

Chapter 6

Inorganic Nanomaterials for the Consolidation and Antifungal Protection of Stone Heritage

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6.1 Introduction

Stone cultural heritage and artworks suffer from weathering due to their interactions with the environment [1, 2]. These significant weathering processes are generally related to an important reduction in mechanical properties and an increase in porosity, pore size, and water absorption [3, 4]. Moreover, the deterioration caused by microorganisms is another critical threat to monuments worldwide [5, 6], especially in tropical areas where high humidity and temperatures encourage the growth of

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microorganisms [7]. So, among different stone treatments, consolidation (i.e., restoring materials' integrity by improving cohesion and binding loose grains) [8, 9] and antifungal treatment protection (i.e., reducing and preventing existing fungal growth) are key phases of conservation practice used to safeguard stone heritage [10, 11].

In this context, the application of nanoscience for the conservation of stone may address a significant number of conservation issues. The possibility to manipulate and control materials at the atomic level and the subsequent understanding of fundamental processes at the nanoscale have led to new research challenges. Within nanoscience, the development of nanoparticles specifically designed for cultural heritage preservation purposes has become of interest to researchers. The reason is based on the unique and sometimes unexpected physical and chemical properties that are present in materials at the nanoscale, such as their increased surface-to-mass ratio, diffusivity, and electrical, optical, and thermal properties [12–14]. However, the application of nanoparticles (NPs) in the stone heritage conservation field requires a multidisciplinary approach which combines material science, petrophysics, microbiology, and cultural heritage conservation along with many other scientific disciplines. In this way, many challenges must be overcome such as studies of the physicochemical properties of materials and how they respond to changing environmental conditions, ultimately providing an exciting opportunity to improve the understanding of their action as conservation treatments.

Therefore, both organic and inorganic nanomaterials have been developed in order to recover the mechanical properties of damaged stone heritage and to provide successful antimicrobial coatings [15–18]. It is essential to note that consolidating and antifungal protective products must take into account fundamental conservation and restoration principles, mainly compatibility, efficacy, and durability [19]. Both treatments are the riskiest conservation and restoration practices due to their irreversible nature and threaten to produce undesirable effects. This is why a wide number of studies have been centered on the design of new stone consolidants and protective coatings with antimicrobial action. A detailed description of the different types of nanomaterials currently used to produce conservation treatments can be found in Sierra-Fernandez et al. [20].

In this chapter, a brief overview about the main synthesis methods and the most common analytical techniques employed for the physicochemical characterization of NPs will be presented. Then, the application of nanotechnology for the stone heritage conservation will be discussed. In particular, a significant portion of the discussion will be devoted to inorganic nanomaterials that specifically focus on stone heritage preservation and the main factors that condition their effectiveness.

6.2 Nanomaterials

6.2.1 *Synthesis Methods*

The term “nanomaterial” can be referred to as materials composed of single elements, such as metals or carbon, or materials made up of several elements, such as metal oxides or composites [21]. In this chapter, when the term “nanomaterial” is used, it is mainly referring to inorganic nanoparticles composed of metals and metal hydroxides and oxides, unless otherwise stated.

The selection of the synthesis method used for obtaining the NPs or the nanostructured materials is a decisive factor used to enhance the suitability of these materials in function of their use for the conservation of artworks. In this way, many synthesis strategies have been developed to obtain inorganic nanocrystals with certain morphologies, particle sizes, agglomeration level, and crystallographic structures. The two commonly used synthetic methods for nanofabrication of NPs or nanostructured materials include “top-down” and “bottom-up” [22]. In the “top-down” approach, the process begins from a bulk piece of material, which is then gradually removed or handled to form materials in the nanometer size range either by milling [23], photo lithography [24], electron beam lithography [25], or anodization [26], among others. The “bottom-up” approach begins with atoms and molecules that get rearranged and assembled to form larger structures [22]. This synthesis approach was the first method used in the field of material science applied to cultural heritage for obtaining calcium hydroxide nanoparticles specifically designed for artwork conservation [27]. Since then, several NPs with different morphologies and structures have been synthesized for application in cultural heritage by using chemical methods mainly due to their potential for scale-up and their lower cost. Examples of widely used chemical methods for synthesis of inorganic NPs include the solvothermal [28], the hydrothermal [29], and the solgel [30, 31] methods. Moreover, alkaline hydroxide NPs were synthesized by using “an ion exchange process between an anionic resin and a calcium chloride aqueous solution operating at room temperature” [32]. Other commonly used synthesis methods for the fabrication of NPs are the pulsed laser ablation method [33], the microwave-assisted synthesis method [34], and the spray pyrolysis method [35]. Most recently, atomic layer deposition (ALD), molecular layer deposition (MLD), and combinations of the two have been used to produce a wide variety of hybrid organic/inorganic materials with innovative properties [36]. These promising techniques are cyclic vapor-phase deposition processes which have been specially adapted and applied to prevent corrosion damage for silver cultural heritage objects [37].

As a remarkable drawback, an important factor to take into account is that they are different procedures, from both the bottom-up and the top-down, to create nanostructures which can be applied according to its specific needs [22]. Using a suitable (compatible with the stone substrate) nanoparticle solution, it is possible to create a

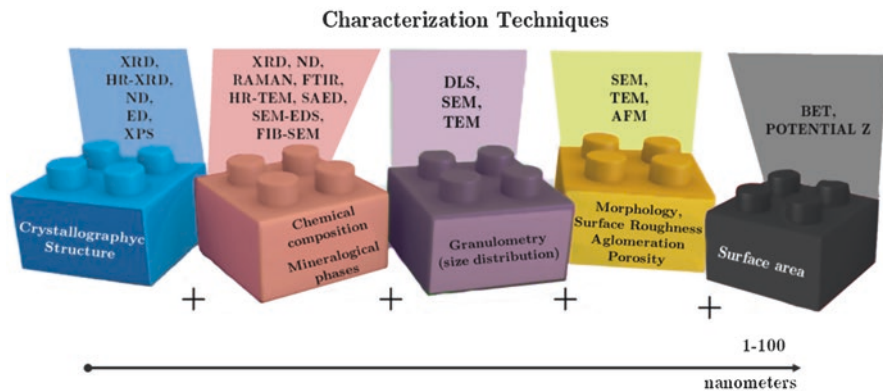


Fig. 6.1 Summary of the analytical techniques most commonly used for the study of inorganic NPs: *XRD* (X-ray diffraction), *HR-XRD* (high-resolution X-ray diffraction), *ND* (neutron diffraction), *ED* (electron diffraction), *XPS* (X-ray photoelectron spectroscopy), *Raman* spectroscopy, *FT-IR* (Fourier transform-infrared spectroscopy), *HR-TEM* (high-resolution transmission electron microscopy), *SAED* (selected area electron diffraction), *SEM-EDX* (scanning electron microscopy-energy-dispersive X-ray spectroscopy), *FIB-SEM* (focus ion beam scanning electron microscopy), *DLS* (dynamic light scattering), *AFM* (atomic force microscopy), *BET* (Brunauer-Emmett-Teller)

consolidating and/or protective product with new properties and characteristics highly compatible with the stone when compared to their bulk counterparts, as shown in the following sections.

6.2.2 Characterization Techniques for Nanomaterials

An extensive number of techniques can be used to evaluate size, shape, polydispersity, composition, purity, and surface properties of nanostructured materials, which have to be studied before their application in stone heritage (Fig. 6.1). Therefore, in this section, some of the most common characterization techniques used to investigate the physicochemical characteristics of nanomaterials designed for their application in the cultural heritage conservation will be briefly discussed.

The crystallography of the nanomaterials is usually studied by diffraction techniques such as X-ray diffraction (XRD), high-resolution X-ray diffraction (HR-XRD), synchrotron radiation X-ray diffraction (SR-XRD), and neutron diffraction (ND). These analytical techniques are very useful tools to investigate, nondestructively, the crystallographic structure, the strains, and the preferred orientations of nanomaterials [38–40]. Specifically, diffraction techniques are also used to measure various structural properties of these nanomaterials, such as defect structure [39, 41], phase composition [42], texture analysis [43], and crystal size [44], allowing many experiments in time resolution. Another fundamental

analytical technique for determining the arrangement of atoms in a crystalline solid and the atomic structure determination is electron diffraction (ED) [45]. Moreover, X-ray photoelectron spectroscopy (XPS) is an analytical method with high surface specificity, widely used for elemental analysis and for investigating the electronic structure of nanomaterials [46].

The presence of impurities may significantly impact the effect or even produce unfavorable results in treated stone substrates. In this way, determination of nanomaterial composition, nature, and/or purity can be accomplished through the analysis of their chemical compositions. In general, chemical composition and purity grade are studied by Fourier transform-infrared spectroscopy (FT-IR) and Raman spectroscopy. These spectroscopic techniques have been shown as powerful techniques for the characterization of inorganic nanomaterials and those modified by the adsorption of molecules with different chemical properties [47, 48]. Furthermore, the energy-dispersive X-ray (EDX) analysis is a technique used in conjunction with scanning electron microscopy (SEM). This type of energy provided by X-ray emission is used to identify the elemental composition of samples and to estimate its proportion at different sample areas. Moreover, transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HR-TEM) are the most commonly used analytical techniques for studying nanoparticles at a spatial resolution down to the level of atomic dimensions (<1 nm) [49]. Thus, imaging, diffraction, and microanalytical information are widely obtained by TEM and HR-TEM. Nevertheless, it is important to take into account the risk of the irradiation damage in the samples caused by the use of electron beams in the transmission electron microscope, which could lead to undesired physical and chemical material property changes or uncontrollable modification of structures [50]. Additional analytical techniques for advanced and precise analysis on different types of nanomaterials are focused ion beam scanning electron microscopy (FIB-SEM) and atomic force microscopy (AFM). These characterization techniques are considered crucial for imaging on the nanometer scale due to their versatility and multifunctionality. While FIB-SEM has shown to be especially useful for studying the three-dimensional morphology of complex material systems [51], AFM allows the visualization and the analysis of individual and groups of nanoparticles, as well as the examination of uncoated and coated surfaces in three dimensions, and can operate in a number of modes, depending on the application and the required information [52, 53].

Among all viable options to measure the particle size of nanomaterials, dynamic light scattering (DLS) and microscopy (SEM and TEM) or a combination of both is important analytical techniques used to determine accurate particle size distribution and polydispersity in a wide range of sizes.

In other words, surface charge potentially affecting “receptor binding and physiological barrier penetration governs the dispersion stability or aggregation of nanomaterials and is generally estimated by zeta potential” [54]. Also, the measurement of the specific surface area (SSA) of nanomaterials is widely determined by the Brunauer-Emmett-Teller (BET) technique [55].

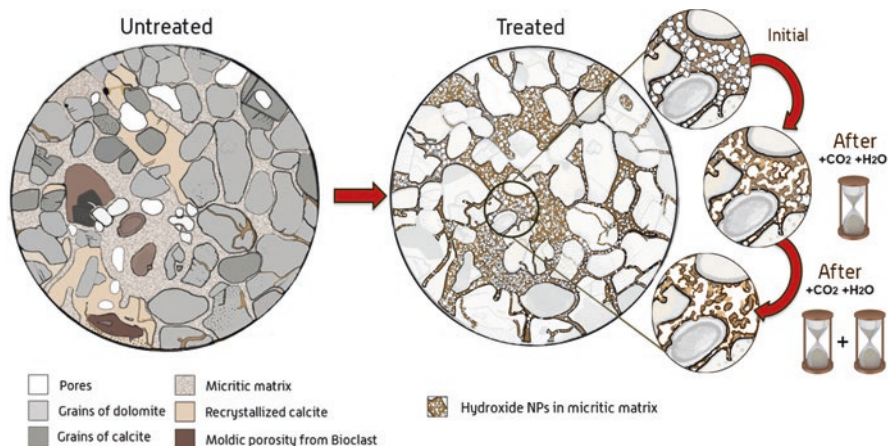


Fig. 6.2 Schematic illustration of a thin film of untreated and treated dolostone by using hydroxide nanoparticles (Reprinted with permission from Sierra-Fernandez et al. [20], Copyright, 2017 Materiales de Construcción. All Rights Reserved)

6.3 Nanomaterials for the Stone Heritage Preservation: Factors Influencing Their Effectiveness

6.3.1 Consolidating Products

The physicochemical compatibility of the inorganic nanomaterials with the stone-built cultural heritage is one of the main advantages of the use of these materials as consolidating products. The reactivity and penetration capacity of a consolidating product within the stone material and, consequently, its effectiveness are potentially increased when its particle size is reduced to the nanoscale. Another significant benefit of nanomaterials is a growth in surface area relative to volume. This greater surface area per unit mass changes or enhances their electronic and optical properties and their chemical reactivity (e.g., carbonation rate) because a greater proportion of atoms are found at the surface compared to those inside.

Significant advances have been made using colloidal nanoparticles based on calcium hydroxide ($\text{Ca}(\text{OH})_2$). In this context, to date, the pioneering products developed by Baglioni and his group [27, 56, 57], and the $\text{Ca}(\text{OH})_2$ nanoparticles designed by Ziegenbald and co-workers [58], are the most commonly used nanomaterials for stone consolidation. The consolidant action of the hydroxide NPs is based on their transformation into carbonate forming through the action of atmospheric CO_2 in the presence of moisture (Fig. 6.2). In this way, the newly formed carbonate phases act on degraded porous substrates, especially those of carbonatic nature, by binding loose grains and/or modifying their porous structure. However, the effect of relative humidity (RH) has been proven to be a clue in carbonation kinetics [59–61]. Thus, different calcium carbonate polymorphs can be produced depending on the relative

humidity, resulting in different physical properties of the treated stony substrate. Therefore, in dry environments (RH values of 33–54%), the carbonation process of the calcium hydroxide NPs is slower, giving rise to the generation of low crystallinity calcium carbonates [62]. However, at RH values of 75–90%, the presence of water acts as an accelerator of the process, and calcium hydroxide is completely converted into calcium carbonate (CaCO_3), developing differences in the nucleation of growth of different $\text{Ca}(\text{CO}_3)_2$ polymorphs within 7 days [60, 63]. Thus, in addition to the generation of calcite and acicular aragonite, unstable polymorphs like vaterite and crystalline hydrated forms (e.g., monohydrocalcite and ikaite) can be formed [59, 60]. Moreover, under high relative humidity conditions, calcium carbonate polymorphs' stability can be affected, generating structural defects that may give rise to dislocations. Differences in the crystalline habit, crystal size, aggregation, and type of atomic-scale structural defects may be expected to affect the polymorphic properties of CaCO_3 and, in particular, carbonation kinetics under specific experimental conditions [64] (Fig. 6.3). Thus, the generation of these different calcium carbonate polymorphs can affect the integrity of the stone substrate, contributing to the loss of cohesion [62]. In this way, questions related to if their physical and chemical characteristics are compatible with the petrophysical properties of the stone to treat or if local environmental conditions to which they are exposed [63, 64] are appropriate for the carbonation process are considered critical.

Furthermore, the NPs' concentration and influence of the dispersions on the effectiveness of hydroxide NPs as consolidants for weathered stone materials should be considered for a successful consolidation treatment. Gomez-Villalba et al. [65] studied the effect of different concentrations of calcium hydroxide NPs obtained by colloidal synthesis ($\text{CaLoSiL}^{\text{®}}$) applied on dolostone samples (Redueña, Madrid, Spain). This study showed that the application of calcium hydroxide NPs in high concentrations (15 and 25%) limited their effectiveness as a consolidating product due to their deposition over the stone substrate, producing changes in the color and the brightness of the treated dolostones. Otherwise, the application of the same product in low concentrations can avoid these secondary effects, resulting in an effective method to improve the durability of stone materials [66]. However, low concentrations may not prevent microbial colonization of biodeteriorative organisms.

Moreover, the most suitable application procedure for each case is another important point to study. Therefore, previous research works were centered on the investigation of the absorption and drying kinetics of magnesium and calcium hydroxide nanoparticle dispersions (average sizes from ~70 to ~260 nm) in pure ethanol, applied by different methods and followed by neutron radiography (NR) over time. These studies revealed a maximum particle penetration of 0.55 cm after 100 min of treatment into the dolostone substrates (Laspra dolostone, Asturias, Spain) treated by brushing. However, during this drying phase, radiographs show the accumulation of nanomaterial just underneath the surface (Fig. 6.4), limiting the penetration depth of the NPs into the dolostone treated by brushing. Recently, Borsoi et al. [67] studied the effectiveness of $\text{Ca}(\text{OH})_2$ nanoparticle dispersions on two lime-based substrates (Maastricht limestone and lime-based mortar) by different

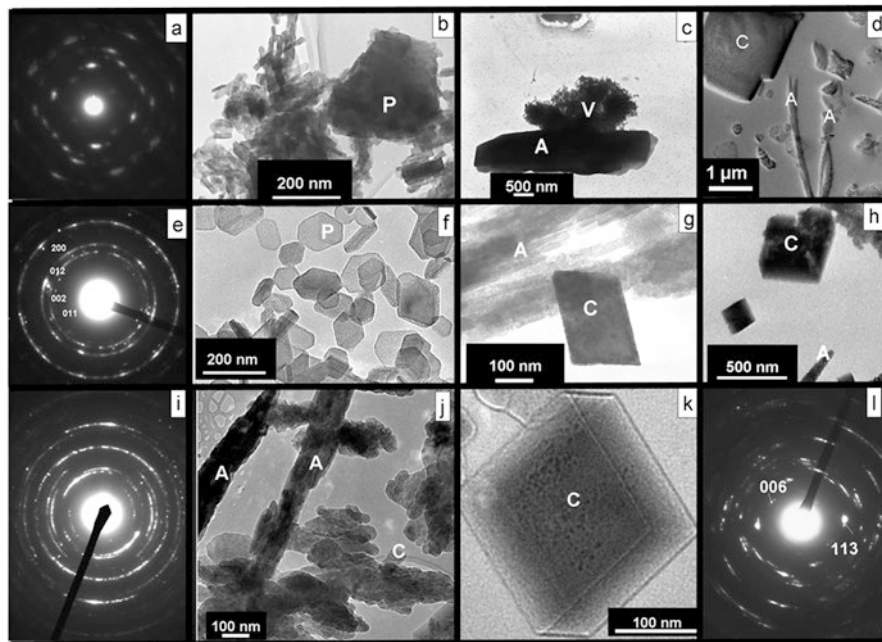


Fig. 6.3 Low magnification TEM images and SAED patterns showing the evolution of the spontaneous carbonation process in two alcoholic colloidal solutions of hexagonal platelike nanoportlandite (solution A (a, b, c, d, i) and solution B (f, g, h, j, k, l)), at 75% RH, for 0 days (b and f), 14 days (c and g), 35 days (d and h), and 2 years (j and k) of exposure. TEM images show the initial portlandite ($\text{Ca}(\text{OH})_2$) (b and f) and nucleation of CaCO_3 polymorphs during the carbonation. Simultaneous presence of calcite and aragonite after 14 days (g) and 35 days (h) in solution B. Metastable vaterite crystals associated with aragonite after 14 days in solution A (c). Small calcite crystals associated with acicular aragonite crystals in both solutions after 35 days (d, h). In solution B, well-crystallized calcite crystals after 2 years (k) always associated with aragonite (j). SAED patterns confirm the initial portlandite in solution A (a) and solution B (e) and the respective CaCO_3 polymorphs for solution A, 14 days (i), and solution B, 35 days (l). P, portlandite; V, vaterite; A, aragonite; C, calcite. Solution A (Nanorestore) and solution B (CaLoSiL) both dispersed in isopropyl alcohol (Reprinted with permission from Gomez-Villalba et al. [64], Copyright, American Chemical Society. All Rights Reserved)

application methods: full and partial saturation capillarity and partial saturation by nebulization. Their results showed how the addition of a small amount of water (5% by volume) in the $\text{Ca}(\text{OH})_2$ dispersion can guarantee moderate kinetic stability so that $\text{Ca}(\text{OH})_2$ NPs can be properly absorbed, favoring their penetration into the stone substrates. According to the methodological application, the authors determined that an effective in-depth treatment of Maastricht limestone can be obtained both with NPs dispersed in pure ethanol and a mixture of ethanol (95% by volume) and water (5% by volume) when they are applied by capillarity until full impregnation. However, their results also showed that the use of calcium hydroxide NPs dispersed in pure ethanol induced a partial back migration of the nanoparticles during drying. In addition, the use of NPs (with particle size range: 50–600 nm)

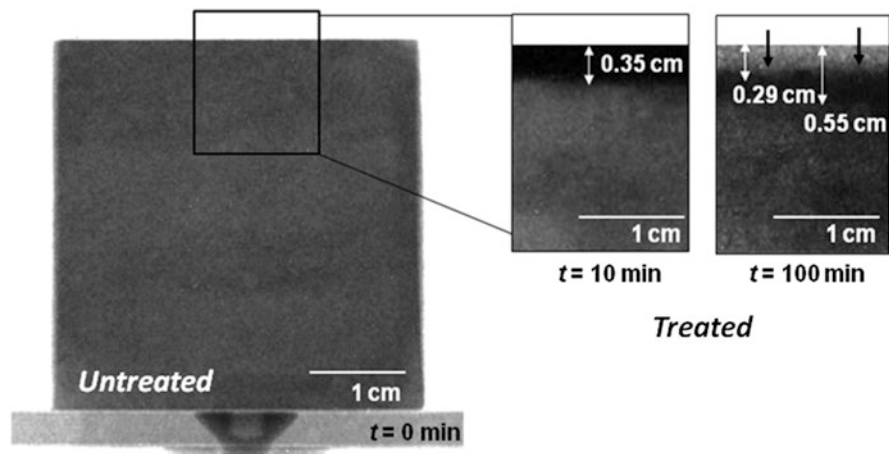


Fig. 6.4 Radiographs of the untreated and treated selected area of Laspra dolostone at different times. The accumulation is visible beneath the drying surface, after 100 min of treatment

dispersed in pure ethanol was recommended when the treatment is carried out through multiple applications by nebulization. The study also suggested the surface impregnation by nebulization as the most suitable application methodology for the treatment of lime-based mortars. The preparation of calcium hydroxide dispersions in mixing solvents, such as butanol with percentages of ethanol, could be considered in the case of the fine porous substrate treatment to improve the penetration depth of this type of nanomaterials [68]. In this sense, while solvents with high boiling points (e.g., butanol or water) would perform better in stone substrates with large pores, solvents with lower boiling points (e.g., ethanol or isopropanol) would reduce the $\text{Ca}(\text{OH})_2$ nanoparticle migration back to the stone surface during solvent drying [68].

On the other hand, some routines have been studied that accelerate the carbonation process of the consolidating treatments based on hydroxide NPs, from the role of solvent [39] to the environmental conditions, such as the use of a fermentation system based on water and a yeast-sugar solution [69]. This simple method generates a microclimate saturated with CO_2 , high humidity, and ethanol which are able to speed up the carbonation process of $\text{Ca}(\text{OH})_2$ NPs.

The kinetic stability of the hydroxide NPs in alcohol dispersions is also another important point to study. Rodriguez-Navarro et al. [39] evaluated the role of alcohol in the kinetic stability and the carbonation behavior of three different calcium hydroxide NP dispersions, which differ in particle sizes and surface areas. The study was focused on $\text{Ca}(\text{OH})_2$ -alcohol interaction and its effect on CaCO_3 polymorph selection and carbonation kinetics, determining that the contact of the particles with alcohol (e.g., ethanol or isopropanol) for long periods of time (>2 months) results in their replacement by calcium alkoxides [39]. The authors suggested that a high conversion of $\text{Ca}(\text{OH})_2$ into calcium alkoxides could be desirable if a preconsolidation

treatment marked by a high CaCO_3 yield with a fast precipitation is required. Most recently, the physical and chemical features of Ca(OH)_2 particles dispersed in ethanol and isopropanol, once subjected to storage for different periods of time at different temperatures (40, 60, and 80 °C), were characterized [70]. It was shown that Ca(OH)_2 particles reacted with ethanol and isopropanol and were partially transformed into Ca ethoxide and Ca isopropoxide, respectively [70]. This conversion into Ca alkoxides also reduced the rate of carbonation of Ca(OH)_2 (nano)particles and induced the formation of metastable vaterite [70].

Another major challenge in an optimal consolidating action of the designed nanomaterials according to the stone substrate is related to their chemical composition. It is important to consider that according to the compatibility, the proportions of magnesium and calcium in limestones and dolostones often differ widely both within a single rock formation and between formations. Therefore, the main research activity has been focused on the synthesis of Mg(OH)_2 and mixed solutions of Mg(OH)_2 and Ca(OH)_2 NPs in search of better compatibility, effectiveness, and durability of the treatment with the calcium-magnesium carbonate substrates. The reason is that the effectiveness of the use of calcium hydroxide NPs is severely reduced in dolostone substrates. The incorporation of the Ca(OH)_2 NPs and their subsequent carbonation on CaCO_3 entail a change in the dolomite crystals of dolostones. In this context, the application of calcium hydroxide NPs in dolostone could regenerate nano-calcite recrystallization, leading the dolomite dissolution due to the calcium ion enrichment [59]. Also, an alteration in the nucleation and crystals' growth may produce aesthetic modifications, such as changes in color and brightness [60].

Thus, Mg(OH)_2 and Ca(OH)_2 NPs have been synthesized by the hydrothermal method and by using the solgel synthesis method, carrying out a detailed study of the main synthesis factors that could influence the properties of the different synthesized NPs [29, 50]. The synthesis of the hydroxide NPs by the solgel method has shown the promise of obtaining highly concentrated dispersions of NPs, which is crucial for the upscale production.

Figure 6.5 shows FE-SEM and TEM micrographs of the magnesium hydroxide and the Mg-Ca hydroxide NPs with different weight ratios (10–90 and 50–50 wt%) obtained via the solgel method. This synthesis method resulted in the formation of highly crystalline and well-defined hexagonal flakes, having an average diameter from ~ 30 to ~ 60 nm. The crystal phases and crystallinity were also determined by X-ray diffraction. Thus, Fig. 6.5g shows the X-ray diffraction patterns of the different hydroxide samples, exhibiting the typical diffraction peaks of the Mg(OH)_2 and Ca(OH)_2 structures and confirming thus the purity and the chemical composition of the different obtained types of hydroxide NPs.

An example of adapting the consolidant composition to the stone substrate is illustrated in Fig. 6.6. In this case, the selection of the nanoparticles as consolidant product in dolostone and limestone substrates was carried out according to the chemical composition and the petrophysical properties of the different lithotypes. Therefore, different types of hydroxide NPs were selected and applied as consolidating products in two types of calcareous stones, widely used in the cultural

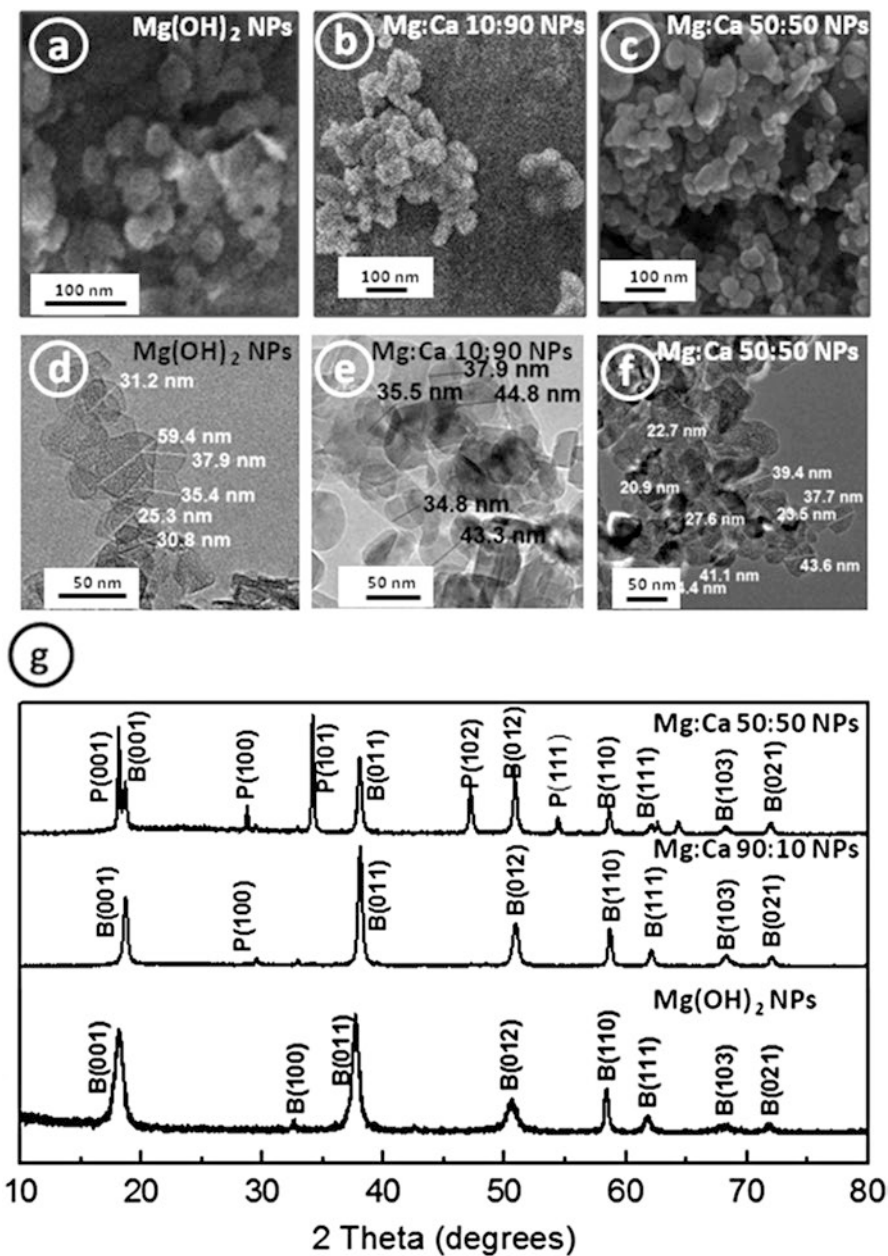


Fig. 6.5 (a–c) Low magnification of FE-SEM micrographs, (d–f) TEM micrographs of Mg(OH)₂, and the Mg-Ca hydroxide NPs with different weight ratios (10–90 wt% and 50–50 wt%, respectively); (g) XRD patterns of Mg(OH)₂ and the Mg-Ca hydroxide NPs with weight ratios of 10–90% and 50–50%. P, portlandite; B, brucite

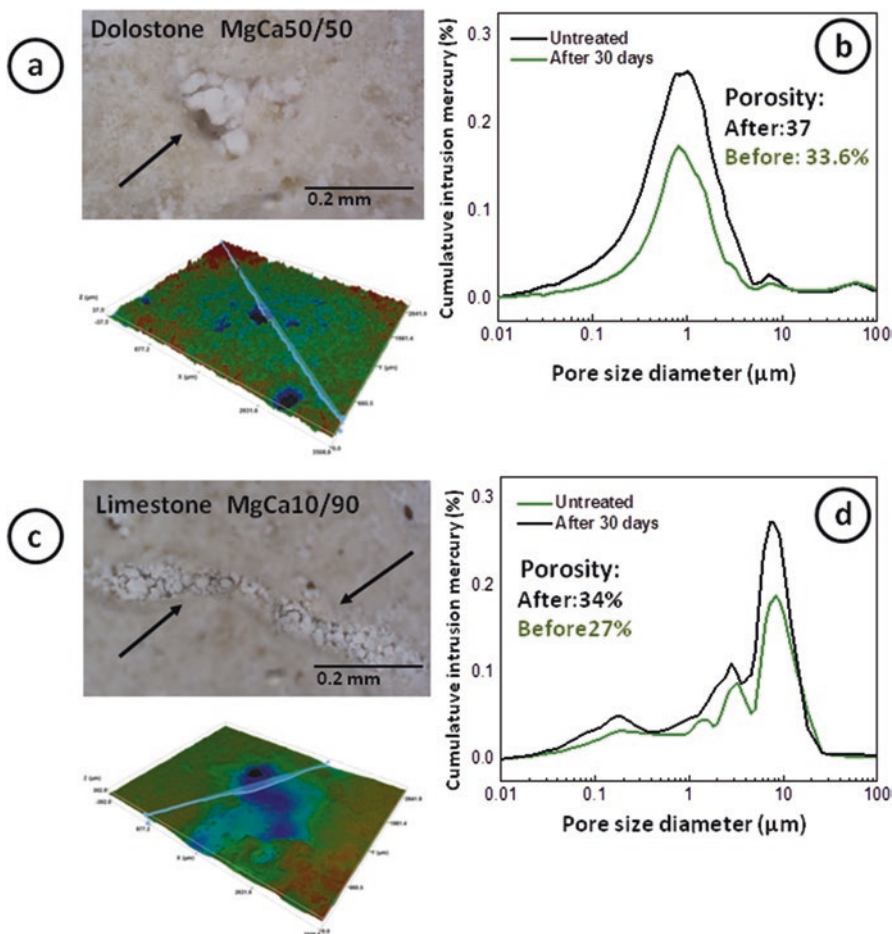


Fig. 6.6 Optical microscopy images and topographic images obtained by confocal microscopy of Laspra dolostone treated by Mg-Ca hydroxide nanoparticles (50/50 wt%) and Conchuela limestone treated by Mg-Ca hydroxide nanoparticles (10–90 wt%) (a and c, respectively). Pore size distribution (PSD) obtained by mercury intrusion porosimetry (MIP) of Laspra dolostone untreated and treated by Mg-Ca hydroxide nanoparticles (50/50 wt%) and Conchuela limestone untreated and treated by Mg-Ca hydroxide nanoparticles (10–90 wt%) (b and d, respectively)

heritage of Spain (Laspra dolostone) and Mexico (Conchuela limestone). Whereas the Laspra dolostone is a fossiliferous dolomicrite composed mainly of dolomite, the Conchuela limestone is a biomicritic limestone with a high content of fossil fragments and is mainly composed of calcite [31]. In order to assure an optimal consolidant treatment and according to their physical and chemical characteristics, both types of stone substrates were treated with different types of hydroxide NPs. Laspra dolostone was treated with a solution of $\text{Mg}(\text{OH})_2/\text{Ca}(\text{OH})_2$ NPs (50:50 wt%) and the Conchuela limestone with a solution of $\text{Mg}(\text{OH})_2/\text{Ca}(\text{OH})_2$ (10:90 wt%)

dispersed in ethanol in a concentration of 2.5 g/L. Figure 6.6a and c shows the optical micrographs carried out in the treated dolostone and limestone substrates, respectively. These results depicted the $\text{Mg}(\text{OH})_2/\text{Ca}(\text{OH})_2$ NPs (50–50 wt%) filled the pores and inter-crystalline dolomite grain contacts in the Laspra dolostone. For Conchuela limestone, the presence of the Mg-Ca hydroxides was detected mainly covering the crystal surfaces, favoring the calcite (CaCO_3) recrystallization. After the consolidant treatment, a decrease in total porosity was detected by mercury intrusion porosimetry (MIP) in the treated dolostone and limestone (Fig. 6.6b and d, respectively) due to the filling of pores by the consolidating product.

6.3.2 Antifungal Protective Coatings

The biotic factors, especially the growth of fungi on the monumental stone, are among the most active microorganisms in the biodeterioration of stone substrates, representing a crucial threat to monuments worldwide. An important group of microorganisms such as cyanobacteria, microalgae, lichens, fungi, and bacteria [5] can grow in different environments by attaching to the surfaces [71]. When attached to the surface, these microbial cells may develop into biofilms, which can produce the microbial deterioration of stone. The biofilms are thus collections of cells on surfaces that are maintained by electrostatic forces (mainly a combination of van der Waals attraction and chemical bonding) and/or extracellular polymeric substances (EPS) [72]. The EPS are composed mainly of a matrix of polysaccharides, which provide crucial functions such as protection for desiccation, erosion, radiation, as well as storage of organic components and nutrients [73, 74].

The microbial communities can colonize on different areas of stone substrates, becoming a convenient habitat for most rock-dwelling fungi [75, 76]. Therefore, fungi can grow as epilithic, on stone's surface, or endolithic communities, within cracks and pores or actively boring into the substratum [77]. These important biological colonizers of stone heritage may cause aesthetic damage originated by their colored patinas [78] and/or biophysical deterioration, which may be occurring due to the pressure exerted on the surrounding surface material during the growth of hyphal networks (biomass swelling or contraction) through the pore system of stone materials [79]. However, different studies determined that the biochemical actions in stone substrates were more important than mechanical attacks [80, 81]. These actions are marked by the generation and excretion of high concentrations of organic acids by fungi during their metabolic activity [79]. In this way, the fungal species can excrete organic acids (e.g., citric, oxalic), inorganic acids (e.g., sulfuric and nitric acids), H^+ , CO_2 , and metabolites and can occur in conjunction with biophysical processes [79, 82], resulting in important degradation processes from the pitting to the complete dissolution (Fig. 6.7).

In addition, while the microbial colonization is marked by the climatic conditions and the anthropogenic contamination of atmosphere [67], the degradation and weathering of stone materials are basically determined by its petrographic

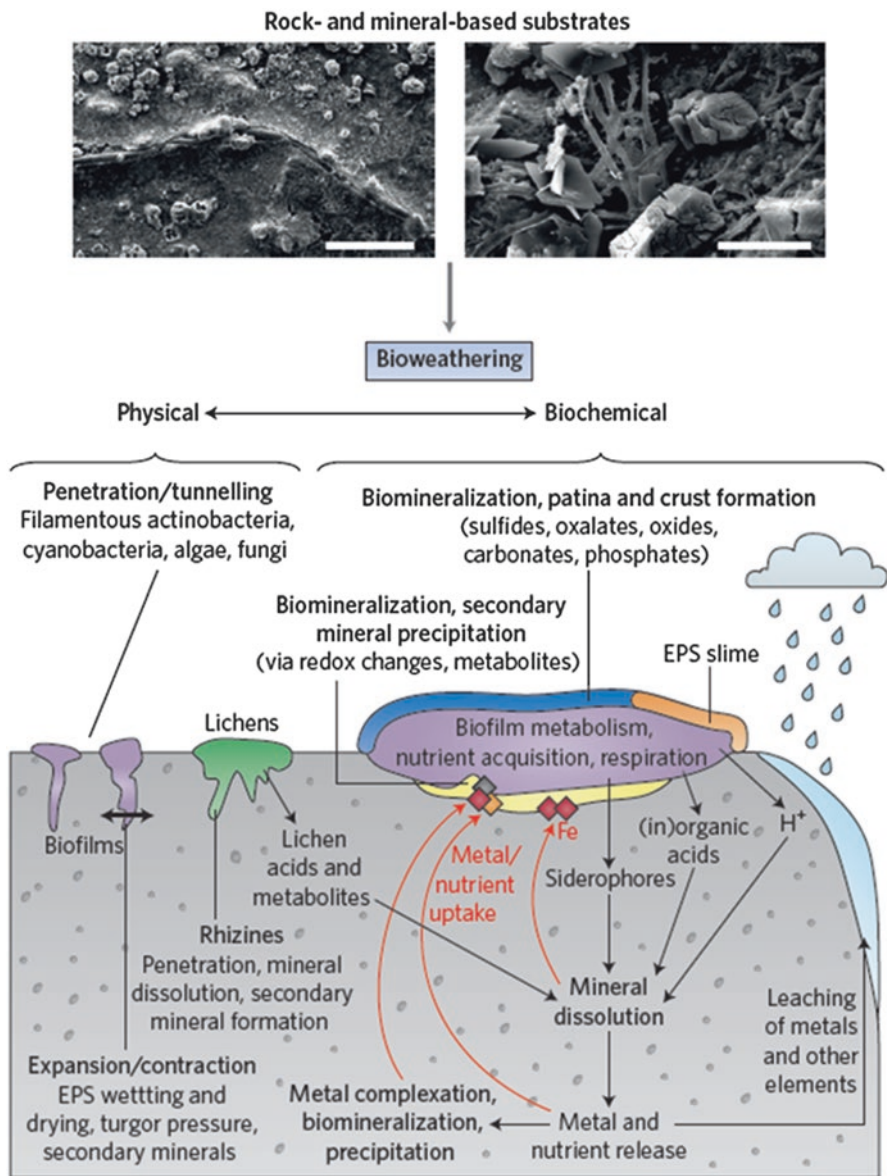


Fig. 6.7 Schematic illustration of the main influences and effects of microorganisms on rock- and mineral-based substrates with the respective SEM images (Reprinted and adapted with kind permission from G.M. Gadd [79], Copyright, Nature Publishing Group. All Rights Reserved)

features (especially the structure and the chemical composition) [30, 76]. Also, the texture and porosity of the material itself are important factors that favor a greater receptivity of the material to be colonized (Fig. 6.8) [76]. The stone microporosity has also been shown as another important factor in the bioreceptivity of stone substrates [31].

In this context, in the last decade, the appearance of nanotechnology has allowed the development of metallic NPs with interesting properties as antifungal agents for stone heritage preservation. To date, it has been reported that metallic NPs, such as Ag [83, 84], Cu [85], and Zn [30, 86, 87], as well as nanostructured metal oxides and combination of both [30, 31], have become attractive alternative sources to combat microbial species that are widely affecting the stone heritage (Fig. 6.9).

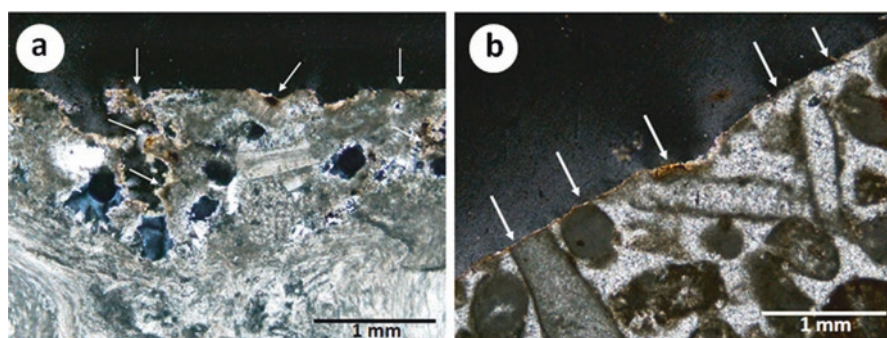


Fig. 6.8 Polarized light optical microscopy (PLOM) images (crossed Nicols) of thin sections from (a) Conchuela limestone (Yucatán, Mexico) showing endolithic fungal colonization penetrating down to the stone along cracks and in the internal stone pores and (b) the presence of fungal colonization confined to the surface of Laja stone (Yucatán, Mexico). To note the different penetration depths of fungal hyphae attributed to the increased porosity of Conchuela limestone (~27%) in comparison with Laja stone (~7%)

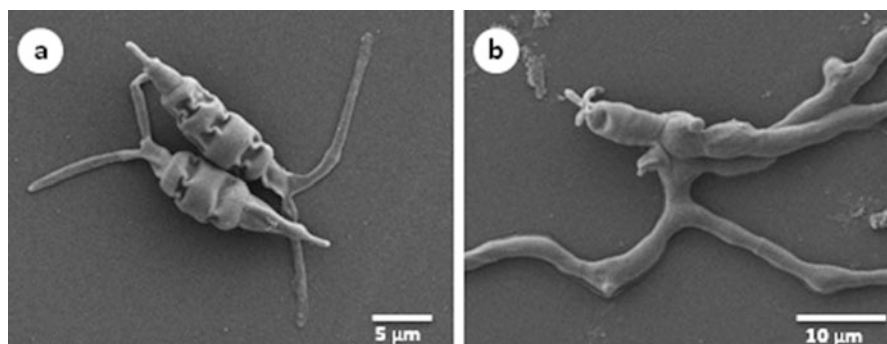


Fig. 6.9 Scanning electron microscopy (SEM) micrographs showing the effect of Zn-doped MgO NPs on conidial germination of *Pestalotiopsis maculans*. (a) Conidial cells and appendages (control); (b) non-germinated conidia with swollen cell and short appendages after exposition of sub-lethal concentration of Zn/Mg oxide NPs

In addition to the compatibility of the nanomaterials with the stone to be treated, another important challenging aspect of antimicrobial treatments is the fact that stone artworks are typically affected by a mixed community of microorganisms. These microorganisms may present different levels of susceptibility toward the used chemical compound. Therefore, nanomaterials with a wide action spectrum are of interest for stone protection. Among the different types of oxide NPs that have been applied as antifungal protective agents for stone heritage, MgO [31], ZnO [86], and TiO₂ [15, 86] are the most commonly used. These nanostructured materials present important physicochemical properties (mainly their inorganic character, greater surface area, and small particle size), which allow them to exhibit a wide spectrum of antimicrobial activity against different bacterial and fungal species, making them of great interest to the scientific community.

Although, in general, the mechanisms of antifungal activity of metallic NPs have not been completely understood, it is important to understand the main factors influencing the antimicrobial activity of NPs. The study of the antimicrobial mechanisms of nanoparticles constitutes a fascinating and open field for future research. Some studies have revealed that the inhibition of fungal growth is carried out by a combination of one or more mechanisms and it may vary based upon the function and chemistry of nanoparticles [87]. The antimicrobial activity of nanomaterials is generally attributed to three models: oxidative stress induction, metal ion release (disturbance in metal/metal ion homeostasis), and non-oxidative mechanisms [88]. Among these modes, the generation of reactive oxygen species (ROS) and the induction of oxidative stress phenomenon are the main modes of antifungal activity of the metal and metal oxide NPs [89, 90]. These reactive species are mainly superoxide anion (O₂⁻), hydroxyl radicals (OH⁻), hydrogen peroxide (H₂O₂), and organic peroxides, which are produced due to the change in electronic properties and the reduction in particle sizes [91, 92]. The ROS are pernicious to microorganisms, causing damage to almost all organic biomolecules and eventually inducing the cell death [89]. Also, different studies have pointed out that the ROS generation depends on the surface area of the NPs, which can potentially increase with a decrease of the particle sizes [93].

Moreover, the oxidative stress may sometimes happen through disturbing microbial process or oxidizing a vital cellular structure or components without ROS production [92]. Thus, other modes of actions to be considered include damage to cell membranes by electrostatic interaction [94], protein and enzyme dysfunction [95], genotoxicity [96], and photokilling [97]. Furthermore, careful attention should be paid to other physicochemical properties of NPs when studying their antimicrobial mechanisms. Several studies have shown that a large specific surface area and atomic ligand deficiency lead the NP aggregation [98, 99]. An increased aggregation state produces a reduction of surface area that could decrease the interaction between NPs and microorganisms and consequently the inhibition or loss of antifungal properties of NPs [100]. In addition, current research works have shown that particle size [93], shape [101], roughness [88], or surface charge [102] of metal and metal oxide NPs can greatly modify their antimicrobial activity. Additionally, the influence of crystal defect content on the antifungal capacity of nanomaterials

should be taken into account. Different studies have highlighted the crucial role of surface defects in the generation of ROS [103, 104]. In this line, recently, Sierra-Fernandez et al. [31] reported the application of Zn-doped MgO NPs as antifungal agents against different fungal species, for dolostone and limestone substrates. Their experimental results have shown that doped Zn greatly improved the antifungal capacities of MgO due to the generation of an increased defect density.

It should be noted that the study of different binding agents to assure an optimal adhesion of NPs being applied over deteriorated stone substrates is an important challenge to reach. In this regard, the use of different systems composed of polymeric matrices and NPs of various concentrations (%w/w) has been the focus of many research works in this field [105–107], in order to obtain coatings with multifunctional properties (hydrophobic, consolidant, and antifungal action) for the stone heritage preservation.

The identification and quantification of microbial communities which are colonizing on stone substrates are crucial steps to implement proper treatments. In this context, different analytical techniques have been used depending on the type of microorganism [108–110]. Moreover, the analysis of affected stone substrates by *in situ* electron microscopy and fluorescence microscopy, sometimes in conjunction with energy-dispersive X-ray spectroscopy, is commonly used [111–113]. SEM and confocal laser scanning microscopy (CLSM) can also be used to study the biodeterioration of stone substrates and/or determine the effectiveness of antifungal treatments based on nanoparticles in stone specimens [114]. Besides, the combination of TEM and SEM is also widely used in order to identify the microorganism types involved in the biodeterioration of stone and their interaction with the untreated and treated stone surfaces [30, 31, 115]. These powerful analytical techniques present the important advantage of evaluating the effectiveness of nanomaterials as antifungal protective coatings without extracting the microorganisms from the stones and interfering with them [115].

Recently, the analysis of biodeterioration and microbial populations has been developed in parallel with the application of molecular techniques based on phylogenetic descriptions for the study of biodiversity in artworks and historical buildings; these techniques can be used to evaluate the effectiveness after the application of the NPs and the temporal stability. Among the different techniques, RT-PCR (reverse transcription-polymerase chain reaction) is typically used. However, in the field of cultural heritage, preservation is still little due to the constraints originated by the sampling methods, the reproducibility, and/or the physiological niches, among others [116, 117]. Moreover, it is important to consider that these techniques present some risks, such as the inability to detect some organisms, reaching in some cases an underestimation of the colonizing population [116]. Therefore, the complementation of these studies with other analytical techniques, such as traditional microbiological techniques and the monitoring of the physiological activity of the microorganisms on and in materials, is absolutely necessary [118]. Besides, further studies are warranted to optimize protocols concerning sample procedures, extraction, PCR (polymerase chain reaction), and PCR amplifications to determine the best techniques to study the microbial communities present in stone heritage [116].

It is important to note that recent genomic and transcriptomic technology offers new solutions to study and understand the activity of whole microbial communities and also the effect of biocide treatments [118, 119]. The development of new cultivation techniques to describe the biodiversity and behavior of microorganisms colonizing the stone cultural heritage is another important challenge to reach [120].

6.4 Conclusion

The design, development, and application of inorganic NPs for the conservation of built heritage constitute an important advance in the application of nanotechnology for stone heritage preservation. Due to the reduced particle size, these advanced nanomaterials present new and enhanced properties, representing a significant improvement on traditional conservation treatments. The key to success is represented by a multidisciplinary research activity, combining material science, petrophysics, ecology, biology, chemistry, and physics and/or cultural heritage conservation. This synergy of different scientific disciplines opens the possibility to engineer materials that are highly compatible, effective, and durable for stone preservation. However, despite all the efforts done so far, the research is still at the beginning of a fascinating endeavor. It is necessary to study a great number of factors that can influence the effectiveness of innovative nanomaterials as consolidating and antifungal protective treatments for stone works of art. Thereby, although these new nanomaterials have been extensively characterized and applied for cultural heritage preservation, little is known about their behavior, effectiveness, and durability in an outdoor environment. Due to the high complexity of real aged stone surfaces, future research should focus on the analysis of the long-term treatment effectiveness after the application on real cases of stone building surfaces. More studies are needed in order to address the stability behavior of different inorganic nanoparticles being exposed to different thermo-hygrometric conditions, with emphasis on their effect on the crystallization kinetics and the development of structural modifications. These studies should be conducted with the aid of advanced characteristic techniques, in multidisciplinary approaches. The advances in the optimization and application of new analytical nondestructive techniques are expected to strengthen the understanding and consequently the effectiveness of new nanostructured materials specifically designed for stone heritage. Moreover, further studies should be centered on exploring the development of hybrid nanomaterials in order to create multifunctional coatings able to exhibit several properties simultaneously (e.g., self-cleaning and biocidal), simplifying and improving the conservative treatments, without visual impact on the surface. Future research is also necessary to shed light on the human health risks and environmental implications resulting from the use of new nanomaterials for stone cultural heritage preservation.

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