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Laser removal of biofilm from Carrara marble using 532 nm: The first validation study

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ABSTRACT

Here we report the first extensive validation study of laser removal of biodeteriogens from marble. The work was carried out on the *Speranza*, monumental sculpture of the 19th century placed inside the English Cemetery in Florence. The statue was covered by diffuse greenish and black-grayish species due to the biological growth, a mix of cyanobacteria, algae and fungi classified as biofilm. Optical microscopy, culture-based methods and CF-PAM imaging (Pulse Amplitude Modulated fluorometry of the Chlorophyll Fluorescence) were used to characterize the biodeteriogens. Laser technique was proposed as an efficient alternative for the traditional cleaning methodologies. Preliminary trials were performed using different wavelengths and pulse durations. The optimization of the laser parameters was performed considering the results obtained from the stratigraphic layers observations of the cross-sections of the stone material and real time CF-PAM imaging of the phototrophic presence.

The study allowed improving, planning and accomplishing of the laser cleaning treatment of the whole statue.

1. Introduction

Exposed stone surfaces represent a substrate on which microorganisms can adhere and grow according to the material bioreceptivity [13,8]. This is indeed the intrinsic characteristic of a given material to be colonized by biodeteriogens and, besides the whole material properties (such as, for example, surface roughness, porosity, moisture content and chemical composition), the bioreceptivity is correlated with the surrounding microenvironment that contribute to the establishment, anchorage and development of colonizing organisms [21]. Cyanobacteria and algae as first colonizers, followed by micro-fungi, lichens and bryophytes, are the natural inhabitants of the stone substratum. They play a crucial role in developing aesthetic and physical-chemical alterations, exerting mechanical destructive forces and causing chemical modifications during their life cycle in the stone material. It was estimated that biological weathering can be even severer than the non biogenic degradation, representing a serious cause of deterioration of the outdoor stone cultural heritage all over the world [21,19,22,6,7].

During restoration interventions, biodeteriogens are usually removed using different methods, whose effectivity can be influenced by extrinsic causes such as previous conservation treatments on stone material and environmental factors [24,25]. Furthermore, the majority of outdoor artworks have to be maintained in place, in most cases without covering structures or other physical protections. For such reasons, the long-term effectiveness of biocide applied on exposed stoneworks is usually scarce, being the future recolonization very difficult to be avoided. The re-growth can involve species that differ from those affecting the artwork before restoration, and it can be really rapid and enhanced by environmental conditions [24,25].

Among the bio-species growing in stone monuments, cyanobacteria are the most complex and challenging to be treated because they are characterized by a great morphological variety and size.

Cyanobacteria, were traditionally considered algae for their ability to perform photosynthesis; but they are now classified as prokaryotes and bacteria, due to many characteristics such as the lack of a cell nucleus or membrane-bound organelles (from single-celled organisms with a diameter of 0.5 μm, to long filamentous forms up to several tens of μm) [1].

Stone deterioration by biofilm (a mix of algae and cyanobacteria) is due by their mechanical and chemical action and is typically

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related to their growth forms. Degradation involves changes in appearance due to the presence of variously colored patinas, greater water retention ascribable to algal films which can aggravate damage by freeze-thaw cycling, facilitation of other organisms colonization, substrate dissolution by chelating agents or organic acids, detachment of flakes of material due to the biomass increase within the material (chasmolithic algae) and the dissolution of the substrate (endolithic algae). Endolithic algae dissolve carbonates to enable penetration within the stone. The characteristics of the produced craters are specific, and correlated to light needs [12]. For all these reasons, the impairment and removal of biofilm from stone monuments should be considered as a priority in restoration interventions.

The periodical removal of biodeteriogens with the help of an eco-effective photochemical system (PS) impairment of biological presence represent fundamental needs for the preservation of outdoor stone cultural heritage.

Since conservative treatments are usually demanding, time consuming and costly, their long-term effectiveness, efficiency and usability must be considered. Moreover, the ethical aspect and the sustainability of the process should also be considered, as the treatments could be harmful to the integrity of the artifact and to the environment. Studies on these topics contributed to the improvement of a responsible management of the conservation of outdoor artworks and allowed to widen the range of suitable methodologies of restoration, as well as their reliability. Among them, laser ablation is a very appealing solution. Although applications of laser cleaning were carried out in the past on stone masterpieces, some issues raised up and are still open. In particular, only few works dealing with the removal of biodeteriogens are reported [9,35,38,28,31–33,20] and none of these involved an extensive validation.

In the present work, the laser treatment of biofilm from Carrara marble was tested for the first time. For the investigation on the irradiation effects, the fundamental (1064 nm) and the second harmonic (532 nm) wavelength of the Nd:YAG laser were employed.

Despite aromatic, aliphatic and OH chemical groups contained in biodeteriogens species exhibit a high optical absorption to the UV laser wavelength, a more energetic radiation, definitely increases the oxidation process. Moreover a deeper penetration of the laser light could be harmful for the marble itself, especially when inorganic particle (such as iron and copper particles) may be found as inclusions inside the stone substrate. In the past, experiments were performed using excimer lasers [37] including *in vitro* irradiation test on a variety of biological growths [11]. However, nowadays excimer lasers are hardly be considered practicable. Recently, further investigations using solid state lasers (Nd:YAG), testing a multi-wavelength approach (UV and IR), were carried out [32,33]. Finally, the laser treatment of lichens using FR Er:YAG (2.94 μm) provided interesting results in terms of thermal deactivation since the complete destruction of the cellular structure was observed. On the other hand, limitations are due to the low ablation efficiency [9]. These are the reasons why we focused our attention to the 532 nm and 1064 nm laser wavelengths.

In this work laser irradiation effects were characterized using optical microscopy and chlorophyll fluorescence measured using pulsed amplitude modulated (CF-PAM) imaging. The results achieved show that 532 nm laser irradiation is significantly more effective than 1064 nm and it can represent a solution for a wider set of biodeterioration problems.

This work represents a rare case where the results of tests applying a novel methodological protocol were then validated performing a restoration intervention of a whole stone monument (in this case the statue the *Speranza*) by using only laser technique instead of the traditional mechanical and chemical approach. In particular, the green laser wavelength at 532 nm was employed for the impairment and the removal of the biodegraded parts; whereas the infrared radiation at 1064 nm for the treatment of black crusts (established methodology).

The optimization of the laser parameters was performed considering the results obtained from the stratigraphic layers observations of the cross-sections of the stone material and real time CF-PAM imaging of the phototrophic presence.

2. Materials and methods

2.1. The statue

The Florentine cemetery known as the *English cemetery* was built between 1827 and 1828 by the Swiss Evangelical Reformed Church outside the city walls, to give a burial to all non-Catholics. The renovation of the city following the proclamation of Florence capital (1865) of the newborn Kingdom of Italy and the implementation of new health regulations, caused the closing of the cemetery and its declaration as monumental site. Since then, the English cemetery became an open-air museum, rich of sculptural works of arts made by the greatest artists of the time, such as Lorenzo Bartolini (1777–1850), Hiram Powers (1805–1873), Giuseppe Lazzerini (1831–1895). They adorned the tombs of illustrious people such as Elizabeth Barrett Browning (1806–1861), Walter Savage Landor (1775–1864), Jean Pierre Vieusseux (1825–1865). The English cemetery is an evidence of the integration and intercultural exchange between Florence and foreign communities as well as the rediscovery of architectural styles of the past and, in particular, neoclassicism.

The *Speranza* statue was sculpted in 1863 by the florentine artist Odoardo Fantacchiotti (1811–1877) for the burial of Reginald Samuel Routh. This is one of the most imposing tombs in the English cemetery, though almost nothing is known about the deceased. The monumental statue, carved in white marble, represents an allegorical figure (about 2 m high) placed on a basement with burial inscription and overturned torches on each edge, representing a symbolic reminder of death. The *Speranza*, as well as the whole artistic work of Fantacchiotti, is characterized by an extreme softness of the drapery and sweetness of the traits, revealing his basic culture deeply founded on classical art [3].

Nowadays the statue was almost completely covered by diffuse greenish and black-grayish patinas, due to the synergistic action of biological growths, urban pollution, and microclimatic factors. To address the critical situation, the restoration of the whole statue was planned in the framework of the project TEMART "Advanced techniques for the material knowledge and the conservation of the historical-artistical patrimony", approved by the Tuscany Region, cofounded by the European Fund for Regional Development (POR CreO FESR 2007–2013).

2.2. Experimental

In order to characterize the state of conservation of the marble, samples constituted by micro-fragments and/or powders were collected from different parts of the statue, following a rigorous criterion of minimal invasiveness. After inspection under the stereomicroscope, five samples were analyzed by X-ray diffraction analyses (XRD) using the silicon ZBP (Zero Background Plate) of a Philips X'Pert PRO PW 3040 diffractometer (Bragg-Brentano geometry) with a CuKα source operating at 40 kV and 40 mA and equipped with a PW3015 X'Celerator detector. XRD patterns were collected by step scanning from 3 to 85°(2θ) with a scan speed of 0.016°/s. Thin sections were prepared after embedding the micro-fragments in polystyrene resin (Mecaprex MA2, Presi, France) and analyzed by means of a polarizing microscope (LEICA