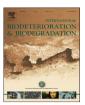
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## Biological nanosilver particles for the protection of archaeological stones against microbial colonization



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#### ABSTRACT

The inhabitation of microorganisms and their subsequent interaction with mineral matrix of the stone substrate under varied environmental conditions encourages deterioration of stones leading to the loss of strength, durability and aesthetic. This study highlighted the synthesis of nanosilver particles (AgNPs) using the biogenic volatiles of the bacterial strain *Nesterenkonia halobia*. The antimicrobial activities of AgNPs were evaluated against the gram positive bacterial strain *Streptomyces parvullus* and fungal strain *Apergillus niger*. Furthermore, the silver particles were mixed with two types of consolidation polymers and were used to coat the external surfaces of sandstone and limestone blocks. The stones treated with silicon polymer loaded with AgNPs showed an elevated antimicrobial potentiality against *A. niger* and *S. parvullus*. Scan electron microscope (SEM) and electron dispersive X-ray spectroscopy (EDX) analysis of treated stones demonstrated the existence of nano-composite structures containing the elemental silver. Polymers functionalized with AgNPs can be used not only as potent biocides but also for the consolidation of the historic monuments and artifacts.

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#### 1. Introduction

Sandstone and limestone are widely used in archaeological building in Egypt, for example sandstone was used in buildings of Egyptian temples. The temple of Edfu is one of these temples. It is located on the west bank of the Nile in the city of Edfu which was known in Greco-Roman times as Apollonopolis Magna, after the chief god Horus-Apollo. At the same time, there are many limestone archaeological buildings in Egypt such as the tomb of Teti's son Tetiankh-km at Sakkara area. The temple of Edfu and the tomb of Teti's son Tetiankh-km are exposed to various environmental and biological factors that result in apparent deteriorations of these monuments (Fig. 1). Heavy microbial colonization can obscure surface carvings, create an impression of poor maintenance and in some cases may promote the decay of the artifacts (Scheerer et al., 2009; Sterflinger, 2013).

Although, stones are extreme environments characterized with insufficiency of nutrients, enormous changes of humidity and

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elevated doses of UV radiation, they can be inhabited by various types of microorganisms causing stones deterioration (Selbmann et al., 2005). Deterioration mechanisms include acidolysis, accumulation of organic nutrients, discoloration, changes in the porosity, vapor diffusion changes and mobilization of ions (Gaylarde et al., 2003; McNamara and Mitchell, 2005).

Physical and chemical characteristics of the stone substratum in addition to the environmental conditions exert a direct impact on the selection of the colonization organisms. Cyanobacteria and algae comprehensively participate in stone biodeterioration via penetration and development of cryptoendolithic communities (Macedo et al., 2009; Hallmann et al., 2013). At the same time, fungi play a crucial effect in the disintegration of stones and archaeological materials due to their enormous enzymatic activity (Scheerer et al., 2009). Due to the high melanization of the fungal cell wall, stones colonized by fungi appear spotty or are even completely sheltered by black films. Moreover, fungi can not be easily killed by biocides or other antimicrobial treatments due to their thick cell walls (Sterflinger, 2010). In the meantime, bacteria are extensively involved in the deterioration of the historic stone structures (Warscheid and Braams, 2000). Different gram negative and gram positive bacterial strains such as Cellulosimicrobium, Stenotrophomonas, Ochrobactrum, Lysobacter, Bacillus, Isoptericola

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Fig. 1. Photographs clarifying the environmental and biological damage of the temple of Edfu in Upper Egypt (a, b, c) and the tomb of Teti's son Teti-ankh-km at Sakkara (d, e, f).

have been isolated from different artifacts (McNamara and Mitchell, 2005; Scheerer et al., 2009; Alisi, 2011). The potential degrading activity of bacteria takes place through the production of acids and surfactants (Stassi et al., 1998). Gram positive bacteria are more resistant to severe environmental conditions due to their capability to produce spores (Fajardo-Cavazos and Nicholson, 2006).

The removal of the microbial growths from stone surface is a complicated process because the conditions of the artifacts as well as the physical and chemical damaging factors must be considered very well. Actions against microbial growth can be mechanical, physical and/or chemical removal biodeteriogens. Many organic and inorganic compounds have been used as biocide agents to eliminate the biodeteriogens from cultural objects (Tiano, 1998).

Silver ions are known with their potent toxicity against broad range of bacteria, fungi, and viruses (Morones et al., 2005; Kim et al., 2007). Moreover, silver-based antimicrobials demonstrated positive properties such as thermal and chemical stability, environmental safety and low toxicity to human cells. These characters make silver-based materials suitable for wide varieties of applications (Lok et al., 2007; Pal et al., 2007).

Nanoparticles are of great interest due to their multiple potential applications (Knetsch and Koole, 2011). The nanometal particles have unique physicochemical properties including ultra small size, large surface to mass ratio, a distinctive reactivity with biological systems (Falletta et al., 2008; Zhang et al., 2011).

Various microorganisms including bacteria and fungi have been reported to produce metabolites that could be used for the synthesis of nanoparticles of different chemical compositions (Du et al., 2007; Verma et al., 2010; Ingle et al., 2011). The biologically synthesized nanoparticles are eco-friendly, cheap and completely safe. The aim of the current study was to evaluate the application of acrylic and silicon polymers loaded with biologically prepared nanosilver structures for the protection of two types of archaeological stones against bacterial and fungal colonization.

#### 2. Materials and methods

#### 2.1. Chemicals

Two types of consolidation polymers were used in this study; Primal AC33 polymer (Dow Chemical Co., USA) and silicon polymer (Wacker BS 1001, Wacker Chemei AG, Germany). The first consists of a mixture of methylacrylate and ethylmethacrylate while the second comprises of 50% silane/siloxane emulsion. Both types of the polymers were diluted to 3% final concentration with water.

#### 2.2. Studied samples and sites

The sandstone and limestone samples were carefully picked up from the fallen fragments of the temple of Edfu and the tomb of Teti's son Teti-ankh-km, respectively. The selected sites for this study are clear example for the environmental and biological negative effect on these monuments.

#### 2.3. Characterization of sandstone and limestone samples

The physical and chemical properties of the tested stones were studied using different techniques. A thin-section examination of the stones was carried out using polarizing light microscopy (PLM) with a Nikon Eclipse (C POL 600) microscope, equipped with an automatic photographic system and a digital camera (Model Nikon Coolpix 950). The mineralogical study included the analysis of samples by X-ray diffraction (XRD) using a Philips PW5 1337 automatic X-ray powder diffractometer with CuK $\alpha$  radiation. Patterns were obtained by step scanning from 3 °C to 75 °C 2 $\theta$  with a count for 0.5 s per step, exploration speed of 7 °C min<sup>-1</sup> and 40 kV and 40 mA in the X-ray tube. At the time, stone samples were studied using scanning electron microscopy (SEM) model JEOL-6400, in order to determine the nature of crystalline texture and microstructure of the stones.

#### 2.4. Preparation of the nanosilver particles

A batch bioreactor was used to prepare the composite metals structures of silver (Essa et al., 2005). The bioreactor composed of two chambers; one was used for bacterial growth (1 L, maintained under aerobic conditions by pumping in filtered compressed air), and the other chamber (100 ml) was used for metal precipitation by passing the culture exit gases through metal solution via a 0.2 μm filter to prevent bacterial contamination. In the growth chamber, about 800 ml of nutrient broth was inoculated with 100 ml of the gram positive bacteria *Nesterenkonia halobia* (formerly *Micrococcus halobius*) in the mid-exponential growth

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