Biotechnology for preventive conservation: development of bionanomaterials for anti-microbial coating of outdoor sculptures

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Abstract

Outdoor sculptures are part of the sociocultural identity of cities, but are extremely vulnerable to deterioration due to exposure to harsh environmental conditions and climate change. Furthermore, deterioration by microorganisms renders urgent need to design protective coatings. This research work proposes an integrated methodology for the development of innovative and sustainable nanofilms for applications in the area of art conservation, very specifically in the preventive conservation of outdoor sculptures. Gathering objective data in the characterization of the surface microbiota is important in order to design strategies that make use of bio or nanotechnology innovative coatings. Methodologies for the characterization of the microbiota present in a granite outdoor sculpture, followed by preliminary results on the application of protective antimicrobial coatings for surfaces of cultural objects are described.

Keywords: preventive conservation, flow cytometry, biocontamination, chitosan, nanofilms

Introduction

The exposure of urban outdoor public sculptures to uncontrollable environmental conditions and high levels of pollution makes these heritage objects highly susceptible to deterioration, and their preservation is a fundamental issue in art conservation.

Conservation solutions have been developed from treatments to eradicate biological agents or prevent their growth on surfaces of these objects. The use of coatings is a classical approach in conservation and restoration; however, most of the currently used treatments use biocides. This leads to environmental and health and safety issues, and there is a limited range of actions available.

The microbiota on outdoor sculptures is complex, with a great variety of genera from several biological groups. Further complexity also arises from deposits of debris from dead cells, dust and pollution. We propose an integrated approach that can be applied regardless of the type of sculpture, location and climate. The proposed methodology allows for quantitative and qualitative characterization of the microbiota with the possibility of differentiation between dead, injured, and healthy cells, which constitutes the fundamental initial step in required for successful development of antimicrobial coatings. To the best of our knowledge, flow cytometry was used for the first time to determine the number of microorganisms and their viability in the context of preventive conservation of cultural objects.

In the recent years, the use of gels and polymeric dispersions for cleaning in conservation has become well accepted (Carretti, Deia, and Weiss 2005) and some of the most innovative solutions for the control of outdoor biocontamination involve nanofilms and gels with antimicrobial activity, mainly based on the incorporation of zinc, copper, and titanium dioxide (Valentini, Diamanti, and Palleschi 2010).

The proposed innovation in this project is in the use of chitosan for the development of antimicrobial bionanomaterials for coating of outdoor sculptures. Chitosan is an abundant polysaccharide that is also inexpensive, biodegradable, antimicrobial, and non-toxic (Kean and Thanou 2010; Rinaudo 2006). The possibility of chemical modification and versatility of rheological films and formulations are very important to conservation, as is the possibility of reversibility of the coating i.e. with enzymes (Rinaudo 2006).

Materials and methods

Sculpture and sample collection

Sol, Lua e Vento (1997) is a grey granite sculpture by Satoru Sato located in Santo Tirso, in the Metropolitan Area of Porto, Portugal and is part of the *Museu Internacional de Escultura Contemporânea de Santo Tirso* (MIEC). The sculpture (Fig.1) is located in a green public area in the city center. Five areas on the sculpture were selected and two samples from each zone were collected by either swabbing with a sterile cotton swab or pressing a sterile disk of a HEMA/MBAm cryogel (Keles *et al*. 2015) onto the sculpture surface. The samples were dispersed in peptone water with 1% Tween 80 and a protocol was applied that involved multiple centrifugation and filtration to eliminate debris (adapted from Anvarian *et al*. 2016). Samples for scanning electron microscopy (SEM) were collected with double-sided carbon adhesive tape and sputter-coated with gold and Fungi-tape™ (Scientific Device Laboratory, USA) was used to collect samples for optical microscopy at 100x magnification. Two granite fragments were also collected from the sculpture with sterile tweezers and a chisel and used for flow cytometry, SEM, and DNA extraction.

Figure 1. *Sol, Lua e Vento* (1997), sculpture by Satoru Sato (MIEC).

Microbial cultivation

Samples were inoculated in duplicate on Plate Count Agar (PCA) and Potato Dextrose Agar (PDA) by the spread plate, and incubated at 25 ˚C in the dark up to 7 days. For the growth of microalgae, BG-11 liquid media supplemented with amphotericin B (7.5 µg/mL) was inoculated and incubated at 20 ˚C with constant light for 14 days, and transferred to BG-11 agar media and incubated under the same conditions.

For aerobiology studies, PDA and PCA (in duplicate) were left open for 10 min in selected sites (near the sculpture and the surrounding vegetation) and incubated at 25 ˚C in the dark for 2 days.

Colorimetry and ATP determination

Colorimetric measurements were performed using a CR-400 chroma meter (KONICA MINOLTA, Japan) on the sculpture surface in the five pre-selected areas, and L*a*b* values were recorded.

Adenosine triphosphate (ATP) was detected by swabbing the sculpture surface and determining the emitted light using a HY-Lite®2 luminometer (Merck, Germany).

Flow cytometry and DNA extraction

BD Accuri™ C6 flow cytometer (BD Biosciences, USA) was used to acquired data without probes in order to calculate the number of autofluorescent microorganisms. Cell viability was determined after addition of thiazole orange (TO) and propidium iodide (PI; BD™ Cell Viability Kit, BD Biosciences, USA).

To identify all culturable and unculturable microorganisms, total genomic DNA extraction was performed with the Ultraclean® Microbial DNA Isolation kit (MO BIO Laboratories, Inc., USA). DNA was quantified by spectrophotometry and fluorimetry and amplified by PCR reaction for Illumina Sequencing of 16S rRNA gene and Internal Transcribed Spacer 2 region, for metagenomic analysis.

Chitosan films

Antimicrobial chitosan films were prepared with a low molecular weight chitosan solution at 0.1% in 1% acetic acid and pH 3.0. 12 g of this solution was weighed and transferred to petri dishes incubated at 30 ˚C for 3 days for film formation. A volume of 0.5 mL of chitosan solution was also poured and spread onto the surface of granite and cement samples and the absorption rate was observed over time.

Results and discussion

The microbiological colonization of *Sol, Lua e Vento* was evaluated. Several types of bacteria and fungi colonies were identified based on their morphology. Zones closer to the ground showed the most variety of colonies with different morphologies. Similar results were observed during aerobiology studies, where the highest variety of colonies was found near the soil. Small colonies of microalgae seemed to be present in BG-11 culture media. Samples collected for optic microscopy also revealed the presence of green biofilms of microalgae and possibly lichens.

Samples collected on the surface of the sculpture, using either swabs or hydrogels, were analyzed by flow cytometry, which allowed for a quick and effective determination of the total number of microorganisms. The presence of autofluorescent microorganisms was detected, being higher in the samples collected with swabs than those collected with hydrogels.

For the same zones, there seems to be a great variation in the number of autofluorescent microorganisms detected, according to the methodology used to collect the samples (swab or hydrogel).

Flow cytometry also allowed the determination of the total number of microorganisms, which ranged between 181-1293 cells/µl for the samples collected with swabs. For these samples, the percentage of live cells varied between 4-24%. Interestingly, the number of microorganisms in the samples collected with hydrogels was lower than in samples collected with swabs.

Microbiological contamination was confirmed by detection of ATP. Highest levels of ATP were observed in accordance with the results obtained with flow cytometry and growth on culture media.

A transparent antimicrobial film can be formed (Fig.2 A) that can be applied in non-absorbent surfaces such as metal and low-porosity stone, like marble, although important effects related to glossiness should be considered especially in rough finish surfaces.

For testing the application on absorbent surfaces, such as stone, a chitosan solution was poured onto the surface of granite (Fig.2 B) and cement samples to observe its absorption rate and possible formation of films. It was observed that both the polished surface of granite samples absorbed the chitosan solution slower than the similar surfaces of cement. These results may indicate that in highly absorbent surfaces, a completely

protective film might not be achieved (Fig.2 C), although the hypothesis of the formation of an endolithic protection is possible. Chitosan films can be optimized for their porosity using various degrees of cross-linking that can be suited for porous materials in order to deal with the possibility of humidity condensation and other water transfer phenomena in highly absorbing materials.

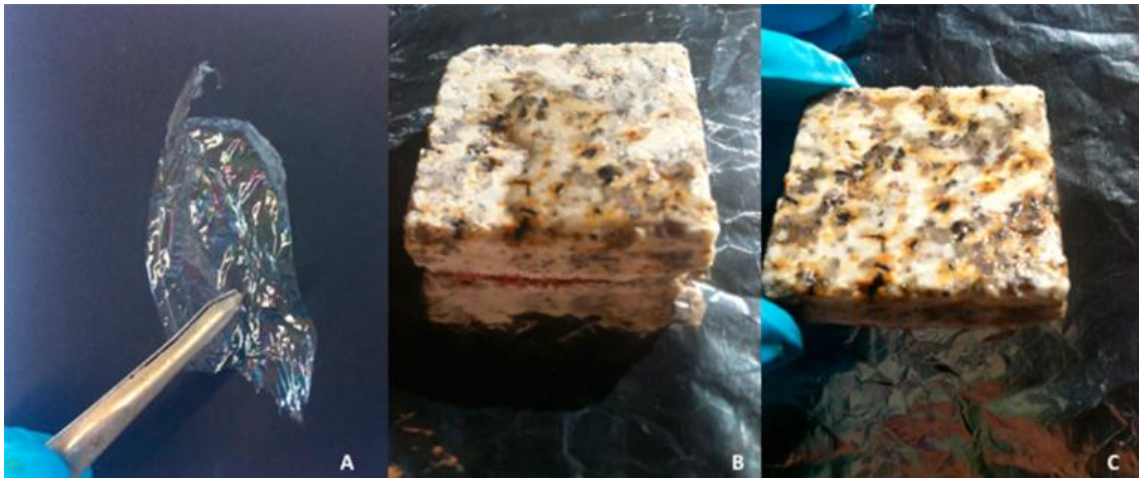

Figure 2. A) Transparent antimicrobial chitosan film. B) Chitosan solution poured onto the surface of granite. C) After chitosan absorption on granite's surface.

The development of new coatings based on chitosan is currently ongoing. In the near future, they will be tested for their effectiveness in preventing the growth of various biological agents as well as the interactions of the coatings with the object materials from a physical, chemical, and aesthetic points of view, important for the conservator-restorer.

Disclosure statement

No potential conflict of interest was reported by the authors.

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