NMR based optimization of bactericidal silicon materials for biomedical devices

Simona Tomaselli[1], Katiuscia Pagano[1], Henriette Molinari[1], Laura Ragona[1]

[1]*SCITEC-CNR, via Corti 12, Milano*

Email: simona.tomaselli@scitec.cnr.it

INTRODUCTION

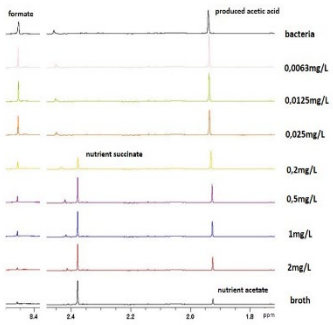
Device associated infections are considered worldwide a major threat to public health as they substantially impact on increased hospital stays and care costs, morbidity and mortality [Shahrour, H., Mat. Sc. & Eng. C-Mat., 2021]. Silicon rubber is the most commonly employed polymer in catheters productions, owing to its mechanical properties and its greater biocompatibility and stability to sterilization and storage conditions. Considerable research efforts have focused on increasing the bactericidal activity of silicon devices through surface modifications: passive or active coatings with bactericidal compounds, and silver incorporation to prevent bacterial attachment and hence biofilm formation [1]. We report here on the feasibility of including 5-chloro-2-(2,4-dichlorophenoxy)phenol (Irgasan or triclosan, IRG), in silicon catheter tubes in order to obtain a biocompatible material preventing *E. coli* and S. *aureus* growth and biofilm formation. Irgasan (IRG), was selected as it is a strong broad-spectrum anti-microbial agent, active against both Gram + and Gram – bacteria, and already in use in many consumer products. In the present paper we introduce an optimized simple swelling procedure providing silicon material active against planktonic and sessile bacterial cells. We further show the efficacy of NMR approaches, here used at different stages of the material preparation, to monitor the upload and release of the bactericidal molecule, thus guiding the optimization of the swelling procedure. Specifically, the analysis of the metabolites secreted by the bacterial cells exposed to the functionalized material has been employed for the first time to detect any minor unwanted leakage (even at sub-lethal concentration) of the bactericidal molecule from the impregnated silicon materials. Viability tests have been used to assess planktonic bacteria survival upon contact with the material and to estimate the effect of biofilm formation on the surface of the silicon tubes. In addition, the properties of the surface of the swelled silicon tubes have been characterized by means of Atomic Force Microscopy (AFM) and contact angle measurements

MIC and NIC

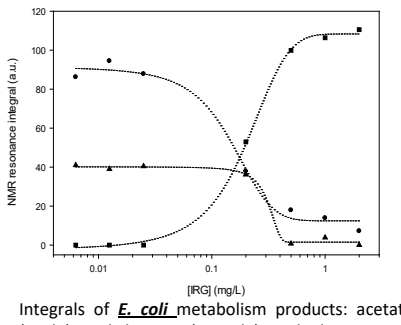
Minimal inhibitory concentration (MIC) represents the inhibitor (IRG) concentration needed to prevent bacterial growth. Non-inhibitory concentration (NIC) is the minimal concentration at which an inhibitor starts to be effective in perturbing bacteria metabolism, without leading to cell death.

Vitality test by NMR

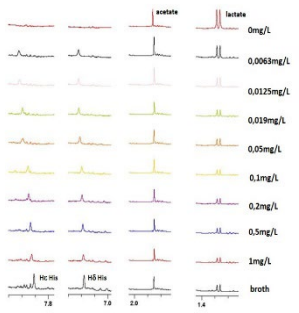
Secreted metabolites integrals are monitored in function of increasing concentration of IRG



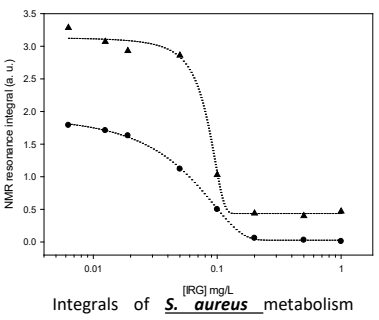
Selected regions of 1D ^{1}H NMR spectra acquired at 500MHz, after 24 hours incubation at 25°C of ***E.coli*** culture in the presence of increasing concentrations of IRG, as indicated by the blue arrow.



Integrals of ***E. coli*** metabolism products: acetate (circle) and lactate (triangle) and the nutrient succinate (square) are plotted as a function of IRG concentration (logarithmic scale). Data fitting to a modified Gompetrtz equation is shown as dotted lines.



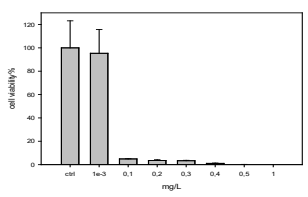
Selected region of 1D ^{1}H NMR spectra acquired at 500MHz, 25°C, after 24 hours incubation at 25°C in the presence of increasing concentrations of IRG as reported for ***S. aureus***.



Integrals of ***S. aureus*** metabolism products: acetate (circle) and lactate (triangle) are plotted as a function of IRG concentration (logarithmic scale). Data fitting to a modified Gompetrtz equation is shown as dotted lines.

Viability tests by CFU counting

Colony forming units (CFU) are counted after incubation of bacteria in presence of different amoutns of IRG



Cell viability of **E.Coli** at different IRG concentrations. The diluted inoculum in presence of ethanol < 0.1% v/v was used as control. Error bars represent the standard deviations from three independent replicates.

MIC, NIC E.coli

NMR: 0.33 ± 0.08, 0.11 ± 0.09 mg/L

CFU: 0.4-0.5 mg/L

MIC, NIC S.aureus

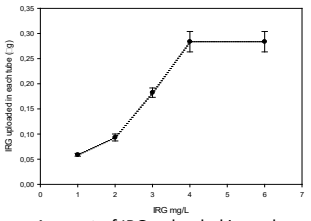
NMR: 0.15 ± 0.02, 0.03 ± 0.02 mg/L

Antibacterial activity Silicon IRG loaded tubes

Silicon catheters were swelled in chloroform in presence of 1, 2, 3, 4 and 6 mg/L IRG. A tube swelled in chloroform but without IRG was used as control (Tubes T0, T1, T2, T3, T4, T6).

Estimation of loaded IRG by NMR

Secreted metabolites integrals are monitored in function of increasing concentration of IRG

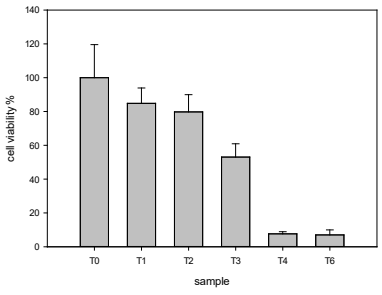


Amount of IRG uploaded in each tube swelled in the presence of increasing IRG concentration.

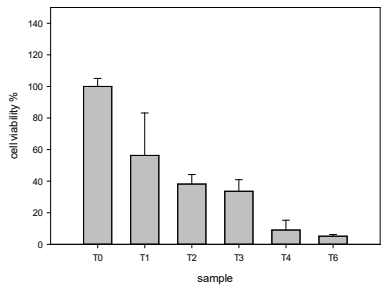
IRG loaded tubes were de-swelled in chloroform and solution dried. The solid residue was redesolved in methanol-d4 and quantified by NMR (ERETIC)

Uploaded IRG represents a 2% of the amount present in the starting bulk solution

IRG loaded tubes bactericidal activity



Percentage of cell viability of **planktonic** ***E. coli*** in the presence of T0, T1, T2, T3, T4, and T6 tubes after 24h contact time

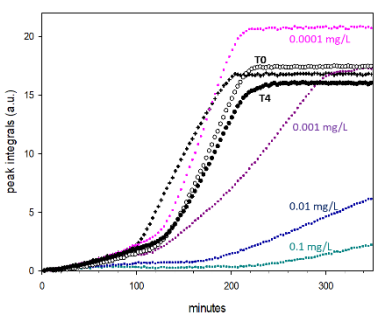


Percentage of cell viability of **sessile** ***E. coli*** bacteria on the surface of T0, T1, T2, T3, T4 and T6 tubes, after 24h contact time

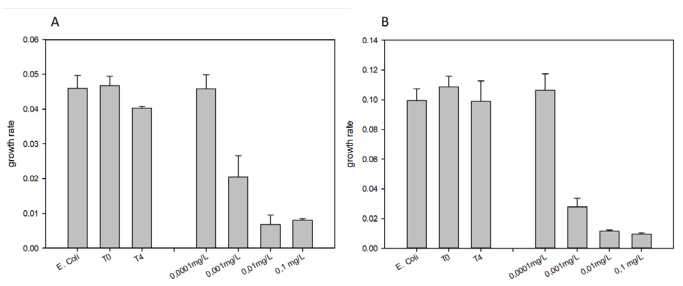
4mg/L as the minimal starting bulk solution concentration required to develop silicon tubes with a bactericidal effect against E. *coli*

Estimate of IRG released from silicone tubes

indirect monitoring of IRG release by following bacteria metabolism in real time. A series of NMR spectra of E. *coli* cells grown in liquid media in the presence of silicon tubes was recorded



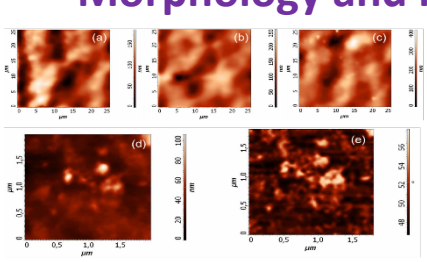
^{1}H acetate resonance integrals observed in bacterial samples in contact with T0 (empty circles) and T4 (black circles) tubes, or in the presence of free IRG at 0.1 (green), 0.01 (blu), 0.001 (dark violet) and 0.0001 (pink) mg/L. The behaviour of bacteria alone is reported for comparison as black crosses.



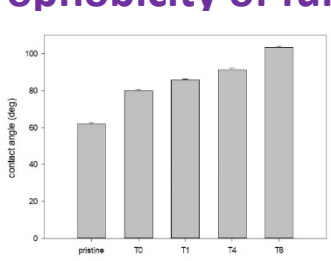
Acetate (panel A) and formate (panel B) growth of E. *coli* alone and in contact with T0 and T4 and in presence of 0.1, 0.01, 0.001 or 0.0001 mg/L free IRG.

Free IRG, eventually released from T4 tube, has a concentration lower than 0.001 mg/L. This estimate is lower than that revealed by direct NMR and UV measurements

Morphology and hydrophobicity of functionalized tubes



AFM height images for the pristine (a), T0 (b) and T4 samples (c). RMS are 36 nm, 26 nm and 45 nm, respectively. Detailed Height (d) and phase images (e) of T4



Contact angles measured on T0, T1, T4 and T6 as well as on pristine tube surface.

Surface hydrophobic nature is progressively enhanced as the presence of IRG on the surface of the tube increases

CONCLUSIONS

NMR is a fast and indirect method to evaluate the release of a bactericidal molecules

IRG show bactericidal mechanism through surface action (contact-killing), based on the exposure of molecules trapped into silicon pores to bacteria

Acknowledgments

POPlab Scitec: M. Pasini, U. Giovanella, E. Kozma, G. Scavia, S. Iannace
SIDAM srl (Mirandola, Italy)

Financial support: Regione Lombardia Project "Piattaforma tecnologica per lo sviluppo di sonde innovative in ambito biomedicale" (ID 244356).
Fondazione Antonio De Marco