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Characterization of Nanostructured Calcium Carbonate Found in Two Ancient Etruscan Tombs

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Abstract. In this work, we have analyzed and characterized a white *patina*, that covers the walls and frescoes of two Etruscan tombs located in the Monterozzi's necropolis of Tarquinia: the *Tomba degli Scudi* and the *Tomba Bartoccini*. The powders have been retrieved from these areas before and during the intervention of the restorers and then SEM\EDX, FT-IR and XRD have been performed for the characterization. These white powders are formed by rod-like and crystalline calcium carbonate, the length of which is a few micrometers, while the thickness has an average value of about 400 nm. This particular structure can be traced back to the moonmilk, a particular form of calcium carbonate, normally found in karst caves or other underground environments. Besides, the presence of some microorganisms in close connection with this material provides an important clue of its biological origin.

INTRODUCTION

The necropolis of Monterozzi is considered as one of the most important archeological sites for the Etruscan historical period for the presence of almost 200 decorated tombs, that represents an incredible document about the pre-Roman painting art and also like a precise look towards the Etruscans' quotidian life and mythology [1, 2]. The *Tomba degli Scudi* was discovered in 1870 and it is one of the largest tombs of the area. It takes its name from the fourteen shields painted in the burial room as a representation of the family power in the military sphere and consists of a central atrium and three lateral rooms, recreating an Etruscan house [3, 4]. Instead, the *Tomba Bartoccini* has a planimeter of four rooms arranged in a cross pattern. It is also called *Tomba dei templari* for the presence of graffiti dated around the thirteenth century, which show a later use of this tomb as a site for some mysterious rites done probably by a group of templars [5].

We previously described the microbiological analysis of the frescoes of the central atrium of *Tomba degli Scudi* during the restoration activity, and the microbial community present in the burial chamber, that was still left untouched [6, 7]. It is important also to claim, that, during the sampling of this deposit, the whole tomb was not opened to the public, and its access was limited to a very strict number of people.

In this work, we have analyzed the samples coming from the burial chamber of the *Tomba degli Scudi* and a lateral chamber of the *Tomba Bartoccini* with different techniques as electron microscopy, X-ray microanalysis (XRD), Fourier transform infrared spectroscopy (FT-IR) and X-ray diffraction (XRD). In both cases, we have found structures nano-rod like of calcium carbonate, with similar crystalline structure, although the powder coming from *Tomba Bartoccini* appears to be rougher in shape.

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MATERIALS & METHODS

Sample Collection

Samples were collected from the walls with sterile cotton swabs, and samples intended for SEM analysis were observed within 24 hours. The temperature in the tombs is monitored all over the year and it remains constant, 16°C; the humidity is 99-100%. This study was performed during the restoration of the two tombs; the white *patina*, which correspond to moonmilk, was completely removed in the *Tomba Bartoccini*, except for a 5-centimetre square left in place, as a control. Samples of the moonmilk from *Tomba degli Scudi* were collected in the burial chamber, not yet restored and where the moonmilk is still in place.

Electron Microscopy and X-Ray Microanalysis

A small portion of the sampled powder has been mounted upon a SEM stub with a biadhesive conductive graphite tape and observed with a FESEM Zeiss Auriga 405, equipped with a Bruker Quantax EDX detector (energy resolution: 123 eV Kα Mn).

X-Ray Diffraction

XRD pattern was acquired using a Philips Analytical PW1830 X-ray diffractometer, equipped with Ni β-filtered Cu-Kα1 (1.54056 Å) radiation, in the 2θ range from 5 to 80° with a step size of 0.02° and a time for step of 3.5 sec. The data were collected with an acceleration voltage and applied current of 40 kV and 30 mA, respectively. The crystalline phases in the resulting diffractogram were identified through the COD database (Crystallography Open Database – an open-access collection of crystal structures) [8]. The average crystallite sizes were calculated with the Scherrer's equation on the most intense phase peak plane.

Fourier Transform infrared analysis

The chemical composition of the as-received samples has been studied by Fourier transform infrared (FT-IR) analysis. Infrared measurements were carried out with a Bruker Vertex 70 spectrometer (Bruker Optik GmbH) equipped with a single reflection Diamond ATR cell. Spectra were recorded with a 3 cm^{-1} spectral resolution in the mid infrared range (400–4000 cm^{-1}) using 512 scans.

RESULTS & DISCUSSION

In our previous report [7], we showed that a white *patina* hides the frescoes of the burial chamber of the *Tomba degli Scudi*. The same situation was present also in the *Tomba Bartoccini*; in order to investigate if this *patina* is similar to that of *Tomba degli Scudi*, we collected samples from *Tomba Bartoccini* during restoration and compared the structure of these *patina*. Figure 1 shows the area where the powder has been sampled.

Although under an optical inspection the samples appear quite similar, under the SEM analysis, the powders coming from the two tombs shows some differences in the morphology (Fig. 2). In fact, in both cases, the crystals appear to be in a rod-like structure, but the ones from *Tomba degli Scudi* have an inferior thickness of about 90 nm, several microns long and a more regular rectangular shape (Fig. 2a and 2b).

Instead, the others result to be almost a micron in thickness and have an irregular form with tapering on the side. These structures have been also described in detail in the work of Caiellau et al. [9], where similar calcite fibers have been found in a cave in Pèrigord (South-western France) and Villiers (Swiss Jura Mountains).

FIGURE 1. Picture of the unrestored part of *Tomba Bartoccini*, where the white *patina* is visible.

In Fig. 3, the EDX spectra and the tables with the quantification, coming from the rods of *Tomba degli Scudi* and *Tomba Bartoccini* are presented.

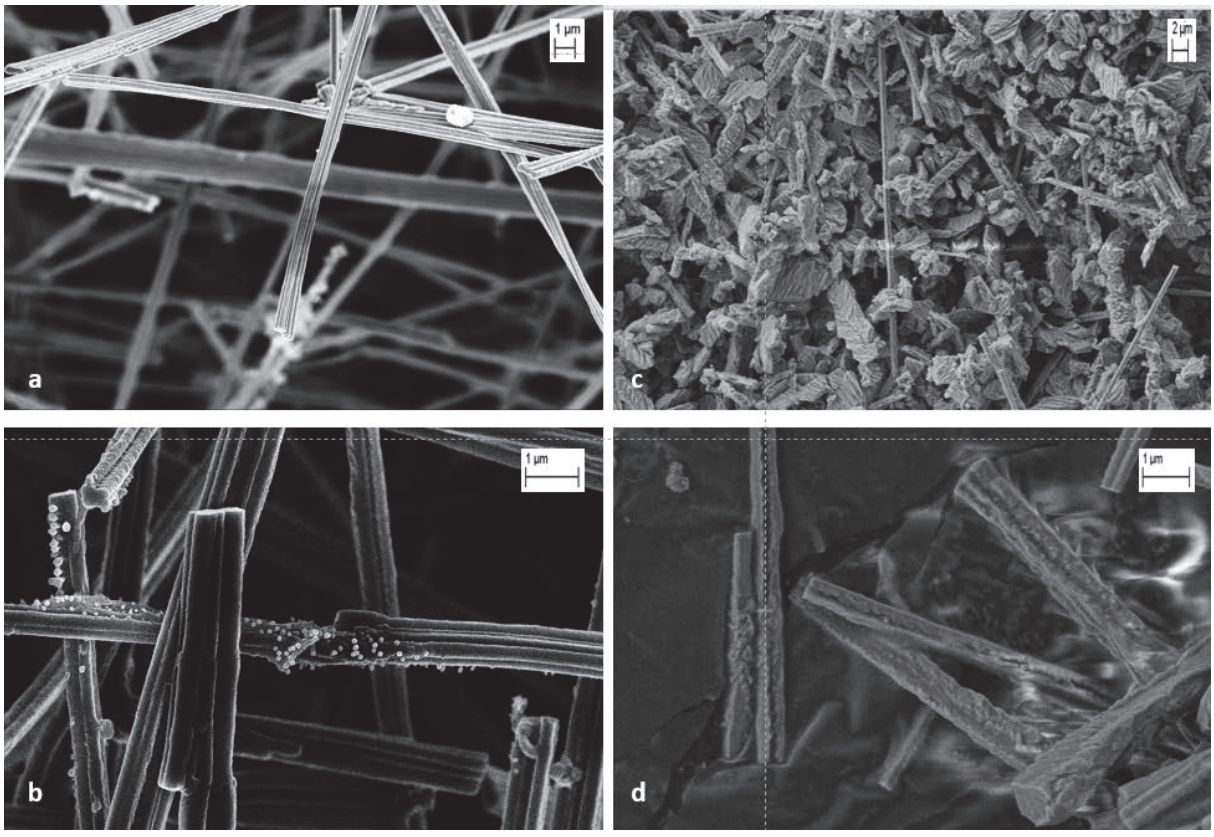
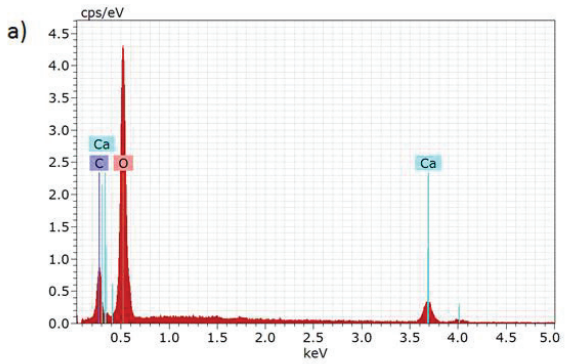
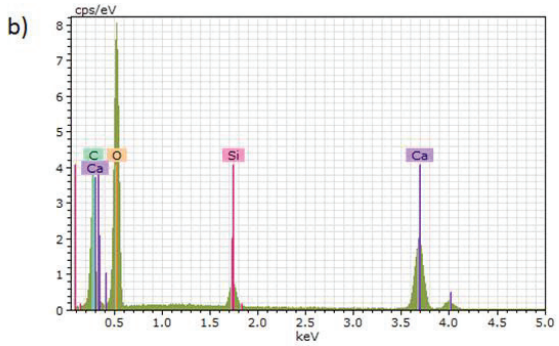


FIGURE 2. SEM images of the rod-like crystals at two different magnifications, coming from the *Tomba degli Scudi* (a, b) and *Tomba Bartoccini* (c, d).

The ratio for C, Ca and O, results to be quite close to the stoichiometry of the calcium carbonate, except for the presence of small quantities of Si, which can be derived from some silica present inside the interaction sphere of the probe.



El	AN	Series	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
O	8	K-series	67.23	75.75	3.98
C	6	K-series	9.05	13.58	0.73
Ca	20	K-series	23.73	10.67	0.50
		Total:	45.51	100.00	100.00



El	AN	Series	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
O	8	K-series	55.61	63.21	8.42
C	6	K-series	15.08	22.83	2.59
Ca	20	K-series	25.94	11.77	1.12
Si	14	K-series	3.38	2.19	0.22
		Total:	100.00	100.00	

FIGURE 3. EDX spectra and compositional ratios of the nanorods found in *Tomba degli Scudi* (a) and *Tomba Bartoccini* (b).

To confirm that these nanostructures are calcium carbonate, we have also performed FT-IR and X-Ray diffraction (Fig.4 and 5).

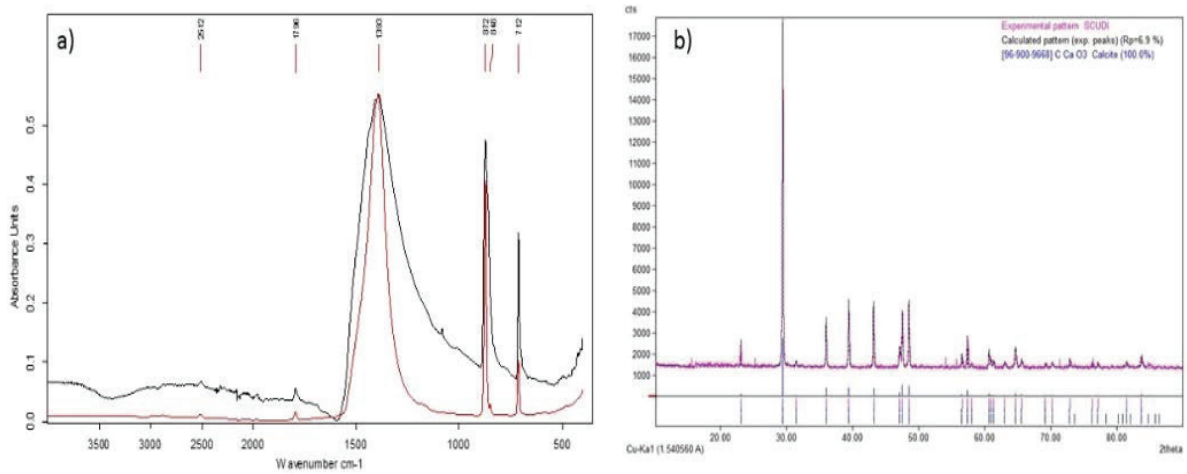


FIGURE 4. FTIR (a) and XRD (b) spectra from *Tomba degli Scudi*. The red line in Fig. 4a is the calcite FT-IR refence spectrum.

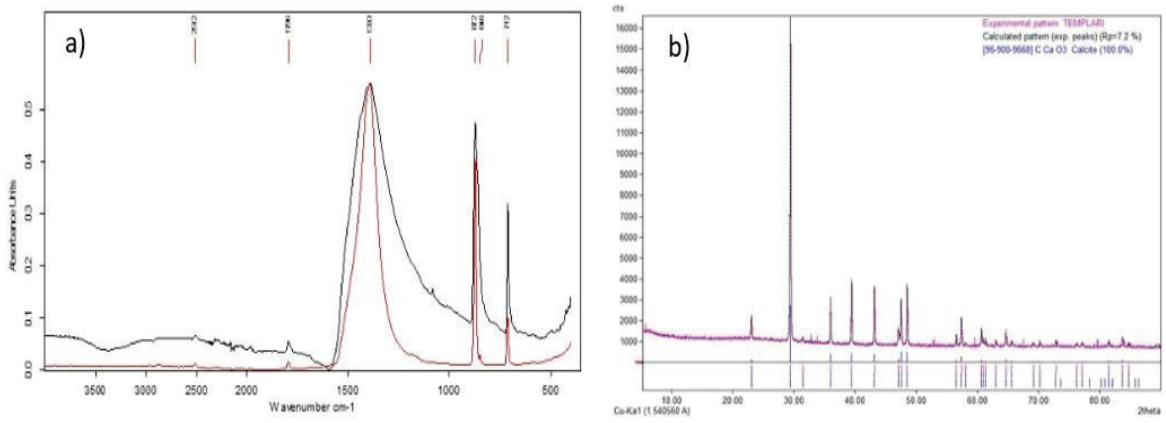


FIGURE 5. FTIR (a) and XRD (b) spectra from *Tomba Bartoccini*. The red line in Fig. 5a is the calcite FT-IR refence spectrum.

Both the samples show in their FT-IR spectra (Fig. 4a and 5a) only the characteristic infrared frequencies of carbonate ion vibrations in calcite at 1796, 1383, 872 and 712 cm^{-1}.

Their composition is also confirmed by the XRD diffractograms (Fig. 4b and 5b), where the patterns provided by these nanorods fit perfectly the reference calcite peaks. The crystallite size for the sample coming from the *Tomba degli Scudi* is calculated as 68 nm, while the one from *Tomba Bartoccini* gives a slightly different result (62 nm), but always near to the previous value.

This result indicates that both the powders are calcite, although different in shapes, as shown in Fig. 2. Using the classification made by Caiellau [8], we can recognize the calcite fibers from *Tomba degli Scudi* as belonging to the smooth type, while the ones from *Tomba Bartoccini* can be considered inside the serrated-edge type. It is largely accepted that the origin of this form of calcite is biogenic, but at the same time, the environment plays a role itself, in the subsequent formation of the final structure; indeed, in both the samples, we noticed the presence of unidentified microorganisms among the nano-structures (Fig. 5). Besides, in our previous work [6], we have demonstrated how some microbial species extracted from the *Tomba degli Scudi* can produce calcite [10]. This process is a physiological adaptation of these microorganisms to fix calcium ions absorbed by the surrounding environment and that are toxic in high concentration [11].

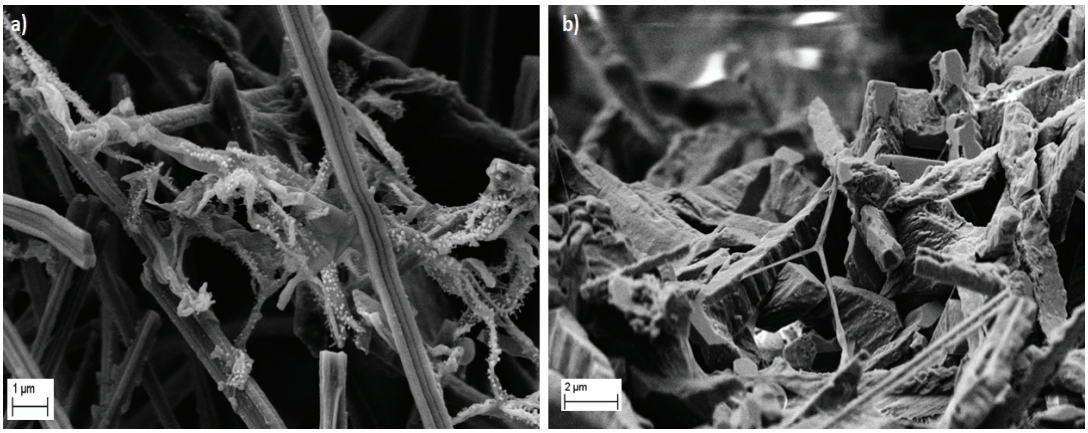


FIGURE 6. SEM images of unidentified microorganisms among the calcite nanorods from *Tomba degli Scudi* (a) and *Tomba Bartoccini* (b).

CONCLUSIONS

In this paper, we have analyzed and compared two samples of a white powder covering the walls and the paintings of two different Etruscan tombs of Monterozzi's necropolis. FT-IR and XRD techniques have shown very similar spectra, demonstrating that, in both cases, this deposit is a crystalline form of calcite with a nanorod-like shape. Nonetheless, the calcium carbonate crystals coming from *Tomba degli Scudi* appear to be finest and better structured in this rod structure, while the other has a rougher and bigger shape. The difference between the two samples can be related both to the presence of different microbial communities, having microorganisms able to produce or eliminate the calcite, and to the surrounding environment in which these deposits are formed. For this reason, a series of analysis about the microbial communities are required, comparing a larger number of tombs of the area and also making an in-depth study of the *macco*, that seems to play a decisive role in the growth process. It's also important to note the mechanisms that lead to the formation of these nanorods is unique in this necropolis, and that this *patina*, covering entirely the walls of the tomb, protect the frescoes rather that damaging them, opening an important future application to the conservation of the cultural heritage.

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