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SCANNING ELECTRON MICROSCOPE (SEM) SHOWS UNDESIRABLE EFFECTS OF ULTRASONIC CLEANING ON RECENT MOLLUSK SHELLS

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ABSTRACT

We evaluated the effects of ultrasonic cleaning on mollusk shells using scanning electron microscopy (SEM) with energy dispersive X-ray (EDX) analysis. The objective was to investigate the effects of this cleaning approach on the organic layer of the shells (the periostracum) and to assess the efficacy of a thin coating of consolidant to protect shells from vibrations. We found that ultrasonic cleaning caused abrasion of the shell surface. Treated specimens showed a flaky exterior, while non-treated specimens showed a more homogenous surface. Pre-treatment with TEOS-based consolidant did not prevent loss of the superficial layer and precipitated solid crystals on the surface. Our findings indicate that ultrasonic cleaning should be used with caution for fragile specimens because it can substantially alter the surface of mollusk shells.

Keywords: mollusk; shell; ultrasonic cleaning; conservation; scanning electron microscope

RESUMO [in Portuguese]

Avaliamos os efeitos da limpeza com ultra-sons em conchas de moluscos usando microscopia eletrónica de varrimento (MEV) com análise de raios-X de energia dispersiva (EDX). O objetivo foi investigar os efeitos dessa abordagem de limpeza sobre a camada orgânica das conchas (o periostracum) e avaliar a eficácia de um revestimento fino de consolidante para proteger as conchas das vibrações. Verificou-se que a limpeza ultra-sónica causou abrasão da superfície da concha. Os espécimes tratados mostraram um exterior mais quebradiço, enquanto os espécimes não tratados apresentaram uma superfície mais homogênea. O pré-tratamento com consolidante TEOS não impediu a perda da camada superficial e precipitou cristais sólidos na superfície. Os nossos resultados indicam que a limpeza ultra-sónica deve ser usada com cuidado para espécimes frágeis, pois pode alterar substancialmente a superfície de conchas de moluscos.

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INTRODUCTION

The anatomical and functional characteristics of mollusk shells have long played an important role in taxonomic studies to identify species (Harper, 1997). However, interest has recently broadened intensively to encompass issues such as developmental mechanisms (Araujo et al., 2014), ultrastructure (see a review in de Paula and Silveira, 2009), and molecular analysis (Geist et al., 2008).

Shells are an efficient tool for surveying biodiversity due to their easy handling and their marked morphological characteristics. Mollusk shells have two layers of natural composition: the periostracum and the ostracum. The periostracum, the outermost layer of shells, consists entirely of organic material, chiefly proteins. As this delicate coating (conchiolin) covering specimens is useful for zoological and taxonomic identification, its conservation is of particular relevance. See Harper (1997) for in depth data regarding the differentiation process and also for specific studies based on the use of SEM technologies to identify species (Korniushin, 2000). Recently developed techniques make the periostracum of mollusks a key character for diagnosing species, in particular in museum specimens (Leonel et al., 2006). The ostracum is the layer below the non-calcified periostracum and it consists of several calcified layers with a wide variation in their microstructures The ostracum often consists of prismatic aragonite crystals, but it is calcitic in most pteriomorph bivalves and in some gastropod taxa (Furuhashi et al., 2009).

Mollusk shells housed in museum collections may suffer various alterations. The most frequent of these is cracking, caused by rapid changes in temperature and humidity (Child and Buttler, 1996; Morton, 2006). Shells may also undergo chemical destructive processes such as efflorescence (also called Bynesian decay; Tennent and Baird, 1985; Sturm et al., 2006; Caracanhas Cavallari et al., 2014). As shells are widely used in biological research, the importance of their long-term conservation cannot be overlooked (Leonel et al., 2006; Sturm et al., 2006).

Removing dust, dirt and biological contaminants (such as mould, fungi, and bacteria) from shells with a cleaning treatment improves conservation (Sturm et al., 2006; De Prins, 2007). The cleaning process also highlights distinguishing features of shells, particularly on the aperture area, which is often covered with soil remnants. One of the most commonly used treatments to remove dirt and soil is mechanical friction. However, as this method is not suitable for micromollusks, cleaning could be performed using an ultrasound tank, as described by other authors (Smith, 1962; Sturm et al., 2006). Studies regarding cleaning of shells, however, are scarce.

The use of an ultrasonic tank is relatively common in the restoration of inorganic materials and preparation of fossil specimens in order to remove dirt and separate the matrix from the fossil (Kuban, 2004). One of the advantages of this technique is the simplified handling of the sample since the vibrational effect driven through a liquid solution induces cavitation, that is, the formation of micro-bubbles that grow and collapse rapidly (Suslick, 1989). These effects cause solid surface erosion or eventual breakage of particles, helping to remove the soil and dirt (Ensminger and Stulen, 2008:327; Santos et al., 2011).

The Natural History Museum of Barcelona (MCNB) recently launched a project to promote citizen participation in the study of Catalan terrestrial malacofauna. A representative collection of this fauna is being assembled as a new resource to provide visitors with reliable information to identify their own samples. As specimens in the reference collection must be as clean as possible, we conducted a thorough cleaning process of the whole collection.

The cleaning treatments followed general conservation criteria (AIC, 1994; ECCO, 2003; ICOM-CC, 2006) and were carried out by one of the authors (MR) during the course of a Master's Degree in Management of Conservation-Restoration Projects at the University of Barcelona in 2013. Throughout this process, the opportunity arose to collaborate with the Scientific and Technologic Centre at the University of Barcelona (CCITUB) and use of their scanning electron microscope (SEM). Because only one SEM session was carried out, we scanned only a small number of specimens. Extensive comparative studies were ruled out because the use of SEM was not planned when the project was developed. We therefore present this experiment as a preliminary work.

The goal of this study was to evaluate the suitability of cleaning micro-mollusk shells (<10 mm) through immersion in ultrasonic baths. Factors taken into account were cleaning effectiveness, the physical integrity of treated samples (the emergence of micro-fissures, abraded surfaces, flaking or desquamation), and the role of a pre-consolidation treatment to protect the physical integrity of the shells.

MATERIALS AND METHODS

For this study we chose three specimens in a good state of conservation (physical integrity) belonging to a common species of terrestrial mollusk *Chondrina tenuimarginata* (Gastropoda: Pulmonata: Chondrinidae - accession number MZB 89-1041 from the MCNB collection; Figure 1).

Each shell underwent different direct conservation processes (Table 1):

Sample 1 (S1): A non-treated sample (control sample) for comparing the effects of cleaning and to observe the natural, unaltered morphology of shells.

Sample 2 (S2): An exclusively ultrasonically cleaned sample.

Sample 3 (S3): A pre-consolidated and ultrasonically cleaned sample.

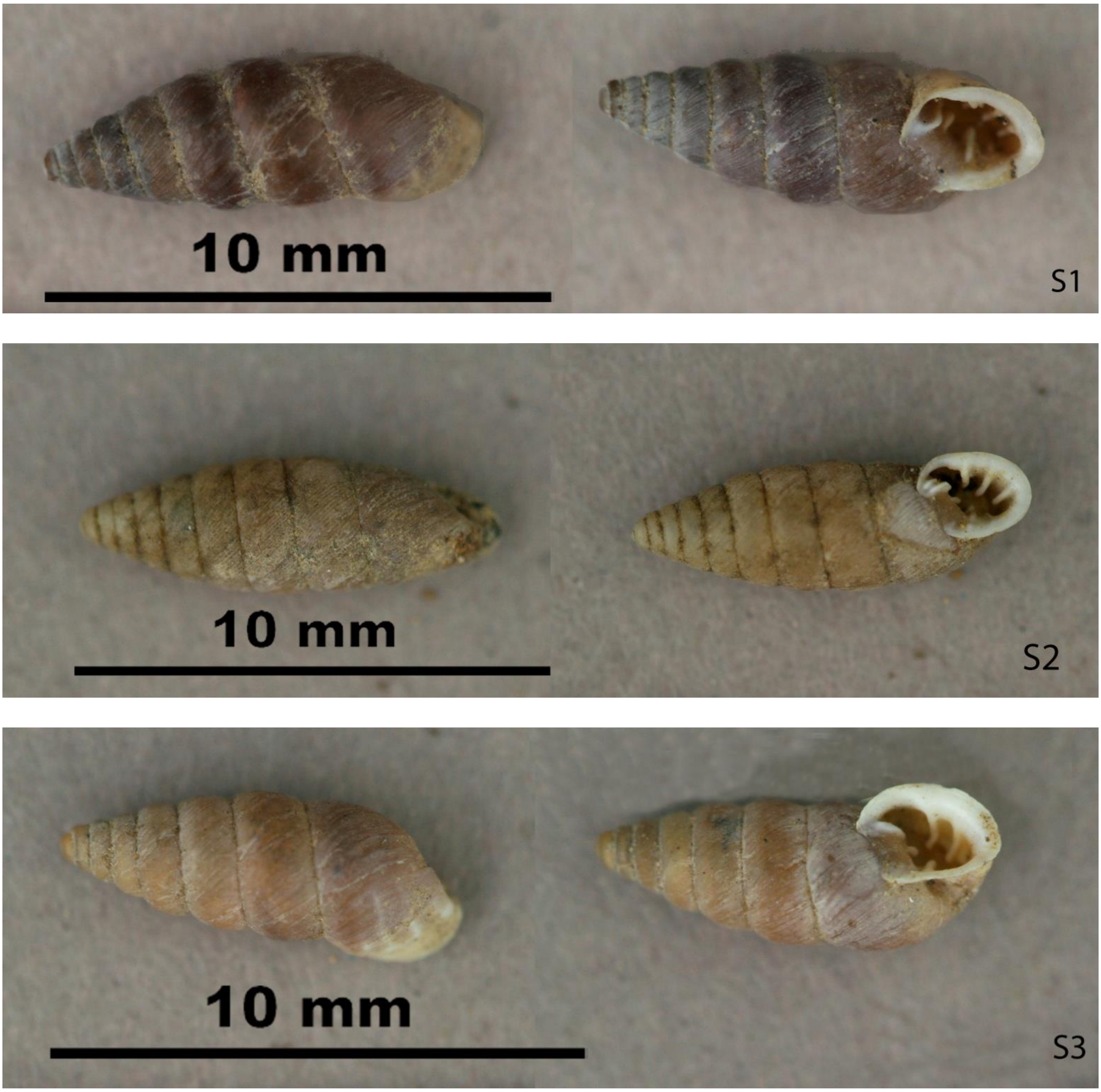

Figure 1 - Images of the three samples *Chondrina tenuimarginata* before treatment. Image by Marina Rull Aguilar.

Table 1 - Summary of treatments applied to samples.

Sample	Consolidation	Ultrasonic cleaning / Neutralization
S1 Control sample	**NO**	**NO**
S2	**NO**	**YES**
S3	**YES**	**YES**

Pre-consolidation treatment (S3) was done using a TEOS-based consolidant, ethyl silicate Estel 1000® (by CTS). The coating was applied using a paintbrush, the most commonly used procedure for consolidation treatments in natural history collections. Before applying the cleaning treatment, the consolidant was allowed to cure for a month (silicate polymerization). The siliceous consolidant was chosen since it allows good penetration due to its formulation and the small dimensions of the monomer seeping into cavities, cracks and pores without a plasticizing effect (Tennent and Baird, 1985; Öztürk, 1992; Morton, 2006; Sturm et al., 2006; De Prins, 2007), allowing superficial cleaning after consolidation. Several studies have shown the effectiveness of TEOS-based consolidants applied on stony supports of archaeological and architectural objects (Price, 1996; Rodriguez-Maribona and Cano, 2000; Meyer and Smith, 2008). TEOS-based consolidants have usually been used in the consolidation of siliceous materials but their effectiveness has also been tested filling narrow voids between calcite grains and, eventually, locking particles undergoing granular disintegration in limestone (Danehey et al., 1992; Price, 1996:55; Ruedrich et al., 2002). Thus, we considered that the use of a TEOS-based consolidant would penetrate the calcareous substratum of the shell.

We discarded the use of other consolidants suggested in previous studies (Sturm et al., 2006; De Prins, 2007). These are usually applied as a protective coating that consolidates by adhesion and creates an external layer similar to varnish with a low penetration capacity. This method does not allow subsequent surface cleaning (see Morton, 2006, for examples of protecting coatings for conservation and prevention of periostracal loss in museum collections). In any case, the use of consolidants and coatings in natural history specimens should be fully justified because it can interfere with future analysis (Sturm et al., 2006).

Cleaning consisted of sonicating samples for 60 seconds in a solution of distilled water with 2% neutral soap Derquim ®LM 02 to break the surface tension of water. Samples were neutralized in a solution of distilled water for 60 seconds. Ultrasound treatment was carried out in an ultrasonic tank Branson 8200 at a frequency of 40Hz and a voltage of 120v. Unfortunately, we were unable to control for other parameters (such as frequency, intensity or temperature) due to the technical limitations of the equipment.

To assess the qualitative dirt level before and after cleaning, we observed the shells using a stereomicroscope Kyowa Optical model SD-2PLQ.

All the experimental samples were directly examined without prior preparation (Cortadellas et al., 2012) using a SEM (model Quanta, 200 XTE 325/D8395 series, manufactured by FEI Company, Netherland) at the CCITUB facilities. The images were taken using the detector LFD at different magnifications (200 X to 1,537 X) as well as with "high vacuum mode" at 20KV of HV (high voltage) and working distance (WD) 9.6 mm, 9.7 mm, 9.8 mm and 10.2.

Up to five microanalysis for sample are performed with Energy Dispersive X-ray (EDX, included to the SEM microscope) and EDAX Genesis as the software for detecting chemical elements.

RESULTS

Observations using a stereomicroscope at 4X magnification allowed a qualitative evaluation of dirt level. After cleaning treatment, the shells presented a low dirt level and alterations were not visible. However, electron microscopy showed clear differences between samples.

Observation of sample 1 under SEM (control sample, Figure 2, S1) showed that the surface was quite homogeneous but irregularities were visible, such as desquamation consisting of subjacent layers in a grainy and crystallized texture. Spot chemical analysis (EDX) also detected superficial deposits, mainly non-metallic elements such as oxygen, carbon, and potassium, with predominance of calcium and very low representation of metallic elements such as aluminum and iron. Dirt and soil appeared chiefly in the sutures and mostly in the aperture. No fissures or cracks were observed.

Sample 2 (ultrasound cleaning treatment only, Figure 2, S2) showed a relatively homogeneous surface but generalized erosion of the outside shell. It presented detachment of the outermost layer, exposing a grainy and crystal texture, visible in the affected zones. Where the outermost layer was conserved, it appeared darker in the images. Small deposits of irregular morphology were seen on the surface. The chemical analysis identified these deposits as a mixture of non-metallic elements such as oxygen, carbon, potassium, sulphur, phosphorus, sodium, and manganese, but with a notable predominance of calcium. These elements may be the remains of external dirt that ultrasonic cleaning treatment failed to eliminate.

Several microanalysis carried out on the grainy surface gave the same result.

The elements identified were oxygen, carbon and chiefly calcium.

The aperture seemed clean, no significant presence of dirt is observed on the rest of the shell. No fissures or cracks were observed.

Sample 3 (consolidant and ultrasound treatment, Figure 2, S3) presented an heterogeneous surface with consolidant deposits discontinuously distributed in a quartered way, very superficially and especially on sutures and in the aperture. Chemical analysis confirmed the composition of the deposits as silica (Figure 3.1). We observed physical degradation on the surface with irregularities, such as desquamation, small orifices and erosion of the outermost layer especially in the exposed areas. Under higher magnification, we observed detachment of the superficial layer that exposed subjacent layers of crystal and a grainy texture (Figure 3). The elements detected in the grainy zones were basically non-metallic: oxygen, carbon, and a notable predominance of calcium (Figure 3.2). No fissures or cracks were observed, nor were superficial calcium deposits seen. Consolidant deposits may cover possible dirt remains, especially on the aperture and sutures. No penetration of consolidant into the shell was observed.

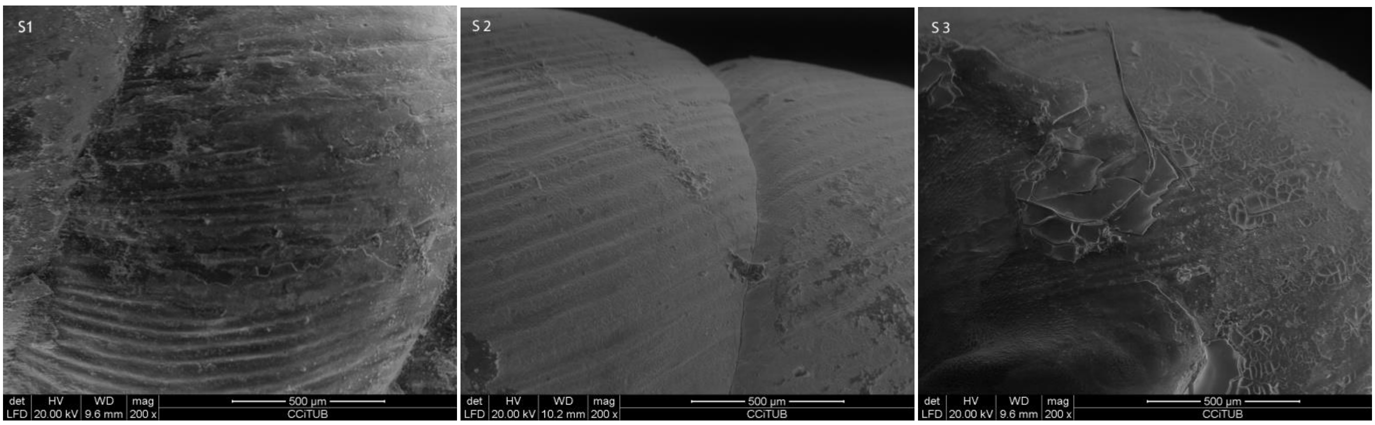

Figure 2 - SEM images of surface morphology of mollusk samples. S1) Untreated surface. S2) Abraded surface after ultrasonic cleaning treatment. S3) Residues of silica on surface after consolidation and ultrasonic cleaning treatments. Images by Cristina Ruiz-Recasens. Section of Conservation-Restoration. University of Barcelona.

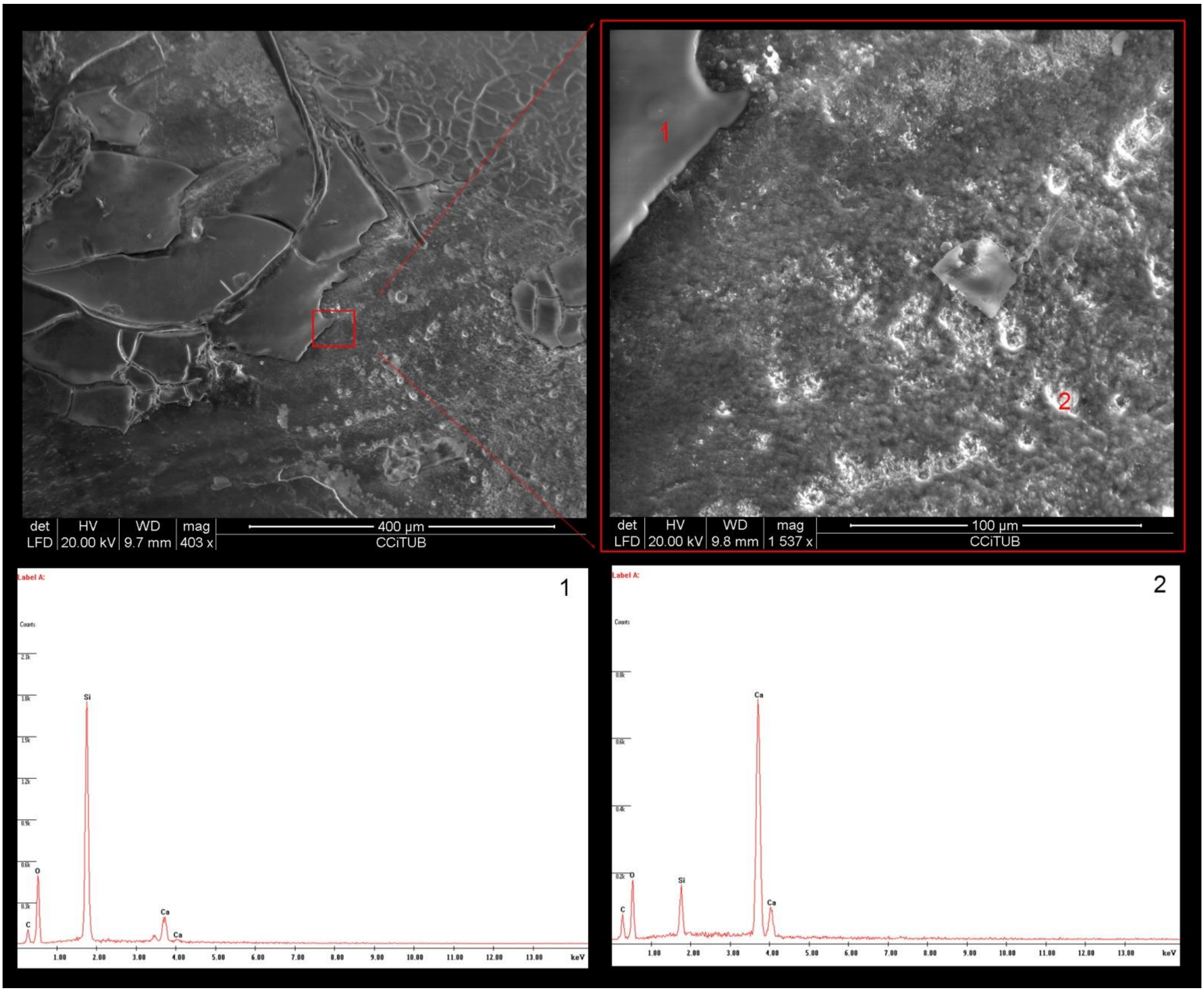

Figure 3 - SEM surface images of sample 3 after consolidation treatment. The numbers indicate the spot positions of the EDX microanalysis 1 and 2. The crystals are solid silica (1) and calcium (2), probably calcium carbonate substrate. Images by Cristina Ruiz-Recasens. Section of Conservation-Restoration. University of Barcelona.

DISCUSSION

Our findings suggest SEM is a good technique to evaluate the effects of ultrasonic cleaning (Stuart, 2007; Juanes Barber and Martín de Hijas Díez, 2008) and show that the ultrasonic cleaning procedure applied in this study had relatively good results with a decrease in dirt remains. However, the cleaning procedure had an undesirable effect on the surface of the shell: degradation was evident on treated samples, although no adverse effects were observed on a structural level (cracks or fissures). Interestingly, no degradation was visible under examination with a stereomicroscope.

The identification of calcium in the EDX microanalysis in the grainy areas suggests that this is the shell calcite. Indeed, five assays in different areas that had the same appearance gave an almost identical spectrum, always oxygen, carbon and a predominance of calcium.

The erosion of the outermost layer of the surface, which is dark in the images and formed by compact successive layers (presumably periostracum), exposes the inorganic part of the shell, the ostracum. The ostracum is lighter colored and grainy and it has a crystal texture. The effects are clear in sample 2, which is particularly affected by abrasion. One possible explanation for this erosion is that the impact of ultrasound waves and the process of cavitation harm the outer layers of the shell through erosion or abrasion. Ensminger and Stulen (2008) argue that some of the chemical and physical effects associated with high-intensity cavitation include production of OH (hydroxyl) and other ions, erosion of metal surfaces, disruption of aggregates, and other effects not producible by any other known means. It should also be taken into account that ultrasound waves increase the pressure and the temperature significantly during the cavitation process and this in turn could increase the effects of degradation (Suslick, 1989; Niemczewski, 2011; Santos et al., 2011; Wagterveld et al., 2011).

Pre-consolidation of sample 3 with TEOS-based consolidant does not avoid this degradation even though the degree of abrasion is lower. The consolidant does not spread out homogeneously and we did not observe penetration into the inorganic substrate. After cleaning, the consolidant appears in the form of isolated deposits. The siliceous nature of the deposit product helps to distinguish between the sample and the consolidant.

Using this method, we were unable to determine whether soil and dirt remain under the layer of consolidant. Certainly, the application of consolidant on the uncleaned specimen has the effect of consolidating any dirt. If the treatment had penetrated into the shell - which unfortunately did not occur in our study - , cleaning of the mollusk shell surface would have been possible. The precipitation of silicate deposits on the surface shell probably occurred during the polymerization process of consolidant, in which case the reaction would have occurred before the ultrasound bath. Öztürk (1992) reported that the drawbacks of the siliceous consolidants are insufficient penetration and formation of shallow and hard surface crusts, formation of soluble salts as by-products in the consolidation reaction, and growth of precipitated crystals.

The poor effectiveness of consolidation may also be due to errors in application, difficulty concerning the penetration of consolidant into the compact crystalline structure, and/or the size of pores of the periostracum (Rodriguez-Maribona and Cano, 2000). Penetration could be improved if the consolidant was applied in a vacuum. The application of TEOS-based consolidant in mollusk shells has not been tested previously. Further investigation is thus needed regarding the distribution and penetration of the consolidant. Different analytical techniques, such as the observation of a cross section, should be applied.

Our preliminary results are illustrative but not representative due to the small sampling size. A future, systematic study is needed to obtain a clear picture of the usefulness of the proposed methods.

To accurately determine the level of degradation caused by ultrasound, it is necessary to carry out further microscopic observations with samples of diverse characteristics and controls for more variables. These observations should be done on the same sample before and after the treatment to establish the effects of duration and intensity of exposure to ultrasonic waves. It would also be interesting to conduct a temporal survey to detect the long-term consequences of cleaning.

CONCLUSIONS

SEM observation highlights damage on mollusk shells caused by ultrasound cleaning that are not detected by stereomicroscopic observation. Thus, we recommend checking the resistance and the physical properties of the specimens in depth before carrying out any cleaning of this type. In spite of the evidence of microscopic degradation, this alteration does not affect the use of these samples for comparative purposes in identification campaigns. The pending question is how this kind of deterioration lowers the suitability for other scientific uses, and what could happen in the long term. Even though this is a preliminary study, we believe the findings are a starting point for further research on the effects of conservation treatment of natural history collections with ultrasound, particularly on mollusk specimens.

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