Promising surface-active ionic liquid coatings for underwater cultural heritage conservation

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***Abstract* – Any submerged inorganic material is promptly colonized by a variety of microorganisms that offer the basis for the settlement of macroorganisms causing the so-called biofouling. The aim of this work is to evaluate applicability and durability of new designed coatings containing Surface-Active Ionic Liquids for the protection of underwater cultural heritage from the first step of biofouling formation. We report here the results of the characterization test performed on limestone and Carrara marble probes (colorimetric and capillary water absorption measurements) and of the UV weathering to evaluate the durability of these coatings. The results have shown that these coatings do not affect the original properties of the stone surfaces and they are stable over time.**

Keywords: Ionic liquids; Antifouling; Submerged inorganic materials.

I. INTRODUCTION

Underwater cultural heritage faces multiple threats that endanger its preservation. Stone surfaces exposed in the marine environment are under the action of physical, chemical and biological factors dependent on exposure conditions. The first cause of deterioration of submerged artifacts is biofouling. Within the first few minutes of submersion, a stone surface is promptly colonized by a variety of microorganisms that make a biofilm or microfouling. Microfoulers (microalgae, bacteria, fungi) offer the basis for the settlement of macrofoulers (macroalgae, crustaceans, serpulids etc.) [1]. The result is the loss of readability of the artifacts and the material itself. Thus, the settlement of microfoulers generates an extracellular polymeric matrix (EPS) (biofilm) that encloses microorganisms which are irreversibly attached to a surface resulting in unavoidable colonization of macroorganisms and deterioration. Biofilm has characteristics such as high resistance to environmental stress and biocide treatments. Therefore, it is critical to implement strategies to prevent this first phase of colonization. In the context of global pressure for more eco-sustainable approaches, it is necessary to develop innovative products and formulations for cultural heritage conservation. For preventive approaches, bio- and nano-technologies represent a significant contribution. Ionic liquids have been proposed as a class of materials with environmental compatibility, thus potentially meeting sustainability criteria [2]. Currently, extensive study of environmental impact, toxicity and biodegradability has made possible wide use of ILs [3,4]. Briefly, ionic liquids (ILs) are a class of low-melting-point organic salts. They have an ionic nature characterized by a cation@anion pair that can be manipulated, while keeping synthetic control of physicochemical and biological properties and functions. The "third-generation ILs" are based on natural and biodegradable ions (cholinium, morfolinium aminoacids, drug anions) [3].

Few data are currently available to support the antimicrobial activity of these preservation products [5,6]. In 2021, De Leo et al. made a series of surface-active ionic liquids (SA-ILs) [7]. The engineered molecules were characterized by mono- and di-choline cations with alkyl chains of different lengths and as conventional halide anions or the surfactant dodecylbenzenesulfonate (DBS). The introduction of surfactant companies within the IL ion pair allowed a control of their bioactivity. In vitro studies with chemically synthesized molecules showed biocidal activity against bacterial and fungal strains isolated from deteriorated stone material. Studies conducted on stone materials with subaerial trials confirmed a relationship between lipophilic and antimicrobial activity, as the more lipophilic products, with long alkyl chains (Fig. 1), showed preventive activity against new microbial colonization [7].

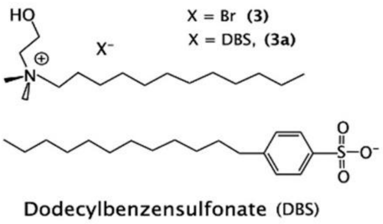

Fig. 1. Schematic representation of cholinium@halides (3) and cholinium@DBS ILs (3a).

Follow up agar diffusion tests against Gram (+), Gram (-), yeast and black fungi, it was determined that the combination of the two best-performing ionic liquids in a molar ratio of 3:1 (ILs MIX) improved their bioactivity.

The site of our interest is situated on the Ionian coast near Crotone (Calabria, Italy). Crotone was one of the most important centers of Magna Grecia for its strategic position on the Ionian Sea. The coastline extending between 'Capo Colonna' and 'Le Castella' was declared a Marine Protected Area in 1991. Several ancient wrecks can be found here, such as the shipwreck, called 'Punta Scifo D' consisting of large blocks and slabs of white marble, some metal artifacts and ceramics. In addition, there are three different shipwrecks loaded with marble columns and other lithic material (e.g. "Cala Cicala"). For this reason, the materials employed in our trials are Carrara marble and limestone, which are the main components of this submerged cultural heritage. In the present work, the effective applicability of silica@ILs MIX coatings on stone samples is evaluated. Nanosilica suspension and tetraethyl orthosilicate as silica precursors were used to promote IL adhesion to the stone surface.

II. RESULTS AND DISCUSSIONS

In order to check that coatings containing ILs MIX combined with silica-based consolidants as binders were applicable on stone materials of different nature with durability over time, the characterization of untreated and treated marble and limestone specimens was carried out. The choice of the number and size of specimens and the tests to be performed were evaluated according to the conditions to be tried and the reference legislation [8]. Limestone and marble probes were treated with only silica-based consolidants, NanoEstel® (NES), TEOS (TE) and Estel 1000® (ES) and NanoEstel® as a binder with ILs MIX as a top layer (Bilayer) (NES+I), Estel 1000® as a binder with ILs MIX as a top layer (Bilayer) (ES+I) and TEOS mixed with ionic species (Monolayer) (TE+I). All the chemicals, unless otherwise stated, were purchased from Sigma-Aldrich and used as supplied. NanoEstel® and Estel 1000® were purchased from CTS S.r.l, Altavilla Vicentina, Italy, and used as suggested by the producer. The syntheses of cholinium precursor N-(2-Hydroxyethyl)-N,N-dimethyl-1-dodecanaminiumbromide (3) and N-(2-Hydroxyethyl)-N,N-Dimethyl-1-Dodecanaminium Dodecylbenzenesulfonate (3a) were performed by following published procedures [7].

A. Applicability of coatings containing SA-ILs

First, colorimetric and capillary water absorption measurements were made to assess any change in the aspect and degree of water absorption of the treated stone surfaces. Marble and limestone specimens were submitted in three replicates for each condition tested. Colorimetric measurements were carried out with ZL 310 colorimeter according to the guidelines outlined in NorMaL 43/93 [9]. The instrument provided data on L*, a* and b* coordinates: L* brightness index (0 = absolute black, 100 = absolute white) and a* and b* chromaticity coordinates (a* is the position between green (a* < 0) and red (a* < 0); b* is the position between blue (b* < 0) and yellow (b* > 0). The color change of the treated surfaces was evaluated by calculating ΔE:

$$\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2} \quad (1)$$

ΔL*, Δa*, Δb* correspond to the differences between different surface conditions. Firstly, colorimetric analysis was performed on all samples before and after treatment. The ΔE values obtained of the samples treated with the same coating were averaged to obtain a single value and standard deviation. The data provided in figure 2 show that the treatment of stone surfaces with silica-based coatings combined with ionic species results in a color change undetectable to the human eye (ΔE less than 5).

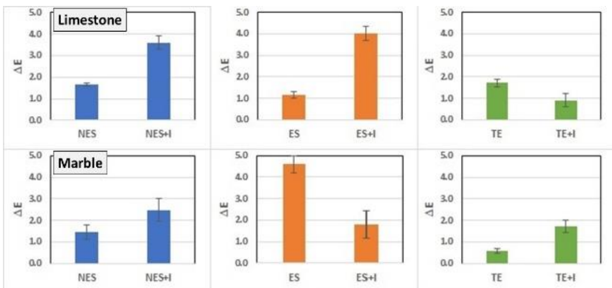

Fig. 2. Colorimetric differences between untreated and treated specimens.

In addition, the degree of water absorption by capillary of untreated and treated stone surfaces with these coatings was evaluated in terms of the amount and rate of absorption. This trial was carried out according to the guidelines provided by UNI EN 15801:2010 [10]. The 3 replica specimens for each condition were weighed and placed on the laying bed with the test surface in contact with the wet paper. The hydrophilic SA-ILs making the stone material highly permeable to the water, so 5 days from the start of the test the specimens showed a saturated state like the untreated specimens (Fig. 3). The amount of water absorbed as a function of time was expressed relative to the unit area by solving the equation:

$$Q_i = [(m_i - m_0) / A] \quad (2)$$

That is the difference between the mass of the specimen at time t_i (m_i) and the mass of the dry specimen (m_0) in relation to the surface area in contact with water (A). Next, the Q_i values of the equally treated samples were averaged to obtain a single value and standard deviation.

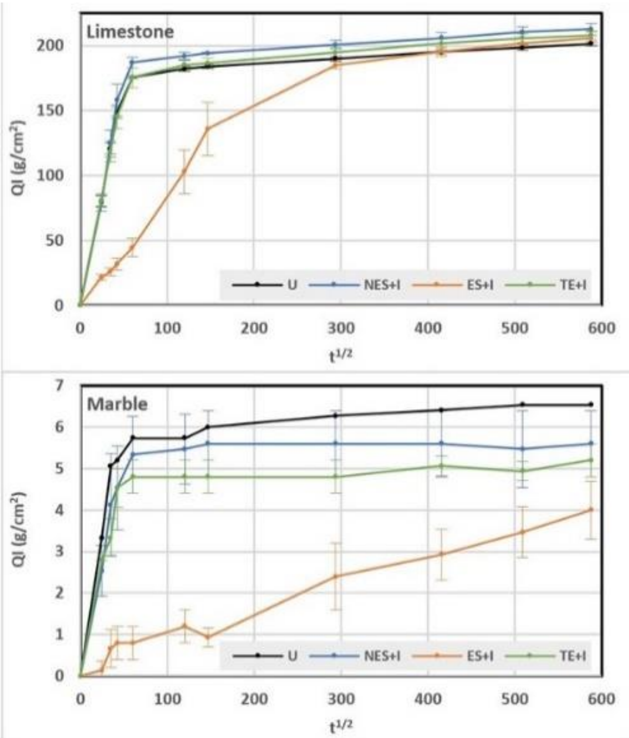

Fig 3. Capillary absorption of untreated and treated samples.

B. Durability of coatings containing SA-ILs

To evaluate the durability of the new developed coatings over time, the samples were next exposed to UV weathering. The samples were tested for UV resistance in the SUNTEST XLS+ chamber to check for changes in material and product properties caused by sunlight. In this way, it was possible to simulate the aging that can occur in the outdoor environment over years. The specimens were left for 507 h under the action of daylight artificial fluorescent lamp combining visible and UV outputs (λ= 300-800 nm, irradiance= 500 W/m^2) at a temperature about 35 °C. Thereafter, the aged samples were again submitted to colorimetric and capillary water absorption degree measurements (Figs 4,5).

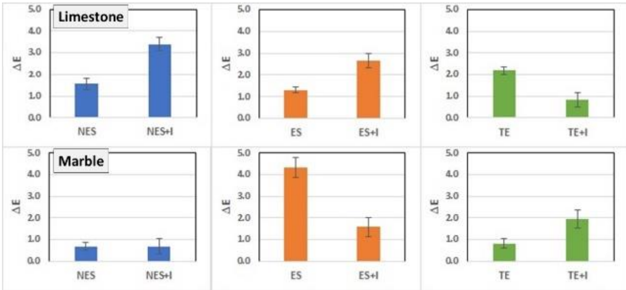

Fig. 4. Colorimetric differences between untreated and treated aged specimens.

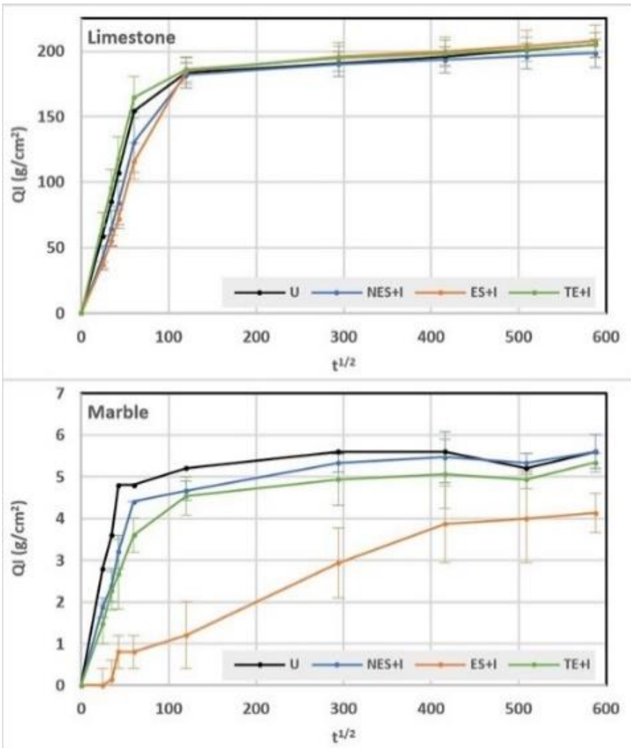

Fig 5. Capillary absorption of untreated and treated aged samples.

The results reported up to now show effective applicability of such coatings on cultural heritage without changing its physicochemical properties and with durability over time. Limestone treated with the combination Estel 1000® and ILs MIX and marble treated with NanoEstel® and ILs MIX after aging show a change of ΔE. Analyzing the L*, a* and b* parameters in detail, the limestone surface shows a not significant change and the marble surface shows an imperceptible negative change of the L* parameter (Fig. 6).

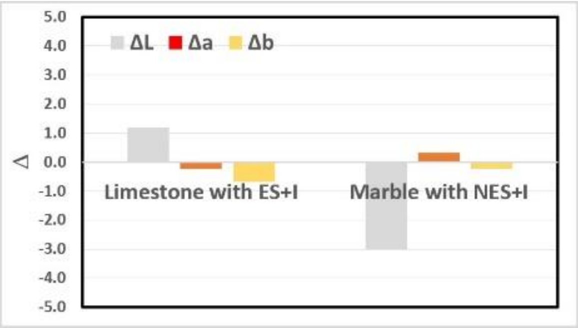

Fig. 6. Variations in L, a*, and b* coordinates between unaged and aged samples.*

The capillary water absorption degree of untreated and treated samples with the coatings under investigation is different in the first 24 hours ($t^{1/2}$ = 293,9). This may be due to the lowering of the porosity, which is filled by the consolidant, or the hydrophobicity of the surface. However, we can say that the behavior of samples treated with TEOS and Nano Estel as binder is quite similar to that of untreated samples. This means that there is no significant decrease in both porosity and hydrophobicity induced by the consolidant. Treatment with Estel 1000 and ILs MIX, on the other hand, induces a significant slowing of water absorption. This result can be attributed to transient hydrophobicity caused by entrapment of solvent in the coating during the curing process. Indeed, analysis on aged samples shows a slight acceleration of the process, due to a " desiccant effect" of UV-daily radiation.

III. CONCLUSIONS

The great challenge for the protection of submerged cultural heritage from the threat of biofouling is the development of specific innovative protocols that meet criteria of eco-sustainability. In this context, Surface-Active Ionic Liquids (SA-ILs) have shown to be antifouling coatings that can fully meet ecological conservation criteria. The test of their applicability on stone material without any change of the original surface properties and of their resistance to the action of UV irradiation allow their possible use on the cultural heritage. These are just the first steps toward the goal we want to achieve. The next challenge will be to test the possible use in the aquatic environment as antifouling coatings. Further tests are planned to assess the degree of wettability of the treated surfaces and the ability to resist from the bioerosive action of water in the marine environment ("washout"). Systems that mimic this process in the absence of microbial communities are currently being set up in order to obtain more information on possible forms of degradation due to seawater.

The effective biocidal and preventive activity against microorganism colonization on stone materials, demonstrated in subaerial tests, makes them promising antifouling agents [4]. It will also be necessary to evaluate the antifouling activity in the real underwater environment. Stone samples treated and untreated with silica-based consolidants combined with ILs MIX were exposed in marine environment. Some samples were taken after 1 hour because the first stages of biofilm formation occur during this time frame of immersion. Other samples will be taken after 1, 6 and 12 months of exposure. The antifouling activity of the coatings will be evaluated by direct analysis of the microorganisms (culture analysis), metagenomic and bioinformatic analysis of DNA extracted from any biofilms present on the surface of the samples.

Holding the samples in the marine environment for one year will allow to confirm the durability of the new designed coatings to the various changing conditions of the real underwater environment.

REFERENCES

[1] S.A.Ruffolo, A.Macchia, M.F.La Russa, L.Mazza, C.Urzì, F.De Leo, M.Barberio, G.M.Crisci, "Marine Antifouling For Underwater Archaeological Sites: TiO2 And Ag-Doped TiO2", Int. J. Photoenergy, vol.2013, Article ID 251647.

[2] S.Lo Schiavo, F.De Leo, C.Urzì, "Present and Future Perspectives for Biocides and Antifouling Products for Stone-Built Cultural Heritage: Ionic Liquids as a Challenging Alternative. Appl. Sci., vol.10, No.18, September 2020.

[3] R.Ferraz, L.C.Branco, C.Prudêncio, J.P.Noronha, Ž.Jetrovski, "Ionic liquids as active pharmaceutical ingredients", ChemMed-Chem, vol.6, No.6, June 2011, pp. 975–985.

[4] M.Petkovic, K.R.Seddon, L.P.N.Rebelo, C.S.Pereira, "Ionic liquids: A pathway to environmental acceptability". Chem. Soc. Rev., 2011, vol.40, pp. 1383–1403.

[5] M.Archismita, I.F.Castillo, D.P.Müller, C.Gonzµlez, S.Eyssautier-Chuine, A.Ziegler, J.M.De la Fuente, S.G.Mitchell, C.Streb, "Poly-oxometalate-Ionic Liquids (POM-ILs) as anticorrosion and antibacterial coatings for natural stones. Angew. Chem. Int. Edit., vol.57, No.45, November 2018, pp. 14926–14931.

[6] S.Eyssautier-Chuine, I.Franco-Castillo, A.Misra, J.Hubert, N. Vail-lant-Gaveau, C.Streb, S.G.Mitchell, "Evaluating the durability and performance of polyoxometalate-ionic liquid coatings on calcareous stones: Preventing biocolonisation in outdoor environments" Sci. Total Environ., Vol.884, August 2023, pp.163739.

[7] F.De Leo, A.Marchetta, G.Capillo, A.Germanà, P.Primerano, S.Lo Schiavo, C.Urzì, "Surface Active Ionic Liquids Based Coatings as Subaerial Anti-Biofilms for Stone Built Cultural Heritage", Coatings, vol.11, No.1, December 2020.
[8] CNR-ICR, "Raccomandazioni NorMal 20/85: interventi conservativi : progettazione esecuzione e valutazione preventiva", Rome, Italy, 1985.
[9] CNR-ICR, "Normal - 43/93: misure colorimetriche di superfici opache", Rome, Italy, 1993.
[10] ICS, "UNI EN 15801:2010 Conservazione dei beni culturali – Metodi di prova – Determinazione dell'assorbimento dell'acqua per capillarità", 2010.