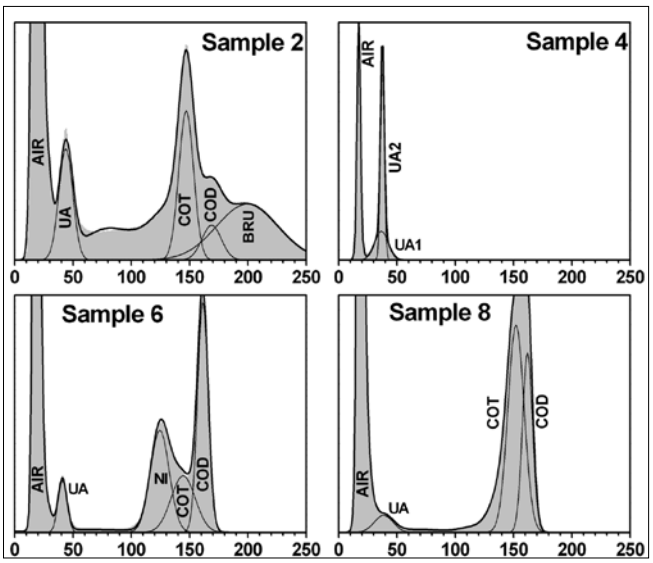

Fig. 3. Examples of histogram analysis for samples 2, 4, 6, and 8. The 3D image histograms (grey) were fitted with a set of Gaussian shaped functions. Gaussians drawn with thin lines represent the pattern compounds. The sum of all fitted Gaussians are marked as thick solid lines. COM, COD, and COT – appropriately calcium oxalate mono-, di-, tri-hydrate; BRU – brushite; UA1, UA2 – different uric acid phases; NI – not identified as a pattern component.

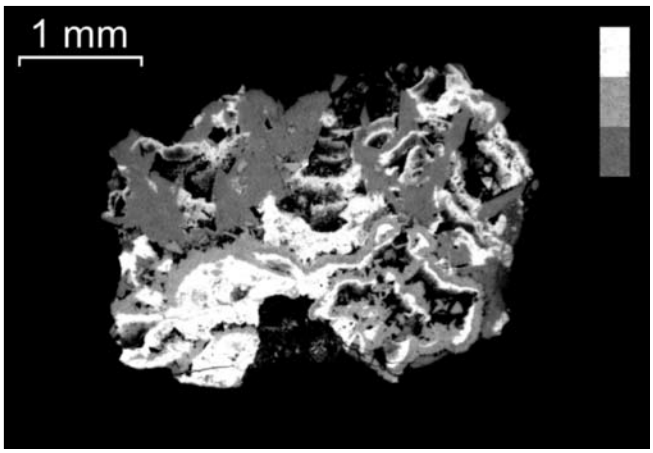

Fig. 4. The sagittal cross-section of the reconstructed 3D image of sample 1. Image contrast was adjusted in order to amplify some futures if compared to Figure 1. The sample was reported from XRD as containing only two compounds: brushite and CaC_2O_4*2.25H_2O. It is obvious on the basis of the reconstruction that the structure is more complicated and contains more mineral types. The legend bar shows from the top: brushite, calcium oxalate dihydrate, and calcium oxalate trihydrate. Uric acid reported from microtomography is not marked in the legend. It takes the dark holes in the sample. Its grayscale is almost the same as the air grayscale.

DISCUSSION

Computed tomography (CT) and particularly helical CT are the best diagnostic choice for urolithiasis diagnosis [1, 5]. In clinical practice it is used to confirm the presence of stones, to discover their locations and to measure their sizes [3]. All this information is crucial, but many authors also emphasize the role of stone structure and composition studies [2, 3, 6-8, 10-14]. The efforts were undertaken to use helical CT in such investigations [6, 12-14]. The usefulness of helical CT in this field was confirmed by the μCT many times [3, 7, 12, 14], but the μCT itself seems to be the best method for precise structure and composition determination as many authors have shown [3, 7, 12, 14]. We proposed the approach based on the histogram analysis of the μCT images. Similar efforts were considered [6], but such works are seldom. The usefulness of proposed solution will hopefully be confirmed in the following detailed discussion of collected data. Also some advantages over the XRD method, considered thus far as the best method for stone composition analysis [8], will be shown.

The common future of all analyzed image histograms, which should be explained first, is a high and narrow maximum at the beginning of the grayscale (Fig. 3). It correlates with air surrounding the sample. The area of the AIR peak should be minimized by clipping the investigated volume as much as possible. If the AIR peak was not minimized it could preclude the observation of the maxima correlating to UA.

In most cases (except for two samples) UA was identified as the component of analyzed stones even if it was not identified it in the XRD measurements (Table 1) and this fact should be explained. UA is the third most frequent stone component and its frequency is estimated at about 10% [1, 8] or even at 30% [11] of all reported stones. Many authors claim, that the most frequent stones are composed of more than one component (which is the case only in about 34%) [1, 8]. What is more, complicated stone compositions and the presence of contaminations even in apparently single-component stones were reported [7]. Because UA is a common component of urine, we believe it might be a contamination in most cases on the concentration level of a few percent, which is below the detection level of the XRD.

Sample 4 is a specific case in our sample set. It is determined by the XRD as a single component calculi consisting of UA only. The peak connected to UA in this sample image histogram (Fig. 3, up and left) is high and narrow at the top, but broad on the bottom and could not be properly fitted with single Gaussian. The fitted curves match well to the histogram if the UA peak is represented by two Gaussians as seen in Figure 3. There are two possible explanations of this fact. First explanation is that the Gaussian shaped peaks are not the best choice. The second possibility, in our opinion more likely, is that there are two UA phases present in the sample. It is quite possible that except from the UA there is also UA dihydrate present in the stone (these two phases were denoted as UA1 and UA2 in Table 1 and in Figure 3). Such a theory is consistent with some data presented [8] where the author stated that UA as well as uric acid dihydrate are frequent stone constituents with a frequency of 10% and 6% respectively.

The difference between the linear attenuation coefficients for UA1 and UA2 recalculated into the GS is about 1. The maxima representing both phases should practically occupy the same position and this fits well to the observed case. The possibility of distinguishing between UA1 and UA2 is the advantage of the μCT approach.

The GS range between UA and calcium oxalates are usually characterized by the flat part of histogram (Figure 3, Samples 6 and 8). This part is fitted with wide and low Gaussians and is probably connected to the organic matter contained in the stone. This is also the region where potential peaks representing the presence of STR and CYS can occur.

In most cases (except for sample 4) some combinations of calcium oxalates were observed, including samples not identified as containing calcium oxalates (see Table 1, Sample 5). The XRD method recognizes such mixtures as COM or a combination of COD and COT (here denoted as the COD/T). In all cases when the XRD reported the presence of COD/T both the COD and the COT related peaks were visible in the image histogram. What is more COD and COT domains were also visible on the cross-sections (see Figure 4).

This is natural that the XRD identified compounds are less numerous than in the case of the histogram analysis. It is because the XRD method is able to demonstrate the compound characterized by larger amount in the sample. Therefore the cases when the compound is reported by the XRD and not reported in the histogram analysis have to be analyzed carefully.

The results for samples 6, 8, and 11 show some discrepancies when considering the COM compound. All mentioned samples were defined as containing COM in the XRD while histogram analysis shows only the COD and the COT compounds. The only possible explanation lies in the changes of calcium oxalates hydration level between the investigations with XRD and with μCT. XRD was performed a few weeks after μCT measurements and during this time, samples were held in the air environment. On the other hand the evidence that the hydration level of calcium oxalates can change in time was not found in the literature. The inhomogeneity of the sample should not be the discrepancies explanation because exactly the same stone pieces were studied in μCT and in powder XRD. In both methods the whole sample volume influences the results.

Also HAP detected in sample 5 was not identified on the histogram. The problem with the HAP is probably connected to the X-ray attenuation coefficient calibration. The μCT system was calibrated in order to investigate lower attenuations than the attenuation of HAP. The GS calculated for HAP is about 406 and is far behind the maximal value of GS for the 8-bit TIFF images. In further investigations, more samples containing HAP should be considered and the calibration problem should be solved.

Not all maxima visible in histograms were assigned to considered pattern compounds (Figure 3, sample 6) suggesting that the set of pattern compounds should be broadened to make proposed method more reliable and useful.

CONCLUSIONS

The method relying on the 3D image histogram of renal stones analysis was described. The images obtained in the μCT investigations were considered in order to investigate stones structure on the level of a few micrometers. The stone structure and composition can be obtained on the basis of the μCT studies. Except from HAP all mineral types detected by the XRD were also detected in the μCT. Calcium oxalates determined in the XRD were reported by the μCT, but some discrepancies in the hydration level were observed.

The proposed method is simple and could be useful because it gives more information about sample composition than the XRD. The histogram based analysis is simpler and no additional equipment is needed if the μCT was performed. As the advantage comparing to the XRD the possibility of distinguishing between different calcium oxalate types should be emphasized. The more complicated analysis (which was not our aim in this paper) of fitted Gaussian areas could give more precise information about the amounts of considered phases.

The only disadvantage of the proposed method is that it could not be used *in vivo*.

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Correspondence

Grzegorz Tatoń
Jagiellonian University Medical College
Department of Biophysics
16, Łazarza Street
31-530 Cracow, Poland
phone: +48 12 619 96 82
mmtaton@cyf-kr.edu.pl