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Abstract. Calciphylaxis is a painful, debilitating, and premonitory condition, which presents as calcified vasculature and soft tissues. Traditional diagnosis of calciphylaxis lesions requires an invasive biopsy, which is destructive, time consuming, and often leads to exacerbation of the condition and infection. Furthermore, it is difficult to find small calcifications within a large wound bed. To address this need, a noninvasive diagnostic tool may help clinicians identify ectopic calcified mineral and determine the disease margin. We propose Raman spectroscopy as a rapid, point-of-care, noninvasive, and label-free technology to detect calciphylaxis mineral. Debrided calciphylactic tissue was collected from six patients and assessed by microcomputed tomography (micro-CT). Micro-CT confirmed extensive deposits in three specimens, which were subsequently examined with Raman spectroscopy. Raman spectra confirmed that deposits were consistent with carbonated apatite, consistent with the literature. Raman spectroscopy shows potential as a noninvasive technique to detect calciphylaxis in a clinical environment. © The Authors. Published by SPIE under a Creative Commons Attribution 3.0 Unported License. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: [10.1117/1.JBO.20.8.080501](https://doi.org/10.1117/1.JBO.20.8.080501)]

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1 Introduction

Calciphylaxis is a painful and debilitating condition affecting 1% to 4% of patients with end-stage renal disease or kidney transplants and its incidence is on the rise.¹⁻⁴ Estimates of the 1 year survival rate and median survival time are about 46% and 1.5 years, respectively.⁵ Patients with calciphylaxis develop calcium deposits in their vasculature and soft tissues that may result in vessel thrombosis and tissue necrosis. Ultimately, patients develop large, open wounds that are painful and at high risk for infection. Current standard of care includes monitoring and managing pain, achieving hormone balance, and proper wound care to prevent potential secondary aggravations, such as infection and sepsis.⁶ Furthermore, the presence of co-morbidities, such as renal failure, may inhibit patients from undergoing multiple surgical interventions.

Since first reported in the literature in the 1960s and 1970s, calciphylaxis has been diagnosed by histological examination of suspicious tissues from at-risk patients (e.g., those with renal failure, dialysis, and elevated serum phosphate and/or calcium levels).^{6,7} However, histological examinations are destructive, time consuming, and require interpretation by a pathologist. Furthermore, debrided sites generally do not heal well, as blood flow to the region is limited due to calcified and thrombosed vessels and the lesion can actually increase in size. While radiographic techniques, such as computed tomography (CT), can detect calcium deposits, they are nonspecific for calciphylaxis, requiring a follow-up biopsy. Alternative diagnostic methods, such as analysis of serum solutes, have unclear utility.⁷ The advent of new treatment options for calciphylaxis patients,⁸ including pharmacologic agents (e.g., bisphosphonates, sodium thiosulfate, and cinacalcet)⁹⁻¹² and hyperbaric oxygen treatment,¹³ demands improved diagnosis.

A primary challenge in detecting calciphylaxis is finding small calcifications within a large wound bed. Previously, calcinosis has been examined in preliminary studies by infrared and Raman spectroscopy.¹⁴ A recent multimodal study of calciphylaxis tissue,¹⁵ including analysis via electron dispersive x-ray analysis, confirmed calciphylaxis composition as carbonated apatite similar to bone mineral (as was hypothesized as early as the 1960s).^{16,17} To reliably detect this carbonated apatite, clinicians need a single noninvasive diagnostic instrument. Noninvasive diagnosis is important because any invasive diagnostic modality, such as debridement, can exacerbate the condition.

Furthermore, in the operating room, surgeons debriding calciphylaxis are not able to determine the complete extent of the disease visually and develop a treatment plan. Often this leads to excessive or inadequate debridement. Currently, hospitals and surgeons are reluctant to treat calciphylaxis for fear of exacerbating the condition. A diagnostic modality that determines the extent of the disease process would allow surgeons to confidently excise the margins of the calciphylactic tissue and assess the extent of the calcified lesions.

We propose rapid, noninvasive, and label-free Raman spectroscopy to improve the detection of calciphylaxis. Raman spectroscopy enables chemical fingerprinting of a sample with micron-level spatial resolution and subsurface probing properties similar to other tissue optical spectroscopies in the deep-red and near infrared regions. Raman spectroscopy has been previously employed in many investigations of calcified tissues, especially bone.^{18,19} These include characterizations of *ex vivo* bone biopsies,²⁰ *in vivo* ectopic bone formation,²¹ and human subject tibial plateaus.^{22,23}

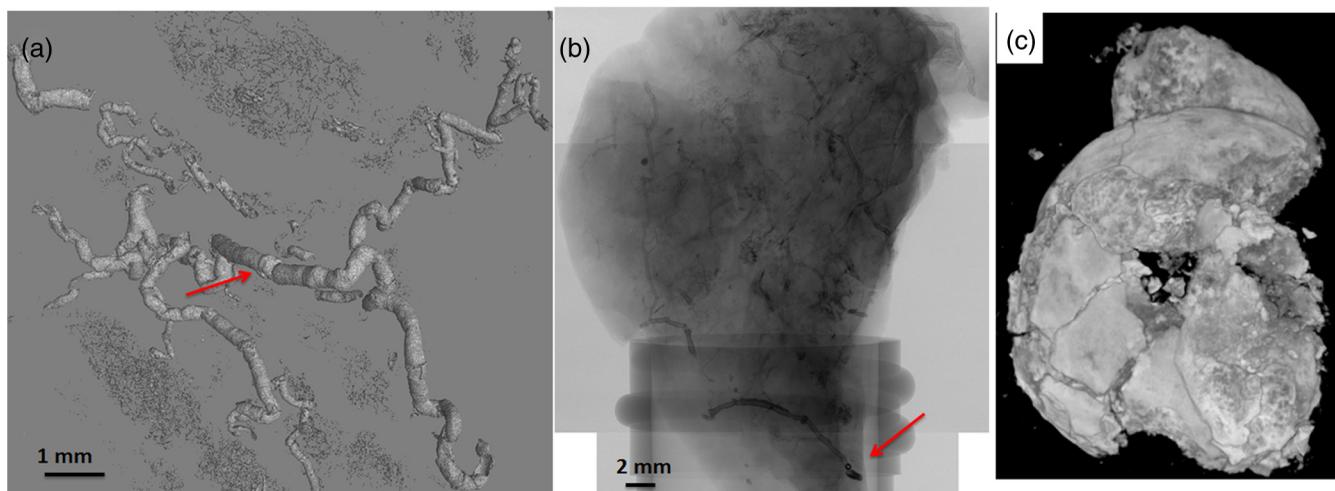


Fig. 1 Representative microcomputed tomography images from calciphylactic tissue. Arrows indicate location of measurements in (a and b) and (c) is close-up of nodule.

Here, we employ micro-CT and Raman spectroscopy to evaluate calciphylaxis biopsy specimens. The study was approved by the Institutional Review Boards at Massachusetts General Hospital and the University of Michigan. Six patients were enrolled. From each patient, a tissue biopsy was obtained, preserved in 3.7% formaldehyde and stored in a 70% ethanol prior to measurement. Micro-CT (Fig. 1; 5- to 12- μm voxel size) confirmed the presence or absence of calcifications in each specimen. Of the six specimens, three specimens had extensive calcification, defined as nodules or regions in which blood vessels were externally calcified. These specimens were examined by Raman spectroscopy. Of the three other specimens, two had small, diffuse nodules embedded in fatty tissue, which is too thick for optical spectroscopy. Only one of these was examined by Raman spectroscopy. The other had no calcification detectable by micro-CT. Importantly, the presence of necrotic tissue did not always indicate calciphylactic mineral nodules.

Raman spectra were collected with either a Raman microprobe (interrogating a tissue area $\sim 0.4 \text{ mm} \times 0.7 \text{ mm}$) or a handheld fiber Raman probe (interrogating a tissue volume $< 1 \text{ mm}^3$). The Raman microprobe system was locally constructed²⁴ (830 nm laser, $< 20 \text{ mW}$ laser power) and operated with 6 to 8 cm^{-1} resolution. The handheld fiber Raman probe measurements were conducted using a filtered N-around-1 probe (EMVision, Loxahatchee, FL) connected to a portable Raman spectroscopy system (Rxn 1, Kaiser Optical Systems; 785-nm laser, $< 20 \text{ mW}$ laser power) operated with 6 to 8 cm^{-1} resolution. The Raman probe was fixed to a locally constructed stand to prevent motion artifacts.

For publication quality, the microprobe and fiber probe integration times were 120 and 60 s, respectively. Probing the entire region of interest is currently limited by collection time. In principle, measurement time could be reduced to 1 to 3 s. Spectra were preprocessed as previously described.²⁵ The carbonated apatite phosphate band at 958 cm^{-1} was used as a signature of calciphylaxis.

Two specimens were examined with the microprobe and the third specimen was examined with the handheld fiber-optic probe. Microprobe measurements were guided by white-light imaging to identify regions of interest. Fiber probe measurements

were guided by manual interrogation to identify hard regions suspected of containing calcified tissue. Then, Raman spectra were collected from suspicious tissue and nearby normal tissue. As needed, tissue specimens were sectioned to gain access to embedded calcifications. Both specimens examined with the Raman microprobe were classified as early stage by micro-CT, with calcified vasculature and calcium precipitate $< 2 \text{ mm}$ diameter. The specimen examined with the handheld fiber-optic probe was classified as late stage by micro-CT, with calcium precipitate nodules $> 2 \text{ mm}$ diameter. These specimens all yielded Raman spectra with phosphate Raman bands at 958 cm^{-1} (Fig. 2). As expected, spectra from nearby tissues lacked a band at 958 cm^{-1} , indicating the absence of calcification. Raman microscopy of small diffuse nodules yielded spectra with strong lipid bands but no measurable phosphate bands.

The current study is limited by the small sample size. Because most debrided tissue is not calcified, most measurements were made on tissue that did not and should not generate a phosphate band. In the context of specimens from patients with calciphylaxis, when a tissue site yielded a strong apatitic phosphate signal, it originated from presence of calciphylaxis.

Our preliminary results suggest that Raman spectroscopy can be developed to improve clinical detection of calciphylaxis. Our previous experience is that spectroscopic evaluation of *ex vivo* specimens does not differ significantly from *in vivo* evaluation of superficial lesions.²¹

A key requirement for clinical translation is development of a handheld fiber-optic probe optimized to detect the clinical presentation of calciphylaxis. The probe must detect ectopic carbonated apatite at most locations, depths, and concentrations that occur *in vivo*. The probe must function despite varying tissue background, including contributions from serum, plaques, eschars, and lipids. Spectra must be collected in $< 2 \text{ s}$ and ideally, no more than 1 s. With rapid measurements, clinicians would be able to map the wound area and characterize wound margins for treatment and debridement. Our early results do demonstrate that noninvasive tissue characterization using Raman spectroscopy can become a viable option for calciphylaxis diagnosis and evaluation of the extent of this debilitating process.

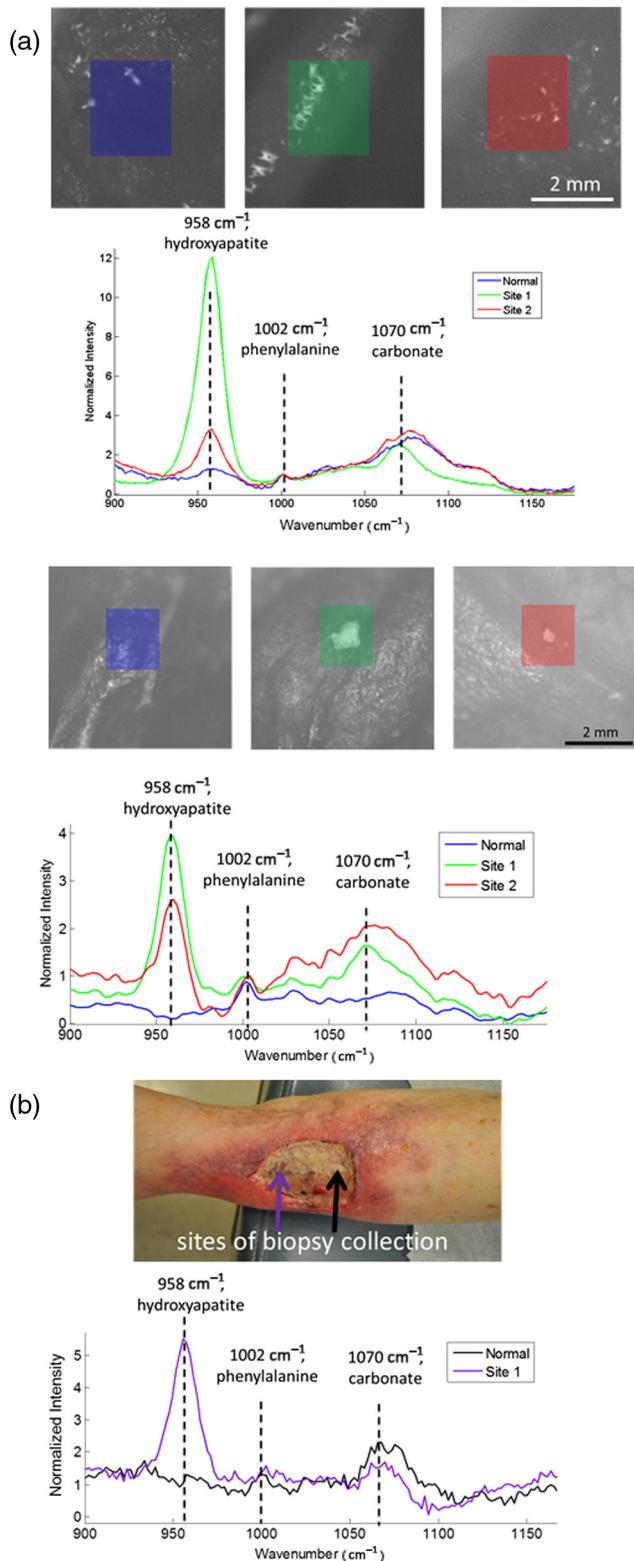


Fig. 2 Resected *ex vivo* tissue from patients presenting with calciphylaxis were examined by Raman spectroscopy. (a) Raman microprobe white-light imaging or (b) fiber-optic probe tactile response guided collocated Raman spectroscopy. Suspicious locations [green and red in (a), purple in (b)] had spectra consistent with apatitic mineral, noted by 958 cm⁻¹ band.

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