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Antifouling eco-filters for water bio-decontamination

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Abstract: Water bio-contamination causes serious environmental and economic penalties and health risks on several applications (e.g. freshwater and seawater circuits). In this work, a non-toxic potential strategy able to control this bioburden through new functional bioactive agents capable of being tethered in polymeric coatings is presented. Econea biocide was successful tethered in a silicone based coating, further used to coat polymeric substrates and monolithic filters. Coated substrates demonstrated promising antifouling effects after being submerged for more than a year at simulated and real conditions. Furthermore, coated monolithic filters showed auspicious growth inhibition and bacteriostatic behaviour for the *S. aureus* MRSA bacteria.

Keywords: biofouling; tethered biocides; non-release coatings; antifouling coated filters; water purification

1. Introduction

Over two-thirds of Earth's surface is covered by water, a vital source for all living organisms, able to provide them with nutrients, as well as transport them to other locations which can offer better conditions for their survival. The progress and sustainability of our society are the most representative examples of this water dependency, from nutrients provision to its own growth and expansion (e.g. maritime transport).

But ironically, what should be a benefit also become one of the biggest cause of problems. The spontaneous colonization by aquatic organisms (ex. waterborne pathogens such as bacteria, fungi, marine organisms) on submerged surfaces, leading to the formation of biofouling [1], has been associated to serious penalties, particularly on engineering surfaces (e.g. pipes for water supply, swimming pools, desalination units, etc.). It gives rise to the known Industrial Biofouling [2], associated to substantial operational consequences (e.g. efficiency losses up to 5% in power plants) [3], substrate deterioration or improper devices function, resulting in severe economic and environmental penalties. It became of particular alarm on fluid circuits systems, such as water purification & distribution, since such systems promote the contact between water and the biofouling attached on surfaces. As a result, fluid contamination occurs, which can lead to subsequent serious or even lethal human infections [1-5], becoming a major public health concern.

Efforts have been widely explored to mitigate this bio-contamination [6, 7]. Hitherto, the most effective strategy follows a chemical approach. It comprises the direct and/or controlled release of toxic substances or disinfectants into the contaminated surface/fluid. In particular, for microorganisms removal from fluids, studies have been focused on the development of filters systems (e.g. structured filters) possessing antifouling properties [8, 9]. The most common approach is based on the deposition or incorporation of bioactive metal ions or metal oxides (e.g. silver, copper based) or even biocides on filters surfaces [9]. However, the major drawback of these approaches is the continuous leaching of those toxic agents into the aquatic environment. Their subsequent accumulation and/or intrinsic ecotoxicity have been associated with serious harmful side-effects [10-12]. Therefore, rigid environmental regulations have been implemented (BPR, Regulation (EU) 528/2012), compromising the permission to use the current or newly available agents.

Recent studies boosted new non-releasing biocide systems, for instance, antimicrobial polymeric materials [7] and coatings with tethered biocides [13, 14]. They evidence that the immobilization of antifouling agents can lead to more controllable and long-term biocidal activity. But, the killed microorganisms remain on the substrate, thus providing a layer to support new ones, which compromise their biocidal action. Innovative approaches are sought to overcome these limitations. As known, such non-releasing biocidal active surfaces or coatings combined with structured filters, such as monoliths, and for biofouling prevention on submerged surfaces have not been explored.

In this study, a novel strategy based on the chemical immobilization of antifouling agents in the most recent foul-release silicone based coatings was developed [14]. It comprised the development of non-releasing biocidal systems by providing new functional reactive biocides capable of being tethered in those coatings, further used to coat monolithic filters. This novel approach is here presented as a potentially long-lasting and eco-friendly antifouling alternative for biofouling prevention in waterborne systems.

2. Materials and Methods

2.1. Immobilization of antifouling agents in polymeric coatings

Commercial Econea® (provided by Janssen PMP, 98%) biocide (4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile) has been selected to be immobilized. It is considered a booster antifouling agent, with high potential to replace toxic antifouling agents, due to its relatively short lifetime (half-lifetime of about 3h is seawater), and a wide range of bioactivity, particularly for hard foulants. For the immobilization of this biocide in polymeric coatings, a prior functionalization process involving its reaction with a diisocyanate reagent in which the biocide functionalized will have a -N=C=O reactive function, capable of being tethered in different and compatible polymeric matrixes (polyurethane and silicone based marine paints, provided by HEMPEL SA) was performed. A detailed description of this process can be found in the recent patent application [14].

The commercial biocide and their functional counterpart were further analysed by Fourier Transform Infrared Spectroscopy (FTIR) (Nicolet Magna FTIR 550 Spectrometer coupled to an attenuate total reflectance unit Smart MiracleTM- Pike Technologies with an individual ZnSe crystal on the 500-4000 cm^{-1} range with 4 cm^{-1} resolution) to evaluate their chemical structure, and in the case of the functional biocides, to confirm the N=C=O functionalization effectiveness. In the particular case of Econea and in order to better evidence the biocide characteristic FTIR bands, the FTIR spectrum was obtained from Econea/KBr prepared waffle.

The R-N=C=O free content was obtained by a standard adapted procedure from the standard ASTMD2572.

Bioactivity evaluation: prior to biocides immobilization in polymeric coatings, and in order to confirm the bioactivity of the functional biocides (e.g. Econea-N=C=O), the antibacterial activity of the developed samples was evaluated using the well diffusion method on Mueller-Hinton agar (MHA). *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212) strains were used as model bacteria for the antibacterial assay of biocides. Briefly, 100 μL of standardized bacterial strain suspensions, corresponding to a turbidity of 0.5 McFarland, were used to inoculate an MHA petri dish, under aseptic conditions. Subsequently, agar wells (diameter = 5.0 mm) were made, filled with 50 μL of the test samples, including the negative control (DMSO) and positive control (Vancomycin), and further incubated at 37 °C for 24 hours. The resulted inhibition zones were reported in millimetre (mm). The tests were performed for each sample and strain in triplicate.

Immobilisation of biocides in polymeric coatings: The biocides were added as additives in a silicone marine based paint (Ref. 87509) gently provided by Hempel A/S. The experimental formulations were developed in the framework of the European FP7 collaborative project FOUL-X-SPEL (grant agreement 285552). Conventional methods (ex. brush) were used for further application, i.e., the coating of substrates.

2.2. Antifouling coatings characterization

Physical-chemical properties of the developed formulations have been assessed in previous works [15, 16], in particular, an eco-toxicity study of those [17].

2.2.1. Antifouling assessment

Proof-of-concept of the most potential coatings with chemical immobilised biocides was provided by antifouling performances assessment at simulated (artificial seawater aquarium, pH 8.3, 23 ± 1 ° C) and real field test conditions (Atlantic sea) of coated polymeric substrates (ex. polyvinyl chloride, PVC). Field tests were performed at relative static conditions in the Estaleiros Navais de Peniche (ENP) pontoon (coordinates 39º21'06.6''N 9º22'10.5'' W) at Peniche, Portugal, and in accordance with ASTM D6990 and D3623-78a standards.

2.3. Coated monolithic filters

Preliminary coating tests of the obtained antifouling/antimicrobial polymeric coatings systems were also performed on ceramic filters supports, such as cordierite based monoliths, by using the conventional dip-coating method. The coated monoliths were further assessed in terms of antimicrobial activity.

2.3.1 Antimicrobial activity

In order to assess the antimicrobial activity of the monolithic filters, a time kill test procedure was adapted for the purpose [18]. Briefly, small monolithic samples (2x2x2 cm) were immersed in a known population of the Methicillin-resistant *Staphylococcus aureus* (MRSA, CIP 1106760) strain (10^6 CFU/mL) for 24 hours at 37°C was used. The microorganism growth was monitored spectrophotometrically along time (each hour) and quantified by absorbance measurements at OD620 nm. In addition, and in order to address the bactericidal and bacteriostatic properties aliquots for each set of inhibition growth assay, was collected and incubated at 37°C for 24h at Muller-Hinton medium. Assays were carried out in duplicate/triplicate.

3. Results

3.1. Functional biocide with linkage ability into polymeric coatings

Econea biocide was successful functionalized (conversions as high as 95%) with a diisocyanate [14, 17], acquiring the N=C=O functionality and thus the covalent linkage ability for compatible polymeric matrix such as silicone based paints. FTIR spectra of the new functional biocide (Econea-

NCO) confirmed that their structure was not modified and the functional group was successfully attached to the expected bridging point (Figure 1). The -N=C=O functionality of the functional Econea-NCO was revealed by the appearance of the new band ranging from 2327 cm^{-1} and 2144 cm^{-1}, which is a characteristic peak of free NCO [19]. This -N=C=O characteristic band is also partial overlapping with the band attributed to the structural nitrile (-C≡N) functional group of the Econea biocide (Table 1).

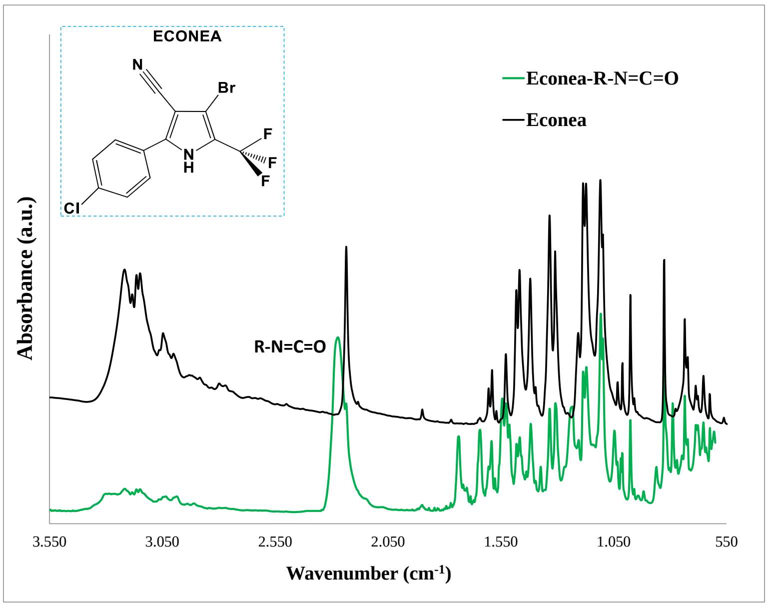

Figure 1. FTIR spectra of Econea and its functional counterpart, Econea-R-N=C=O.

Table 1. Main obtained bands from infrared spectra (FTIR) analysis of Econea biocide and its functional counterpart Econea-R-N=C=O.

Econea biocide	
Main characteristic bands	**Attributed group**
Group of intense broad bands: 3357-2852 cm^{-1}	Amines, amides, O-H bond, H
Intense narrow band: 2260-2152 cm^{-1} Maximum at: 2235 cm^{-1}	Nitrile, -C≡N
Band 1290-1335 cm^{-1}	Aromatic amine
Double intense bands: 1110-1180 cm^{-1}	CF3
Narrow band with a maximum at 827 cm^{-1}	C-Cl (substituted benzene)
Econea-R-N=C=O (Econea-NCO, functionalized)	
Intense broad band: 2327-2144 cm^{-1} Maximum at: 2254 cm^{-1} e 2237 cm^{-1}	Assigned to -N=C=O together with Nitrile, -C≡N
Narrow band with a maximum at 815 cm^{-1}	C-Cl (substituted benzene)
Double intense bands: 1110-1180 cm^{-1}	CF3

On the other hand, an NCO content of 9 ± 2 wt. % was obtained for the Econea-NCO, showing that one NCO group may be incorporated in the biocide structure. Deviations on the NCO content are associated with the presence of impurities and moisture, which is impossible to remove completely during the preparation procedure. Moisture, in particular, is highly reactive with the NCO group, therefore a limited amount is expected to react with free NCO, reducing its content and reactivity.

3.1.1. Bioactivity evaluation of biocides

Bioactivity assessment of the Econea and functional Econea-NCO biocides (Figure 2, Table 2) showed that for the tested bacteria both biocides possess antimicrobial activity.

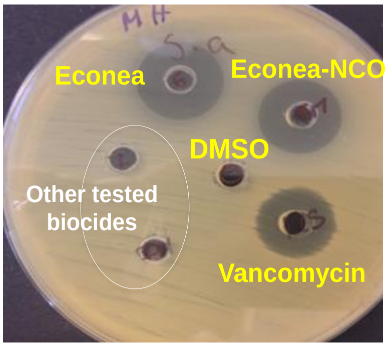

Figure 2. Representative Well Diffusion test for the assessment of the antimicrobial activity of Econea biocide and its functional counterpart (Econea-NCO), against *Staphylococcus aureus*. Dimethyl sulfoxide (DMSO) was used as the negative control and Vancomycin as the positive control.

Tabela 2: Antimicrobial activity of the Econea biocide and its functionalized counterpart against Gram-positive bacteria.

Biocides	***Enterococcus faecalis* (mm)**	***Staphylococcus aureus* (mm)**
Econea	20	17
Econea-R-NCO	21	17
DMSO (negative control)	5	5

3.2. Antifouling assessment

Proof-of-concept of the most potential coatings with chemical immobilized or tethered Econea biocide was provided by antifouling performances assessment at simulated (aquarium). Figure 3 shows representative coated PVC substrates (6x4 cm) after submersion in an artificial seawater aquarium for more than a year (1.5 years). Nonetheless, and in order to confirm the behaviour of the above-mentioned formulations, at hardest or aggressive conditions in terms of biofouling formation promotion, real field tests have been performed on coated PVC substrates (10x10 cm) (Figure 4).

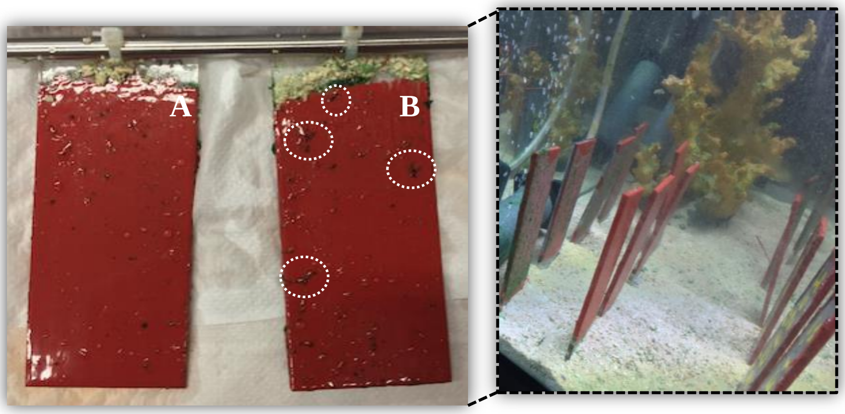

Figure 3. Representative Silicone based coatings with immobilized Econea biocide (<0.6 wt.%) (A) and respective Reference (B) without biocide after 1.5 years in an artificial seawater aquarium.

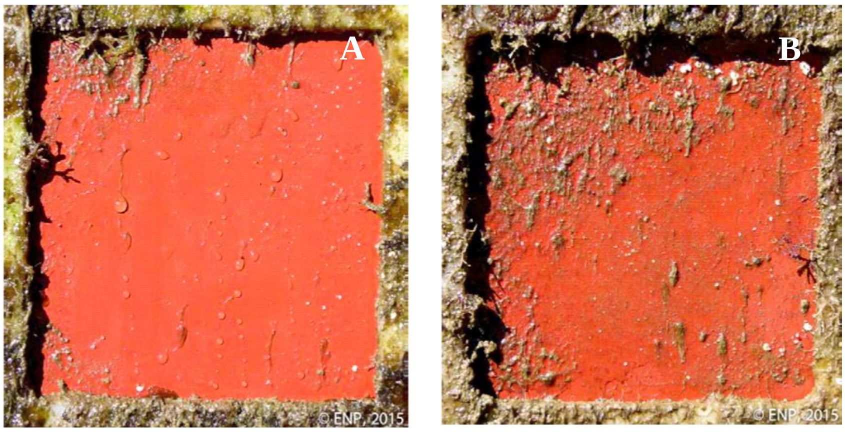

Figure 4. Silicone based coatings with immobilized Econea biocide (<0.6 wt. %) (A) and reference (B) without biocide after 45 weeks (about 11 months) submerged in Atlantic sea at the pontoon of Estaleiros Navais de Peniche (ENP), SA, Portugal. Photos gently provided by ENP.

3.3. Antimicrobial activity of coated monolithic filters

Cordierite monolithic filters have been coated for the first time with the prepared non-release antifouling/antimicrobial polymeric systems. Uniform and well adhered polymeric films were obtained (Figure 5), allowing proceeding with the first antimicrobial tests, through the analysis of the coatings effect on the structured support on the bacterial growth over time performed for *S. aureus* MRSA strain (Figure 6 and 7).

Figure 5. Ceramic monolithic filter (200 cpsi) coated with a silicone based coating containing tethered Econea (< 0.6 wt.%).

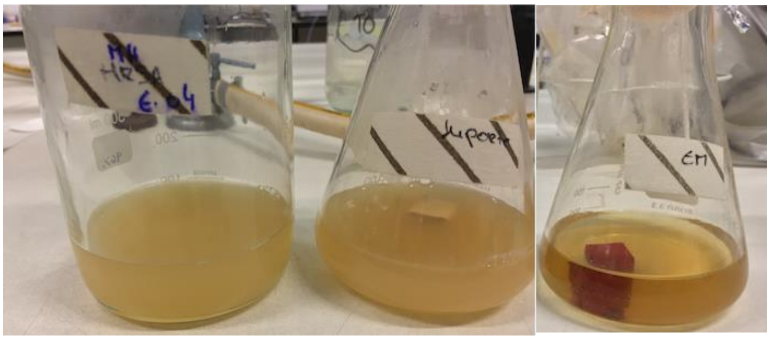

Figure 6. Illustration of the Inhibition growth behaviour on the bacterial strain *S. aureus* MRSA CIP 106760 culture for the uncoated and coated monolithic filter with a silicone based coating containing tethered Econea biocide (< 6 wt.%).

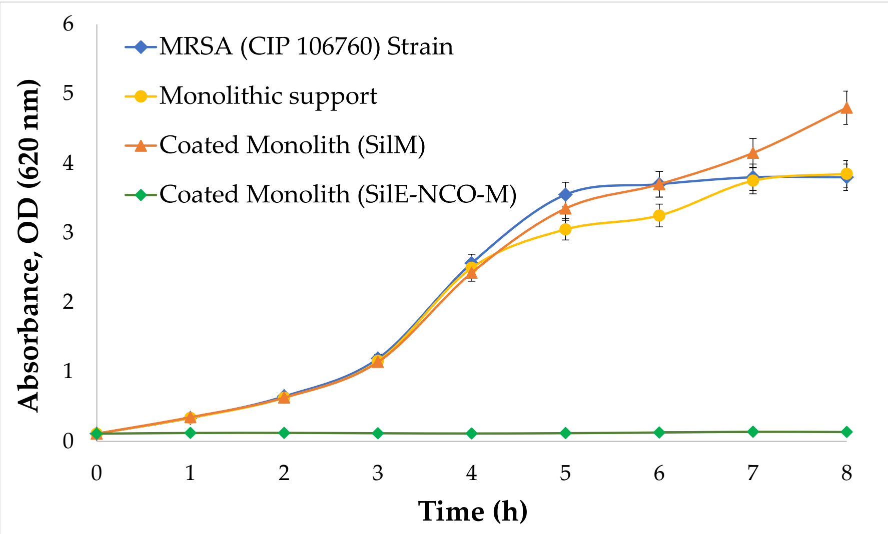

Figure 7. Inhibition growth behaviour of bacterial strain *S. aureus* MRSA CIP 106760 for the uncoated and coated monolithic filters: SilM – coated with a silicone (PDMS) based coating and SilE-NCO-M – coated with a silicone based coating containing tethered Econea biocide (< 6 wt.%).

The bactericidal and bacteriostatic properties of the coated filters have been also evaluated by performing the incubation at accurate conditions for aliquots of each inhibition growth assay (data not shown).

4. Discussion

A recently developed functionalization process [14] for bioactive agents has been used for the Econea biocide functionalization with an isocyanate reactive functionality.

FTIR spectra analysis revealed the presence of this reactive function in the biocide spectra, confirming that its structure was not modified and that the isocyanate functional group (band range: 2327-2144 cm^{-1}) was successfully attached to the expected bridging point of biocide structure (-NH). NCO contents determination of the obtained functional Econea (Econea-NCO) are in accordance with the expected degree of bridging point substitution (-NH) with the isocyanate functional group.

Bioactivity assessment of the Econea and functional Econea evidenced antimicrobial activity, particularly against *S. aureus* and *E. faecalis* bacteria, suggesting that the biocide properties were not significantly affected by the functionalization process.

The efficacy of this immobilization strategy was further evaluated by the tethering of Econea antifouling agents in polymeric matrices, such as a silicone marine based coating. The immobilization effectiveness has been confirmed in previous works [17], where an eco-toxicity study performed in accordance with the European Standards, and for leaching waters obtained from coatings containing the immobilized biocide, allowed the classification of those coating formulations as non-toxic for the environment. In addition, the developed non-release silicone based formulation appears to also promote a better anticorrosion protection when compared to its reference counterpart without biocide [16].

The antifouling behaviour of the obtained antifouling silicone based coatings at simulated conditions (Figure 3), suggested, that the coating formulation containing tethered Econea (A) can provide a better antifouling effect than its reference counterpart without biocide (B), since for this last formulation more and bigger spots of biofouling formation were found on the coated substrate surface are after an immersion period of more than a year.

Furthermore, and under real conditions (Atlantic seawater), promising antifouling effects were obtained for the non-releasing antifouling coating (A), which corroborated with the tests at simulated conditions. The coated substrate (A) remained clean after 45 weeks (about 11 months) submerged in Atlantic sea.

In the second stage of this work, and aiming to combine this non-releasing biocidal active coatings with a structured filter for the bio-decontamination of waterborne systems. The best non-toxic Econea based coating was used to coat cordierite monoliths. The first antimicrobial assays performed on those coated monoliths against *S. aureus* MRSA CIP 106760 strain had shown auspicious inhibition effects on the bacteria growth. A complete growth inhibition was observed on a monolithic filter coated with a silicone based coating containing tethered Econea (SilE-NCO-M) when compared with an uncoated filter (Figure 6 and 7) or even with a coated filter with the silicone coating reference (SilM) (Figure 7). Also, an *S. aureus* MRSA growth increase on the silicone coated monolith (SilM) was observed. This is an expected behaviour already observed by others [20, 21] on silicone or polydimethylsiloxane (PDMS) based surfaces, which also attempted to improve the microbial resistance of those PDMS surfaces, mostly by following surface functionalization strategies.

After the bacteria inhibition assays, the bacteria medium was incubated in order to address the Bactericidal and Bacteriostatic properties of the uncoated and coated monoliths. After the incubation, the bacteria growth for all cultures (data not shown). These results confirm a bacteriostatic behaviour for all tested monoliths, meaning that the previous found bacteria growth inhibition in the medium containing the coated monolith (SilE-NCO-M), with the non-release Econea silicone based coating, prevents the biofilm formation rather than killing the bacteria. This behaviour is desirable since it minimizes the selective pressure on bacteria to evolve treatment resistance.

4. Conclusions

The developed non-toxic antifouling strategy based on the tethering of antifouling agents, such as Econea biocide, in polymeric protective coatings, able to provide non-release biocidal coatings acting by contact, thus avoiding the biocide releasing into the environment or aqueous environment, prove to be not only a potential environmental friendly alternative for the protection of filters surfaces, and therefore for the purification of waterborne systems where they are applied. This approach also evidenced to be capable to provide a long-lasting cleaning effect than the conventional toxic agents releasing strategies.

The auspicious antimicrobial and bacteriostatic achieved behaviour for coated monolithic filters with a silicone based coating containing tethered Econea biocide, is undoubtedly a key result for further research in the field, in particular for the water bio-contamination burden, far to be overcome.

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Author Contributions: The research work presented in this paper was carried out with the collaboration of all authors. Specifically, Elisabete R. Silva conceived and designed the experiments; Olga Ferreira performed the experiments; Elisabete R. Silva, Olga Ferreira, and Patrícia Rijo analyzed the data; Maria J. Calhorda and João C. Bordado contributed with reagents/materials/analysis tools; Elisabete R. Silva wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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