Raman spectroscopy for label-free identification of calciphylaxis

William R. Lloyd
Shailesh Agarwal
Sagar U. Nigwekar
Karen Esmonde-White
Shawn Loder
Shawn Fagan
Jeremy Goverman
Bjorn R. Olsen
Dolrudee Jumlongras
Michael D. Morris
Benjamin Levi
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1 Introduction

Calciphylaxis is a painful, debilitating, and premorbid 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.

Keywords: Raman spectroscopy; calciphylaxis; near-infrared light; light scatter; tissues.

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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 ∼0.4 mm × 0.7 mm) or a handheld fiber Raman probe (interrogating a tissue volume <1 mm^3). The Raman microprobe system was locally constructed (830 nm laser, < 20 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 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. 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 mm diameter. The specimen examined with the handheld fiber-optic probe was classified as late stage by micro-CT, with calcium precipitate nodules >2 mm diameter. These specimens all yielded Raman spectra with phosphate Raman bands at 958 cm^-1 (Fig. 3). 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.

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 s 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.
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References


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 apatic mineral, noted by 958 cm⁻¹ band.