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Detection of silica and calcium carbonate deposits in granulomatous areas of skin sarcoidosis by μFourier transform infrared spectroscopy and Field Emission Scanning Electron Microscopy coupled with Energy Dispersive X-ray Spectroscopy analysis

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ABSTRACT

Sarcoidosis is a multisystem inflammatory disease affecting different organs particularly lung, skin, eyes and joints. Characterized by noncaseating epithelioid granulomas, sarcoidosis is considered to be caused by a complex interplay between genetics and environmental agents while it still remains a disease of unknown etiology. 10 skin biopsies from patients with cutaneous sarcoidosis were included in the study. After polarized light examination (PLE) through optical microscopy, these skin biopsies have been investigated through μFourier transform (FTIR) infrared spectroscopy and Field Emission Scanning Electron Microscopy coupled with Energy Dispersive X-ray Spectroscopy (FE-SEM/EDX). Three biopsies showed a refractive material at PLE. FTIR and FE-SEM/EDX analyses indicate the presence of silica at the center of the granulomas in these three biopsies. Another striking result is related to the presence of calcite, a calcium carbonate at the periphery of the granulomas. To our best knowledge, this is the first time that the presence of this calcium carbonate has been reported. Such description at the submicrometer scale paves the way for a better understanding of the physicochemical processes related to sarcoidosis and will help clinicians to develop new diagnostic tools.

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1. Introduction

Sarcoidosis is a multisystem granulomatous disease affecting the skin in 25–30% of cases reported in [1]. Cutaneous lesions are widely variable; papular sarcoidosis, annular sarcoidosis, maculopapular eruption and lupus pernio are more common [2]. Disease onset peaks during the third and fourth decades of life have a higher incidence among women and are seen more frequently in Blacks than in Caucasians [2]. Sarcoidosis is characterized by non-caseating epithelioid and giant cells granulomas, the hallmark of the disease. The granulomatous inflammation observed in sarcoidosis is thought to be caused by a complex interplay between a genetic background, environmental agents, infectious antigens and T lymphocyte immune reactions [3].

Cutaneous sarcoidosis preferentially affects sites with a prior injury such as tattoos or scars and a polarizable material has been reported in almost 25% of cases suggesting that foreign materials could be a nidus for granuloma formation and a potential trigger for the disease [4–7].

Sarcoidosis, as observed in other granulomatous diseases, is associated with calcium metabolism disorders. High serum calcium is seen in 5–10% of patients [8], mainly due to a dysregulated production of calcitriol by activated macrophages forming the granulomatous lesions [9]. These metabolic disturbances may lead to deposit of calcium in various organs, including the skin, as seen in calcinosis cutis [10].

Sarcoidosis is a disease of which the causes are still unknown, although the role of environmental mineral particles is strongly suspected on the grounds of epidemiological data [11,12]. Nowadays, there are increasing interest and recent developments in nanotechnology in order to understand the effects of nanoparticles on living tissues [13]. Therefore, the importance of morphology and chemical analysis of pathological calcifications has been clearly underscore in different organs but never recognized in sarcoidosis [14–17]. The present study has been designed to investigate the physico-chemical characteristics of 1) intragranuloma polarizable foreign materials and 2) tissue deposits in biopsy samples of 10 cutaneous sarcoidoses. In order to describe their structural characteristics as well as their chemical nature at the subcellular scale, two different techniques were used: Field Emission Scanning Electron Microscopy coupled with Energy Dispersive X-ray Spectroscopy (FE-SEM/EDX) [18–20] and μFourier Transform Infra-Red (μFT-IR) [21–27] spectroscopy.

2. Material and methods

2.1. Patients

Ten samples of formalin fixed, paraffin embedded biopsies from cutaneous sarcoidosis patients, stained with hematoxylin and eosin (H&E), were retrieved from the Pathology Department of Hôpital Tenon APHP Paris (Pr I. Brochériou). Sarcoidosis patients were followed in the Dermatology Department (Pr C. Francès) or the Internal Medicine Department (Dr C. Bachmeyer) of Hôpital Tenon APHP Paris. The diagnosis of sarcoidosis has been made on clinical data and histopathology results [4]. In accordance with French legislation, no written informed consent was necessary. Clinical characteristics of patients and histopathology of biopsies are summarized in Table 1.

The first stage of this investigation employed optical microscopy and PLE analysis.

2.2. FTIR microspectroscopy

FT-IR data were used to localize and determine the chemical nature of the pathological deposits in the skin biopsies. As discussed in previous reports [20,23], skin biopsies were deposited on low-e microscope slides (MirrIR, Kevley Technologies, Tienta Sciences, Indianapolis). All the FT-IR hyperspectral images were recorded with a Spectrum Spotlight 400 FT-IR imaging system (Perkin Elmer Life Sciences, France), with a spatial resolution of 6.25 μm and a spectral resolution of 8 cm^{-1}. Each spectral image, covering a substantial part of the biopsy, consisted of about 30,000 spectra. All biopsies presumed to contain crystal deposits were analyzed with the Spotlight 400 FTIR imaging System in the mid

Table 1
Skin biopsies analyzed: clinical characteristics of the patients and histopathology.

n	Patient		Clinical presentation[b]		Biopsy		
	Age	Gender[a]	Site	Morphology	Site	Granulomas location	PLE[c]
1	69	M	Toes	Papules	Toe	Superficial and deep dermis	Negative
2	43	F	Nose Upper limb	Angiolupoid Plaque	Elbow	Superficial and deep dermis	Positive
3	31	M	Temple	Annular lesion	Temple	Superficial and deep dermis	Negative
4	42	M	Upper limbs Tattoo	Papules	Upper limb	Superficial dermis	Positive
5	42	M	Back	Papules	Back	Superficial and deep dermis	Negative
6	61	F	Upper limbs	Papules	Upper limb	Deep dermis and subcutis	Negative
7	49	M	Lower limbs	Papules	Lower limb	Subcutis	Negative
8	54	M	Lower limbs	Nodules	Lower limb	Deep dermis and subcutis	Negative
9	23	F	Forehead	Papules	Forehead	Superficial and deep dermis	Positive
10	21	H	Lip	Macro-cheilitis	Lip	Superficial and deep dermis	Negative

[a] M: male; F: female.
[b] Clinical presentation of the cutaneous lesions.
[c] PLE: polarized light examination.