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Nano-hydroxyapatite for the conservation of Serena stone

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***Abstract* – In past decades, interest in using nanomaterials for the preservation of valuable cultural heritage was rapidly increased due to their excellent properties. In the present study, a biomimic method for the consolidation of sandstone (i.e. Serena stone) by hydroxyapatite was investigated. The strategy is to mimic the growth of bone like crystals: calcium (as $Ca(OH)_2$ nanoparticles) and phosphorus (as diammonium hydrogen phosphate) are introduced into stone substrate and then, mineralized in-situ at room temperature. Before the treatments, $Ca(OH)_2$ nanoparticles have been synthesized and characterized by different methods: scanning electron microscopy (SEM), transmission electron microscopy (TEM) and dynamic light scattering (DLS). In addition to that, the conservation efficacy was ascertained by measuring physical-structural properties, especially the resistance to weathering induced by salt crystallization.**

I. INTRODUCTION

The conservation of artifacts is an essential and important practice due to their historical relevence, value and also from an economic point of view. In the traditional restoration and conservation processes, synthetic polymers have been largely employed as adhesives and conservation agents and the use declined due to several defects: a lower compatibility with substrates, the lack of reversibility, excessive colour changes, poor durability, and a drastic reduction of water capillary absorption and surface wettability [1-4]. In last decades, nanotechnology introduced new methods and techniques, which provides scientific advances and new products that are smaller, faster, stronger, and more reliable. Most benefits of nanotechnology depend on the fact that it is possible to tailor the essential structures of materials at the nanoscale to achieve specific properties. As a result, the use of nanomaterials to preserve valuable artefacts have been considered as an alternative method [5]. For instance, titanium dioxide (TiO_2), zinc oxide (ZnO), and zirconium dioxide (ZrO_2) nanoparticles are highly employed in this field due to their excellent properties (non-toxic, readily available, good natural color, and chemical and microbial resistance) and scientist have reported that these materials, mixed with binder (e.g., siloxane polymers) can provide multifunctional properties to the stone based artefacts [6-9]. Apart from these materials, different types of hydroxide nanoparticles (calcium hydroxide, barium hydroxide, magnesium hydroxide and strontium hydroxide) dispersed in an alcoholic medium were studied for the conservation of wall paintings as well as for different types of stone substrates (limestones, mortars, and calcareous substrates) [2,10-12]. This method can introduce more nanoparticles (NPs) in a single application than the other suspensions, but multiple applications are still needed.

Considering all the interesting features as well as scarcity of available conservation products, we have studied a different method (respect to the other available methods) for the conservation of stone substrates, in particular, sandstones. In this method, $Ca(OH)_2$ nanoparticles were introduced into the stone substrate as the first treatment and then, it was treated with diammonium hydrogenphosphate ($(NH_4)_2HPO_4$) as the second treatment to produce hydroxyapatite by in-situ reaction at room temperature. Interestingly, as reported in the literature, apatite coatings were found on ancient monuments (it may be originated by ancient milk-based treatments) and this type of minerals showed durable properties to the stone substrates [12-13]. Moreover, Yang et al. 2011 and Weththimuni et al. 2018 have reported that the biomimic growth of bones like hydroxyapatite represents a very promising method for the conservation of calcareous materials (e.g., Lecce stone) [12, 14]. On the other hand, this type of studies can be seen rarely in the literature, partucularly for sandstones [15]. During the mineralization process, a porous and interlinked apatite

phase is formed, which can reunite broken parts inside the sandstones. It ultimately lead to conserve the stone by acting as fillers in conservation purpose. This study is focused on the application of this method to a particular sandstones, i.e. Serena stone (SS). Serena stone, also known as quarry stone, mainly contains grains of silicates and/or Quartz, and has quite low porosity (~5%) [16-17]. Throughout the centuries, Serena stone has been important architectural and ornamental material in Italy. The Etruscan used quarry stone from Fiesole (quartz-feldspar turbidities from Oligocene) for building city walls, temples and necropolis [18]. Later on, quarry stone was employed to create architectural ornaments or as building material for the city needs. Unfortunately, in the 19th century, the extraction of Serena stone ceased completely due to deterioration problems [18]. The difficulties encountered have favored the research and development of new conservation treatments for consolidation of the precious stone finds [18].

$Ca(OH)_2$ nanoparticles have been synthesized in laboratory and characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and dynamic light scattering (DLS). In addition to that, the conservation efficacy was ascertained by measuring physical-structural properties such as water capillary absorption, permeability to water vapour, chromatic variation, and the resistance to weathering effect induced by salt crystallization. Morphological and microstructural behaviors as well as distribution of the deposited phase into the stone substrate have been examined by SEM-EDS.

II. MATERIALS AND METHODS

A. Materials

Calcium chloride dihydrate ($CaCl_2.2H_2O$), Sodium hydroxide (NaOH), 2-propanol and diammonium hydrogenphosphate (DAHP: $(NH_4)_2HPO_4$), and sodium sulfate (Na_2SO_4) were supplied by Sigma Aldrich and used without further purification. Water has been purified using a Millipore Organex system (R⩾18 MΩ cm). Serena stone (squared specimens of 5×5×1 and 5×5×2 cm) were provided by Favret Mosaici S.a.s. (Pietrasanta, Lucca, Italy). Before treatments, stone specimens were smoothed with abrasive, carbide paper (No: 180 mesh), and washed with deionised water. Then, the samples were dried in oven at 60 °C, stored in a desiccator to reach room temperature and measured the dry weight, according to UNI 10921 Protocol [19].

B. Synthesis of $Ca(OH)_2$ nanoparticles and characterization

$Ca(OH)_2$ NPs were synthesized in the laboratory following the literature method [1-2], which can be summarized as below: 200 mL of 0.6M NaOH solution was added dropwise into the same volume of 0.3M $CaCl_2{\cdot}2H_2O$ solution contained in a round bottom flask at about 90 °C. The system was stirred continuously during the addition of NaOH to the $CaCl_2$ solution (20 minutes), then stirring was stopped (24 hours). After 24 hours, several deionized water washing were performed to remove the produced NaCl and then, $Ca(OH)_2$ NPs in 2-propanol dispersion (5 gL^{-1}) was prepared in a rotary evaporator at 80 °C. The final dispersion of $Ca(OH)_2$ in 2-propanol was kept under nitrogen gas to avoid carbonation process. The synthesized NPs were characterized using different techniques in order to understand the particles size, shape and other properties: Dynamic Light Scattering (DLS measurements were performed by a MALVERN Z590 apparatus), transmission electron microscopy (TEM acquired using electron microscope Jeol JEM-1200EXII, Olympus digital camera, Megaview G2), and scanning electron microscopy and energy-dispersive X-ray spectroscopy (SEM-EDS were collected by using a Tescan FE-SEM, MIRA XMU series, equipped with a Schottky field emission source, operating in high vacuum, and located at the Arvedi Laboratory, CISRiC University of Pavia, samples were gold sputtered using a Cressington sputter coater 208HR).

C. Application of consolidating agent

In order to evaluate the conservation efficacy of biomimetic hydroxyapatite, Serena stone specimens were treated with $Ca(OH)_2$ NPs and DAHP by brushing (a small paintbrush with 1 cm width) until the surface saturation was reached (i.e., the surface remained wet for 1 minute). All the analyses were done using only one surface (5x5 cm) of stone specimens and all the treated samples were used for the salt crystallization test. Producing hydroxyapatite by biomimic method is two steps reaction: First step: $5gl^{-1}$ $Ca(OH)_2$ NPs dispersion was introduced into the SS specimens by brushing; Second step: 24 hours after the application of $Ca(OH)_2$, 5.0% DAHP solution was introduced into the samples by brushing. After that, stone specimens were dried at room temperature for 3 weeks. The weight differences of stone specimens before and after treatments were calculated after fully drying the specimens. Furthermore, Serena stone specimens were named as SSA after treatments.

C. Analysis of physico-chemical properties and the strengthening effect of treated samples

The morphological and micro structural differences in stone substrates due to treatments were investigated by performing SEM-EDS analysis. The water absorption by capillarity is an important test to analyze changes inside stone materials due to the treatments, because the water absorption coefficient is closely related to the pore structure by the pore radius and porosity of the stone material. The amount of absorbed water as a function of time was determined on 5x5x2 cm samples according to the UNI EN 15801 protocol [20]. Water vapour

permeability was determined on 5x5x1 cm stone specimens according to UNI EN 15803:2010 protocol [21]. Chromatic variations were measured by a Konica Minolta CM-2600D spectrophotometer, determining the L*, a* and b* coordinates of the CIELAB space, and the global chromatic variations, expressed as ΔE* according to the UNI EN 15886 protocol [22]. Five measurements on each specimen were performed and all the given results are average values.

The chemical weathering effect of salt crystallization test was done following the Spanish standard test of UNE-EN (12370) [2, 12, 23]. The percentage of dry weight lost (DWL%) due to the 15 cycles of test was calculated in both stones (SS and SSA).

III. RESULTS AND DISCUSSIONS

A. Characterization of synthesized NPs

To investigate the particle size of synthesized $Ca(OH)_2$ and its distribution in alcoholic dispersion, several analyses were performed. DLS analysis reveals that NPs have average size around 69 nm and the most of the particles are in the range of 40-120 nm, suitable for consolidation treatments. In addition, TEM analysis suggested that the presence of 30-50 nm sized $Ca(OH)_2$ particles displaying almost spherical shape (Figure 1a) as also confirmed by the SEM observations (Figure 1b). Although most particles have spherical shape, some hexagonal shaped particles as well as aggregated particles can be observed in the alcoholic dispersion as reported in our previous papers [2, 12].

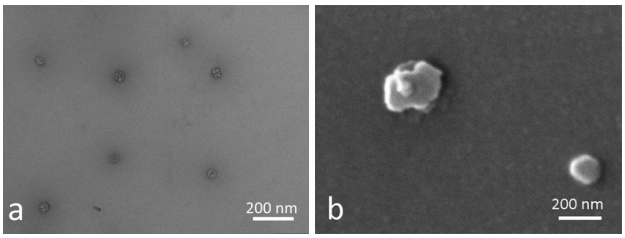

Fig. 1. Microscopy images of NPs: (a) TEM, and (b) SEM

B. The physicochemical properties of treated samples

Three weeks after the treatments, all the analyses were done on Serena stone specimens in order to evaluate the conservation efficiency of newly formed hydroxyapatite. According to the chromatic measurements, the original colour of SS changed (ΔE*=3.8±0.5) moderately due to the formation of hydroxyapatite crystals on the poorly porous SS surface. However, this chromatic change is lower than the standard value of overall chromatic variation that can be detected by naked eye (ΔE* < 5) [12, 17]. In addition, water capillary absorption measurements showed that there is a slight reduction of water absorption in Serena stone after treating by apatite (SSA) due to the effect of treatments. Moreover, amount of water absorbed in 96 hours (Qf) reveals that there is only slight changes for long time absorption compared to the untreated Serena stone (SS). This behaviour suggests that the biomimic apatite could be a suitable consolidant for SS, because it does not dramatically alter the water suction behaviour of original stone materials. On the other hand, the formation of hydroxyapatite induced the reduction of the water vapour permeability value around 20% (Table 1: 84 g/m^2 24h for SS and 68 g/m^2 24h for SSA), indicating that the treatment affects the breathing function of the stone to an acceptable extent.

Morphological and microstructural behaviour of treated stone matrix with respect to the untreated one was investigated by performing SEM-EDS analysis. In order to identify newly formed crystals in stone matrix, the cross-section analysis was performed in different depth from the stone surface. For instance, Figure 2 summarizes the results obtained from cross-section analysis of SSA at 1mm depths from the surface (the image of SS used as a reference). After application of $Ca(OH)_2$ nano-dispersion and diammonium hydrogen phosphate, they penetrate inside the stone matrix through pores and then, they converted to apatite. The new crystalline phase is morphplogically similar to the structures observed in bones (Figure 2c), and its chemical composition obtained by EDS analysis indicates presence of Ca, P, and O as the main elements (Figure 2d). Hence, the newly formed crystals can be ascribed to the thermodynamically most stable phase of hydroxyapatite (with chemical formula $Ca_{10}(PO_4)_6(OH)_2$) as explained in the literature [24]. Furthermore, the gradual formation of hydroxyapatite from $Ca(OH)_2$ and DAHP on treated stone has been also confirmed by XRD analysis [12]. Due to its bone-like structure, the newly formed hydroxyapatite can provide a strengthening effect by reconnecting each-other the stone components that lost cohesion due to deterioration processes. The new phase may also induce filling of the pores with a consequent porosity reduction.

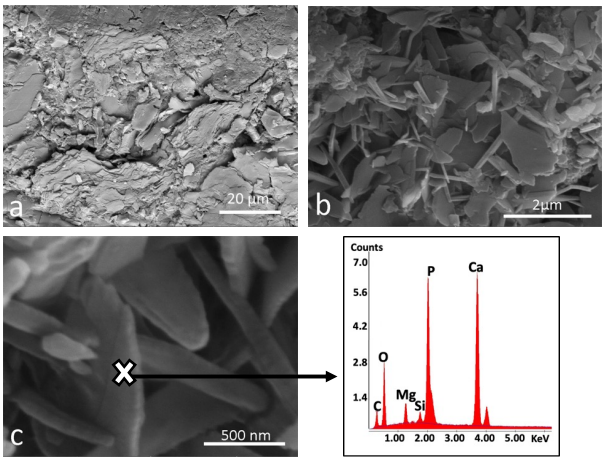

Fig. 2. SEM-EDS images of Cross-section of Serena stone (Det:BSE): (a) untreated, (b) treated with apatite around 1mm depth from the surface; (c) at very high magnification of b; and (d) EDS analysis of c

C. The strengthening effect of treated stones

Salts, and particularly sodium sulfate, are known to among most destructive agents in stones weathering [25]. Therefore, the study of crystallization process is very important to fully understand their effect particularly on porous network. The salt crystallization test was performed to investigate the consolidation efficiency of hydoxyapatite in Serena stone accoding to the literature method (as explained in the experimental section) and 15 test cycles were done in both SS and SSA specimens.

Serena stone (both SS and SSA) showed very good behaviour for salt crystallization test until around 7 cycles, acting as a strong stone material with high tensile strength due to its original substrate. Stone materials is poorly released after the first cycles, suggesting that only a few amount of salt are absorbed due to the low porosity of the Serena stone.

Table 1. The result of water vapour permeability measurement and DWL% after salt crystallization test (15 cycles)

Samples	Permeability (g/m^2 24h)	DWL%
SS	84 (±2)	74.35 (±0.81)
SSA	68 (±4)	46.44 (±1.87)

SS specimens started to damage rapidly after 10 cycles may be due to the super saturation of salt inside stone matrix. On the contrary, treated stone specimens (SSA) showed better performance than untreated stone and started to damage after 15 cycles of the salt crystallization test (the difference of DWL at the end of the test was around 38%), confirming the good consolidation effect provided by hydroxyapatite (Table 1).

IV. CONCLUSIONS

A biomimic method involving the in-situ formation of hydroxyapatite was evaluated for the consolidation of Serena stone. Physico-chemical studies that included chromatic measurements, water capillary absorption and water vapour permeability experiments showed that the original properties of stone substrate are not dramatically altered by the treatment.

Serena stone specimens treated by this biomimic method showed an enhanced resistance to the decay caused by salt crystallization. Therefore, owing to the strengthening effect provided by hydroxyapatite this method can be considered a promising consolidation technique for sand stones.

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