UNIVERSITÀ DI PAVIA
Department of Drug Sciences

Activity evaluation of supramolecular compound based on N-acetylcysteine for the treatment of *P. aeruginosa* biofilm

Guerini Marta; Grisoli Pietro; Perugini Paola
Department of Drug Sciences, University of Pavia, Via Taramelli 12-14, 27100 Pavia, Italy, marta.guerini01@universitadipavia.it

INTRODUCTION

Cystic Fibrosis (CF) is a genetic pathology causing mucus obstruction, neutrophil-dominated airway inflammation and bacterial infection (1). *Pseudomonas aeruginosa*, a gram-negative bacillus, produces a biofilm, that prevents the penetration of antibiotics. Furthermore, the microorganisms inside are increasingly aggressive (2) (3). This work focused on the evaluation of effectiveness of a supramolecular compound based on N-acetylcysteine (NACESOL™), against *P. aeruginosa* biofilm. The prevention activity was evaluated both by microbial assay and by Scanning Electron Microscopy (SEM) associated with the X-Ray Spectroscopy (EDS) to determine the elemental composition of surface films in the SEM (4).

MATERIALS AND METHODS

For the production of the biofilm, P. *aeruginosa* ATCC 10145 was cultured overnight in TSB under aerobic conditions at 37°C; then a stainless-steel disk was immersed in the bacterial suspension for 48h at 37°C. To be able to determine the actual presence of the biofilm on the disk, a count of the bacterial suspension after mechanical stresses and sonication of the stain-less steel surface was performed.

Then, different weights of NAC and NACESOL™ were put into bacterial suspension in order to have different concentrations of N-acetylcysteine (0.5 mg/ml, 2 mg/ml, 4 mg/ml). For each analysis, a control was made without active. Results of the biofilm prevention assays are expressed as percentage values of biofilm reduction using a non-treated metal disk as a control.

SEM analysis on the disk was performed operating at 20 kV (TESCAN, Mira 3 XMU, Brno, Czech Republic). Comparison of biofilms with different thicknesses was made using both SE (Scattered Electrons) and BSE (Back-Scattered Electrons) detectors. Semiquantitative composition microanalysis was determined by energy dispersive spectrometry (EDS, EDAX Ametek Inc).

The antioxidant activity of NAC and NACESOL™ was evaluated through their capability of scavenging DPPH radical (6).

Solid physicochemical analysis were also made through thermal analysis, FTIR measurements and XRPD measurements.

RESULTS

Eradication of Biofilm assay

Free NAC at 0.5 mg/ml reduces the number of colonies by 19.42% +/- 4.28%, at 2 mg/ml the reduction percentage is the 20.87%, and at 4mg/ml the reduction percentage is 89.52 +/-5.83% .

NACESOL™ at 0.5 mg/ml of NAC is able to reduce the number of colonies by 51.41 % +/- 22.10, at 2 mg/ml the reduction percentage is 68,98% +/-24,30%, and at 4mg/ml the reduction percentage is 48.00 +/-31.14% .

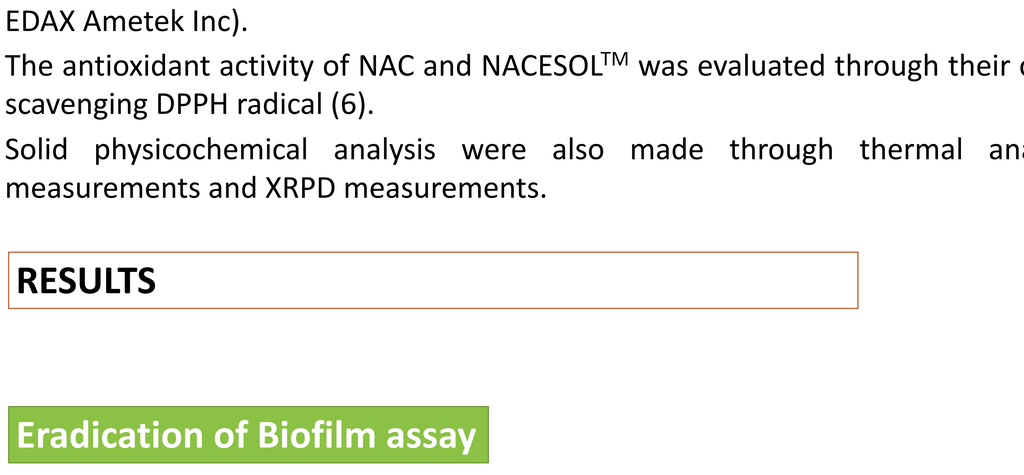

Figure 1. Percentage of biofilm reduction at 0.5 mg/ml of NAC

Scanning Electron Microscopy

SEM analysis using BSE detector permitted to highlight the distinction between the biofilm presence and the clean steel. By increasing the concentration of the active, the biofilm thins more and more, until only the clean steel layer was seen.

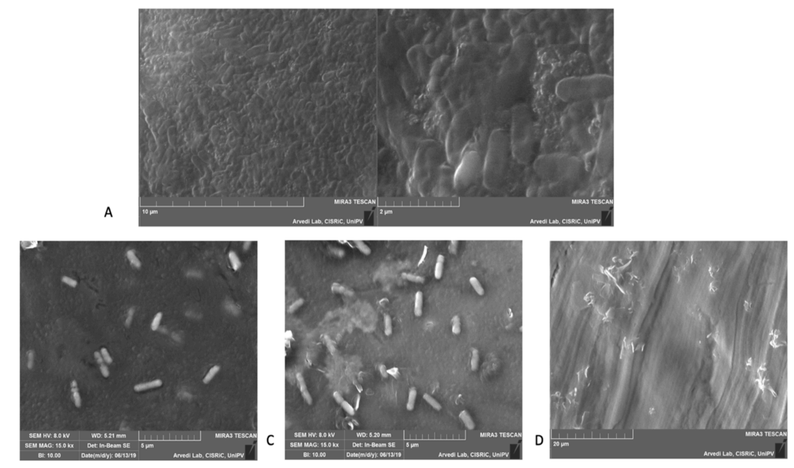

Figure 2: Higher magnification of SEM pictures of a Pseudomonas aeruginosa Biofilm on an untreated steel-surface of: (A) untreated biofilm; (B) biofilm with 0.5 mg·mL⁻¹ of NAC; (C) biofilm with 2 mg·mL⁻¹ of NAC; (D) biofilm with 4 mg·mL⁻¹ of NAC.

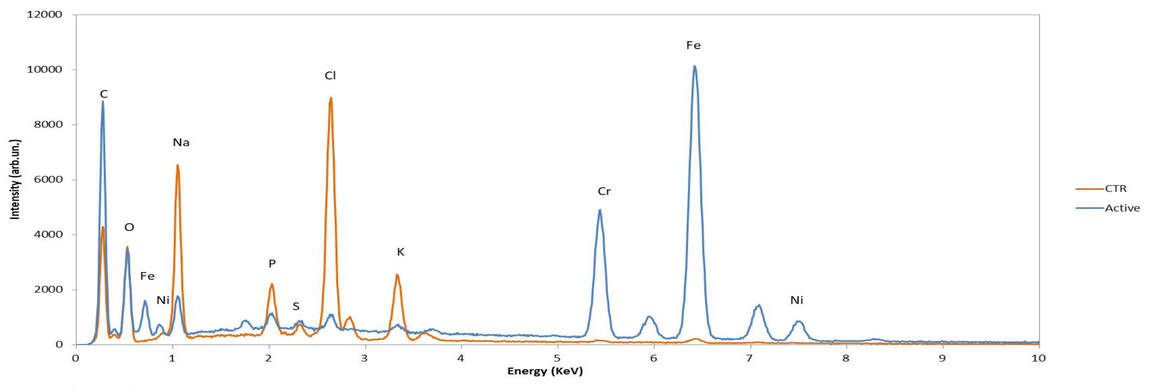

Figure 3. Plotted spectra of the control and active-treated disk

Antioxidant analysis

The comparison between free NAC and NACESOL™ (Fig. 4) show that the antioxidant activity of the NAC is independent of its crystalline or amorphous structure: in fact, NACESOL has an antioxidant activity comparable to the free NAC.

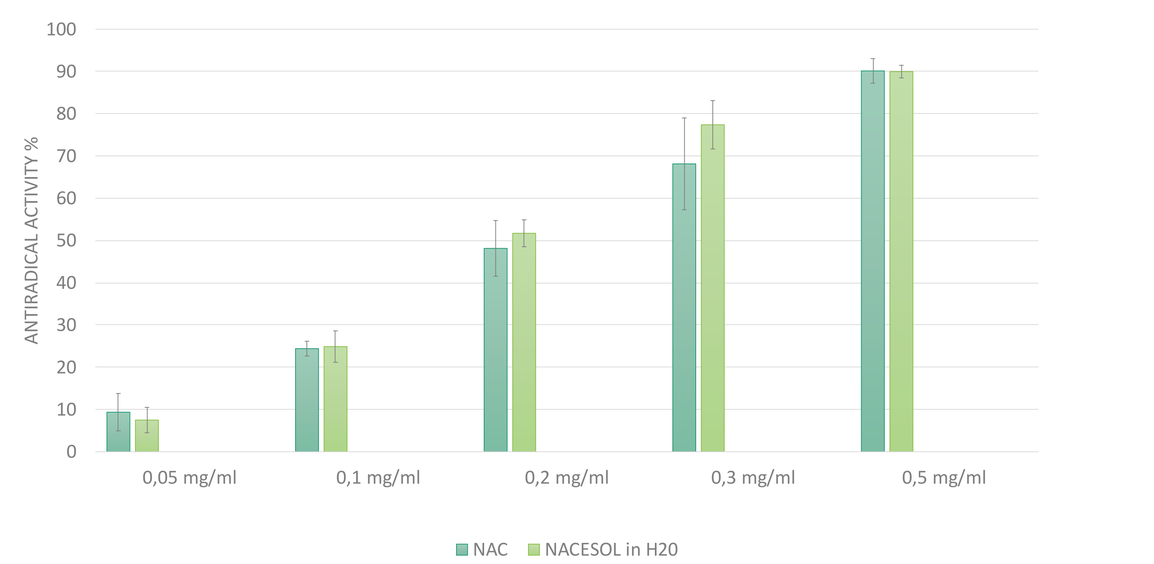

Figure 4. NAC and NACESOL™ antioxidant activity.

CONCLUSIONS

Through the high resolution of the SEM it was possible to have a clear view of the bacteria inside the biofilm, the thickness, the morphology of the same and its gradual eradication with the increase of the concentration of active. We demonstrate also the effective increase of the biological activity of the NAC / α-CD / Resveratrol as shown in the figure 1. Moreover, the DPPH assay demonstrate that the antioxidant activity at 0,5 mg/ml of NAC is the same, and therefore that the active in the multicomposite does not lose its activity, as shown in figure 4.

1) Hartl D. J. of Cystic Fibrosis. 11, 363–382 (2012). 2) Bjarnsholt T.. Pediatric Pulmonology. 44, 547–558 (2009). 3) Hentzer M. Alginate. J. of bacteriology. 5395–5401 (2001). 4) Calheiros L., Gomes J. M. . Scanning. 1-77, (2017). 5) Carli F. Corvi Mora P. Canal T. Patent number WO 03/097012 A1, 2003
6)Brand-Williams W., Cuvelier M. E., Berset C., LWT- Food Science and Technology. 28, 1, 25-30, 1995.