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Original article

Applying quantitative micro-Raman spectroscopy to analyze stone compositions extracted from ureteroscopic lithotripsy urine

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ABSTRACT

Objective: In this study we applied quantitative micro-Raman spectroscopy (MRS) in binary stone compositions extracted from ureteroscopic lithotripsy (URSL) urine. Urolithiasis is a severe disease with a great impact. For ureteral stone patients, a basket retractor is used to catch the stone for analysis when patients undergo URSL. However, this can cause potential ureteral-related complications. The identification of the composition of a urinary stone is important for metabolic evaluation, decision making, and diet control. In this study, we applied a quantitative MRS method in a clinical setting to measure the composition of binary urinary stones in urine extracted via URSL.

Materials and Methods: This study was approved by the Institutional Review Board of Taipei City Hospital, Taipei, Taiwan from November 2008 to November 2009. Urine samples from fragmented stone sites in the patients' ureters were collected via the URSL procedure by drawing at least 10 mL of urine with a syringe. The urine samples from 30 patients were analyzed using an MRS-based analysis method, both qualitatively and quantitatively.

Results: The post-URSL urine powder from the samples was successfully analyzed using a quantitative MRS-based method, which was based on a linear equation developed according to the ratios of the relative intensities of the Raman bands corresponding to binary mixtures of known composition. Fourteen urine samples from 30 patients disclosed binary composition, and the ratio percentages were obtained using the equation along with a quantitative MRS-based method.

Conclusion: We successfully applied the quantitative MRS-based method clinically to analyze the stone compositions extracted from URSL urine. This method decreases the need to use a basket to catch macrostones for Fourier transform infrared spectroscopy analysis, while also still providing quantitative and quantitative stone analysis information by using the microstones in urine.

1. Introduction

Urinary stones have been a major issue for centuries. Its prevalence rate is 8–15%,[1–4] with rising incidence rates in the past 30 years.[5] There is no doubt that urolithiasis is a significant problem worldwide, accounting for a considerable degree of morbidity and mortality. For frequent stone formers, treatment can be a painful experience. The exact mechanism of urinary stone formation is not fully understood; it may be caused by multiple factors.[6] Apart from medical treatment, the prevention of recurrence is also very important. Statistical surveys show that 50–70% of urolithiasis patients experience a recurrence within 5 years, and 3% of patients experience renal failure due tourolithiasis.[7] The medical therapy, metabolic evaluations, and diet control of urolithiasis patients are based on the results of exact stone analysis.[8] Exact stone analysis represents an effective metaphylaxis of residual and recurrent stones, and is thus important for therapy. Currently, urinary stone analysis is mainly performed on stones that are at least visible to the naked eye in clinical settings.[9] However, in recent years, the treatment of urinary stones has been completely revolutionized, and it is now unusual for a patient to undergo open surgery as the first line of treatment. With improvements to ureterorenoscopic lithotripsy (URSL) and extracorporeal shock wave lithotripsy (ESWL), stone management has become more effective, less invasive, and more comfortable than before. In a URSL procedure, a

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basket retractor is used to retrieve the stone for Fourier transform infrared spectroscopy (FTIR) analysis, but associated complications (such as ureteral trauma, ureteral convulsion, ureteral degloving, and even renal loss) can occur. Resident doctors undergoing their training periods are particularly vulnerable to such complications. Previously, stone analysis was not possible because urine could not be analyzed using a FTIR-method for the URSL procedure. The previous analytical methods employed for qualitative and quantitative analysis of macrourinary stones included FTIR, wet chemical analysis, X-ray diffraction, and scanning electron microscopy with energy dispersive X-ray.[10] X-ray diffraction and FTIR provide reliable results, but remain qualitative in nature and may require prohibitively large amounts of samples for measurement. FTIR is the most practical method, but is vulnerable to interference by water. Potassium bromide is used for pellet optimization, and the pellet thickness can affect sample absorbance. In addition, traditional FTIR spectroscopy requires extensive sample preparation. Recently, attenuated total internal reflection FTIR is used for the analysis of small stones, but it still requests a pellet to work before spectroscopy analysis.[11]

Recently, Raman spectroscopy (RS) has been extensively studied for applications in mineral analysis, cell dynamics monitoring, and biomolecular detection. RS includes both qualitative and quantitative measurements. RS allows real-time, nondestructive analysis, without requiring labeling technologies, and using noninvasive measurements. Best of all, RS can be used as a fingerprint tool for the sample, providing both quantitative and qualitative measurement information. It also only requires minimal sample preparation. RS has opened up a new field in the area of real-time qualitative and/or quantitative mineral, cell, and tissue analysis.[12] In 1997, Kontoyannis et al[13,14] first developed the quantitative analysis of calcium oxalate monohydrate/calcium oxalate dehydrate (COM/COD) and hydroxyapatite (HAP)/COM from the calibration curve of pure synthetic materials. In our previous study, we applied a microRS (MRS)-based approach to analyze the composition of urinary stone sediment directly from the extracted urine after the URSL procedure. The MRS-based approach provided a quick and convenient method for qualitative microurinary stone analysis.[15] In this study, the goal was to apply the MRS-based quantitative analysis technique for binary urinary stone powder compositions after URSL, with no loss of information compared with previously-used techniques.[16]

2. Materials and methods

Approval for this study was granted from November 2008 to November 2009 by the Institutional Review Board of Taipei City Hospital, Taipei, Taiwan. The patients involved in the study all signed informed consent forms. Urine samples from fragmented stone sites in the ureter were collected with an URSL procedure by drawing at least 10 mL of urine with a syringe. The 30 urine samples were analyzed using the quantitative MRS-based analysis method. Figure 1 shows the diagram of the quantitative MRS-based system for post-URSL urine powder composition analysis, which has been implemented in clinics.

In the previous study, we used an MRS-based approach to measure seven common standard compounds, including COM, COD, HAP, dicalcium phosphate dehydrate (DCPD), uric acid, and magnesium ammonium phosphates (struvite). The COM and HAP compounds were manufactured by the Fluka Corporation, Everett, WA, USA; the DCPD and magnesium ammonium phosphate compounds by the RDH, Sigma–Aldrich, Darmstadt, Germany, and the uric acid compound by the Acros Organics, Morris Plains, NJ, USA. The COD was prepared based on the following procedure:

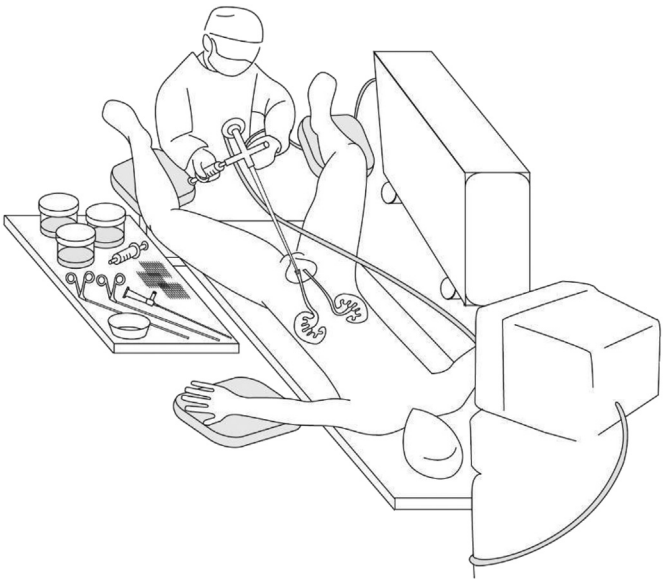

Figure 1. Micro-Raman spectroscopy-based analysis of post-ureteroscopic lithotripsy (URSL) urine powder with the URSL procedure, from bench to bedside. The URSL laser provided the energy to fragment the ureteral stones; the micro-Raman spectroscopy system then analyzes the quantitative results of the urinary stones after the syringe/filter procedure.

32 mL of 0.2M calcium chloride was added to 400 mL of 0.01M sodium citrate and magnetically stirred at 100 rpm at 23°C. Then, 16 mL of 0.05M sodium oxalate was rapidly added to this mixture, and stirring continued for 10 ± 15 minutes. The precipitate was then filtered off and washed repeatedly with distilled water. The residual solvent was removed by drying to constant weight at ambient temperature over calcium chloride granules and in a vacuum.[16]

For the detection of two mixed urinary stone compositions, we derived the calibration curves for each mixed group. We analyzed the seven most prevalent urinary stone mixtures based on their prevalence from clinical surveys.[10] We then established standard calibration curves according to the known mixture concentration from the pure synthetic compound, thus producing an equation for quantitative measurement.[16]

A 20 mW helium–neon laser, 632.8 nm, was used for Raman excitation. The laser spectral line width was further spectrally purified with a laser line band-pass filter. A notch filter (HSPF-632.8-1.0; Kaiser, Ann Arbor, MI, USA) was used to block the excitation light while allowing the Raman signals to enter the spectrometer system. An 80-cm focal length spectrometer system (HR800, Jobin Yvon, Longjumeau Cedex, France) was used with an 1800 g/mm holographic grating to provide spectral resolution at 1 cm^{-1}. A liquid nitrogen-cooled charged-coupled device two-dimensional array detector was used to measure the Raman signal by integration at 1 second, with a total of 4 seconds for the complete scanning from 400 cm^{-1} to 1800 cm^{-1}. The standard urinary stone compounds were measured on a microscope slide with a 50× microscope objective lens and a 1000-μm confocal hole. After evaluation of the standard mixtures, we applied quantitative analysis to the clinical urinary stone powders in the extracted urine after URSL. For post-URSL urine powder measurements, we drew 0.5-mL urine from the bottom of the 10-mL urine sample, and placed it on a microscope slide. Then, we used a hair dryer to dry the sample before the MRS measurement. This specimen was photobleached for 30 minutes before Raman analysis. Table 1 discloses the equation of the quantitative RS analysis to show how to perform the quantitative analysis.[16]