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Analysis of Moonmilk Nanofibers in the Etruscan Tombs of Tarquinia

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Abstract. The moonmilk is a secondary calcite deposit (speleothem) formed by nanofibers of calcite and commonly found in karst caves. While its biogenic origin is widely accepted, the mechanism of calcite nanofibers production remains unclear. Crystallization and formation mechanism of calcium carbonate nanostructures are of high interest in different fields, such as geomicrobiology (rock-microbes interactions), astrobiology (biosignatures), medicine (antibiotics producing bacteria), nanotechnology (calcium carbonate nanofibers) and recently, archeology. The discovery of the moonmilk in the hypogeal tombs of the ancient necropolis of Tarquinia (Italy), provides a unique opportunity to compare moonmilk crystal shapes that originated from two type of rocks, *Macco* (a calcarenite) and *Sabbione* (a hybrid sandstone). We analyzed, through a SEM analysis, moonmilk samples from twelve tombs in the necropolis area and found that rocks provide an ideal environment for the formation of the nanofibers; the *Sabbione* promoted the growth of a finer and thinner carbonate nanostructures, probably due to a slower growth kinetics caused by a low calcium value present in this rock.

INTRODUCTION

The ancient Etruscan Necropolis of Tarquinia is an UNESCO World Heritage site (Viterbo, Italy), in which more than 200 painted hypogeal tombs (dated from the 7th to the 2nd century B.C.) were discovered. The tombs were excavated in a yellow bioclastic calcarenite, called *Macco* [1, 2], formed during the Pliocene era (5 million years ago). A hybrid sandstone rich in fossils called *S*abbione, is also present in a small area of the Necropolis [2]. The walls and ceilings of the tombs of Tarquinia were covered by a white patina, referred to as moonmilk [3, 4]. This calcite precipitation is induced by the metabolic activities of a hypolytic microbial community, which live in environments with high calcium content [5, 6, 7, 8, 9, 10]. In a previous study, we described that this massive, widespread calcite deposition on the walls preserved for centuries the Etruscan mural paintings, and once removed, revealed new information on the ancient Etruscan mode of life [11]. Unfortunately, the mechanism of the calcite nanofibers formation remains unknown and until now these fibers cannot be reproduced in laboratory conditions. Thus, the discovery of the moonmilk in many tombs of the Necropolis allowed to sample in an area of several kilometers and to compare the rocks in which the tombs were carved, evaluating not only the influence of the surrounding environments in their deposition, but also to hypothesize the possible steps in the formation of the nanofibers.

FIGURE 1. Map of the Tarquinia's necropolis, where the analyzed tombs have been enlightened with a different colour according to their underneath rock substrate, red for *Macco* and blue for *Sabbione*.

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MATERIALS AND METHODS

Sample Collection and Environmental Parameters

Moonmilk deposits were collected from the 12 tombs of the Monterozzi Necropolis [12] (Tab.1) using sterile scalpels (about 0,5 g) and kept in 10 ml sterile tubes. Samples intended for SEM analysis were processed within 24 hours from collection. Due to the archaeological importance of this site, we were allowed to extract only one sample from each tomb. The relative humidity in the tombs typically varied in the range 95-98% and the temperature between 16-18°C.

Scanning Electron Microscopy

SEM micrographs were performed using a Field Emission Scanning Electron Microscopy (FESEM) Zeiss Auriga 405, with a chamber room that maintains a pressure of about 10^{-5} - 10^{-6} mbar. Before mounting the samples inside the microscope, the specimens were coated with 20 nm of chromium using a Quorum Q150T sputter.

RESULTS AND DISCUSSION

We previously reported a direct correlation between the calcium content of the rocks in which the tombs were carved, and the thickness of the biogenic moonmilk deposit: in Macco, with high calcium values, a thick deposition was observed, while in the Sabbione, with low calcium content, a very thin moonmilk layer was present [3]. We also observed a variability in calcite shapes in the moonmilk samples from the Tomba Bartoccini and Tomba degli Scudi, which are carved in *Macco* and *Sabbione*, respectively [4], We hypothesized that the different composition of the rock substrates could also influence the shape of the moonmilk nanostructures and to verify this hypothesis we sampled moonmilk from 12 tombs, which are representative of the entire area of the Etruscan Necropolis (Fig. 1). Three out of twelve tombs, Tomba del Cardinale, Tomba dell'Orco and Tomba degli Scudi, were carved in the *Sabbione*, while the remaining tombs were carved in *Macco* (Tab. 1).

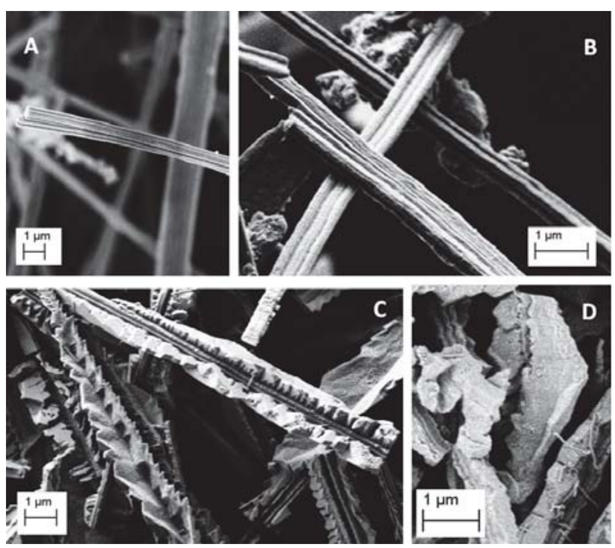

FIGURE 2. SEM micrographs of different moonmilk shapes: a) single smooth nanorods; b) multiple smooth nanorods; c) serrated-edge type; d) micrometric rough structure.

The moonmilk samples were analyzed through the SEM analysis and categorized following the work of Cailleau et al. [7], listing the nanofibers from very thin and smooth nanorods to more complex structures [13]. According to Cailleau, single or multiple parallel units of nanostructures (Fig. 2A 2B) can be associated to the first steps of moonmilk formation [7], and indeed, they are abundant in many samples and in particular in the moonmilk deposits

TABLE 1. List of the analyzed Etruscan tombs, with their position, expressed in decimal degrees, rock substrates and Moonmilk shapes, described according to Cailleau classification [7].

Tomb's name	Position	Rock	Observed Moonmilk shape
Tomba Bartoccini	42.249536, 11.771456	*Macco*	Single and multiple smooth nanorods, rough micrometer serrated edge types
Tomba Bertazzoni	42.239539, 11.791551	*Macco*	Multiple smooth nanorods and rough micrometer serrated edge and complex types
Tomba dei Leoni Rossi	42.240358, 11.794708	*Macco*	Single and multiple smooth nanorods, rough micrometer serrated edge types
Tomba Maggi	42.248375, 11.769660	*Macco*	Single and multiple smooth nanorods, rough micrometer serrated edge types
Tomba delle Pantere	42.241230, 11.795545	*Macco*	Single and multiple smooth nanorods, rough micrometer serrated edge types
Tomba dei Pigmei	42.241230, 11.795545	*Macco*	Rough micrometer serrated edge and complex types
Tomba delle Sculture	42.248050, 11.770867	*Macco*	Single nanorods and rough micrometer serrated edge
Tomba dei Vasi Dipinti	42.246032, 11.784392	*Macco*	Single and multiple smooth nanorods and rough micrometer serrated edge and complex types
Tomba del Vecchio	42.246022, 11.784386	*Macco*	Single and multiple smooth nanorods and rough micrometer serrated edge and complex types
Tomba del Cardinale	42.248172, 11.778390	*Sabbione*	Single and multiple smooth nanorods
Tomba dell'Orco	42.246699, 11.780554	*Sabbione*	Single and multiple smooth nanorods
Tomba degli Scudi	42.247597, 11.777425	*Sabbione*	Single and multiple smooth nanorods

from the tombs carved in *Sabbione*. On the contrary, in samples taken from the *Macco* area, these nanostructures are in minority, and the moonmilk fibers have a micrometric thickness, with a prevalence of serrated edge or with more complex shapes (Fig. 2C & 2D).

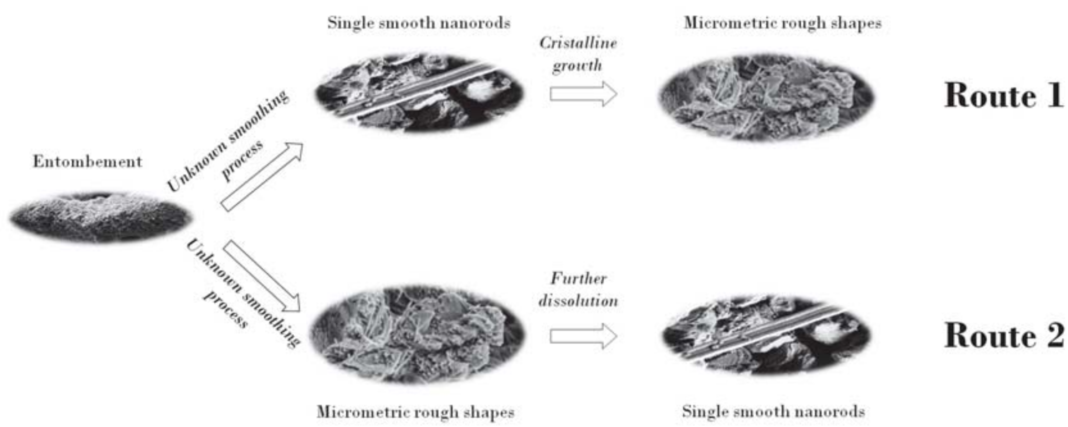

FIGURE 3. Possible routes for the moonmilk formation

The number of moonmilk samples, in which these structures are observed, provide clues to explain the process that regulates moonmilk formation in this area. In fact, as already demonstrated [14], the calcium content in the rocks is the most important parameter responsible for the thickness of the biogenic moonmilk deposition; as illustrated in Fig. 3, two routes can be proposed to explain the moonmilk formation.

The entombment of microbial species can be considered as the first step of the process (Fig. 4), as previously proposed by Cailleau [7]. Subsequently, the thin smooth moonmilk formation is promoted by the microbial community

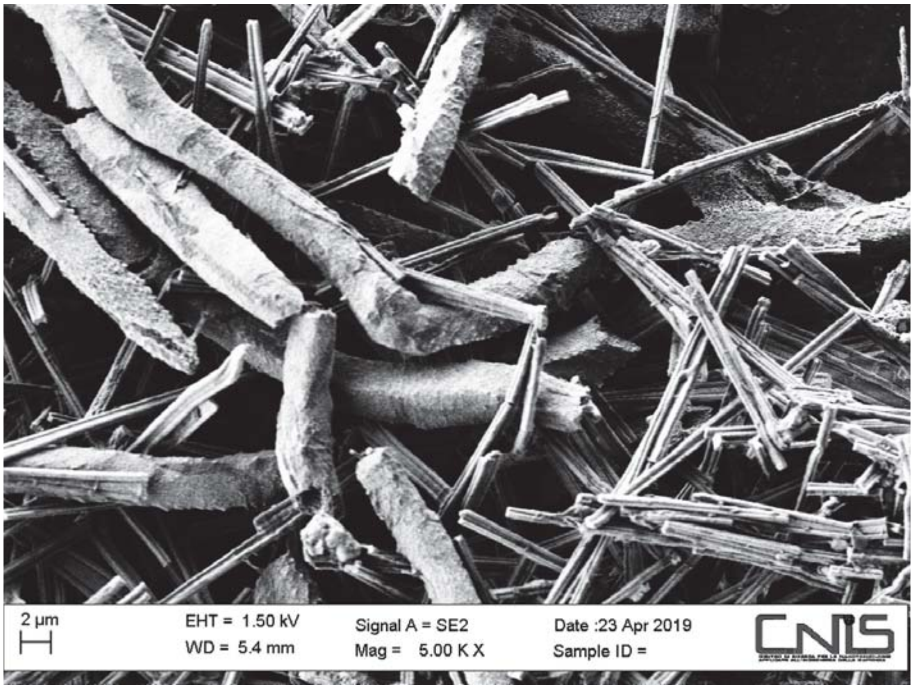

FIGURE 4. SEM micrographs of entombment formations in the *Tomba dei Vasi Dipinti*.

and by the chemical-physical environmental parameters present in these tombs that afterwards, by crystalline growth, could result in complex shapes formation (route 1). A second route can also be suggested: after the entombment, the calcium carbonate is subject to dissolution, leading to rough micrometer structures at first, and subsequently to smaller and thinner shapes of calcite as a final product. Indeed, from the moonmilk microbial community, many bacterial species able to dissolve calcium carbonate were recovered [11]. To verify these non mutually exclusive routes, a depth knowledge of microbial metabolism and the chemical elements and microfluidic properties of the rocks will be necessary.

CONCLUSIONS

In this work we had the possibility, for the first time, to collect and compare several moonmilk samples from the underground tombs in the Tarquinia's Necropolis, in a large area. From these observations, we propose two non-mutual exclusive hypotheses, for the different steps in moonmilk formation. Both argue for a direct correlation between the nanofibers size/shape and the calcium content in the rocks. Thus, in addition to a specific microbial community which promotes the first steps of moonmilk formation, an important role in this process is the rock substrate which influences the thickness of moonmilk deposition.

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