PRELIMINARY COMMUNICATION

For reprint orders, please contact: reprints@futuremedicine.com

Future MICROBIOLOGY

Exposure to extremely low-frequency magnetic field affects biofilm formation by cystic fibrosis pathogens

Giovanni Di Bonaventura*[1,2], Arianna Pompilio[1,2], Valentina Crocetta[1,2], Serena De Nicola[1,2], Filippo Barbaro[3,4], Livio Giuliani[5], Enrico D'Emilia[5], Ersilia Fiscarelli[6], Rosa Grazia Bellomo[7] & Raoul Saggini[4]

SUMMARY **Aims:** To evaluate the *in vitro* effects of extremely low-frequency magnetic field (ELF-MF) on growth and biofilm formation by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Burkholderia cepacia* and *Stenotrophomonas maltophilia* strains from cystic fibrosis patients. **Materials & methods:** The motion of selected ions (Fe, Ca, Cu, Zn, Mg, K, Na) was stimulated by the ion resonance effect, then influence on growth and biofilm formation/viability was assessed by spectrophotometry or viability count. **Results:** Generally, exposure to ELF-MF significantly increased bacterial growth and affected both biofilm formation and viability, although with differences with regard to ions and species considered. **Conclusion:** Exposure to ELF-MF represents a possible new approach for treatment of biofilm-associated cystic fibrosis lung infections.

KEYWORDS
• biofilm formation
• *Burkholderia cepacia*
• cystic fibrosis • extremely low-frequency magnetic field • lung infection
• *Pseudomonas aeruginosa*
• *Staphylococcus aureus*
• *Stenotrophomonas maltophilia*

Pseudomonas aeruginosa, *Burkholderia cepacia* and *Staphylococcus aureus* are the most common bacterial pathogens isolated from the airways of cystic fibrosis (CF) patients where they cause chronic infections responsible for high morbidity and mortality [1,2]. However, the extensive use of antipseudomonal antibiotic therapy exerted a relevant selective pressure on pulmonary bacterial populations recently leading to an increasing number of reports involving potentially emerging and challenging pathogens [3]. This is the case of multidrug-resistant *Stenotrophomonas maltophilia* whose isolation from CF airways is recently reported with increasing prevalence and incidence [3,4].

Physicians treating CF patients are increasingly faced with infections caused by multidrug-resistant strains. Efforts are also hampered by the high microbial adaptation to the CF pulmonary environment, resulting in an increased ability to form biofilms, sessile communities embedded in a self-produced extracellular polymeric substance (EPS), intrinsically resistant to antibiotics as well as toward the host immune defense [5].

Novel strategies that could replace or complement current therapies are, therefore, needed to counteract chronic infections in CF patients.

Magnetic fields are widely used in medicine, in diagnostic (i.e., MRI) as well as in therapeutic (i.e., magnetic stimulation of brain areas, magnetic drug targeting, treatment of pressure ulcers

[1]Department of Experimental & Clinical Sciences, G. d'Annunzio University of Chieti-Pescara, Chieti, Italy
[2]Center of Excellence on Ageing, G. d'Annunzio University Foundation, Chieti, Italy
[3]Prometeo S.r.l., Padova, Italy
[4]Department of Neuroscience & Imaging, G. d'Annunzio University of Chieti-Pescara, Chieti, Italy
[5]INAIL, Workers Compensation Authority, Research Center of Monteporzio Catone, Rome, Italy
[6]Bambino Gesù Children's Hospital & Research Institute, Rome, Italy
[7]Department of Medicine & Ageing Sciences, G. d'Annunzio University of Chieti-Pescara, Chieti, Italy
*Author for correspondence: Tel.: +39 0871 3554812; Fax: +39 0871 3554822; gdibonaventura@unich.it

Future Medicine part of fsg

and bone regeneration) practices [6–8]. The rationale for these applications comes from the results obtained in several studies designed to explore the interaction between extremely low-frequency magnetic fields (ELF-MF) and more complex biochemical structures, such as enzymes, amino acids, protein, genes and DNA [9–13]. The plausibility of these interactions derives from the discovery of the inner structure of water, one of the consequences of the quantum electrodynamics [14,15].

Recently, exploiting the 'Liboff–Zhadin effect' [16–20] in order to develop a biotechnology based on the modulation of ion currents, within the cytoplasm and through the cell membrane, ELF-MF have been applied to induce maturation and differentiation of stem cells [21–23] or tumor cells [24,25], and to treat heart failures, tumors or degenerative age-dependent diseases [26]. Furthermore, we recently provided the first validation for clinical use of external pulsed electromagnetic fields in the rehabilitative treatment for lower back pain [27].

In recent years, several studies were focused on the interaction between weak magnetic fields and living systems in the frame of microbiology. In this regard, particular attention has been recently given to bactericidal effects associated to magnetic field exposure [28–32]. However, despite the antibacterial effect of magnetic field exposure against planktonic cells being extensively investigated, the effect on the bacterial adhesion and its subsequent growth as biofilms remains significantly unexplored. In this regard, at the best of our knowledge, there is no available literature dealing with the effect of electromagnetic fields on CF pathogens.

The purpose of this work was, therefore, to determine for the first time whether application of ELF-MF could influence growth and biofilm formation by Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa, B. cepacia* and *S. maltophilia*) pathogens causing chronic lung infections in CF patients. Thus, we used a device properly designed for *in vitro* experiments at room conditions able to produce a sequence of weak ELF-MF, each one tuned with ion cyclotronic resonance (ICR) frequencies related to specific metal ions.

Overall, our results showed that exposure to ELF-MF, despite stimulating bacterial growth, significantly reduces biofilm formation thus suggesting it could be relevant to consider this technique for preventing CF chronic lung infections.

Materials & methods

• Bacterial strains & growth conditions

Three strains each of *P. aeruginosa* (Pa1, Pa5, Pa7), *B. cepacia* (Bc6, Bc9, Bc11), *S. maltophilia* (Sm122, Sm139, Sm143) and *S. aureus* (Sa3, Sa4, Sa7) were tested in the present study. Strains were collected from respiratory specimens obtained from patients admitted to the CF Operative Unit, 'Bambino Gesù' Children's Hospital and Research Institute of Rome, considering one isolate for each patient. Strains were selected for multi-resistant phenotype, and the ability to form high biofilm amounts on a polystyrene surface. Multidrug resistance was defined in the case of resistance to at least three of the following classes of antibiotics: aminoglycosides, macrolides, fluoroquinolones, trimethoprim-sulphamethoxazole, tetracycline, and β-lactams or β-lactam + β-lactamase inhibitor combinations. All strains were strong biofilm producers, according to Stepanovic *et al.* [33]. Isolates were speciated by API® System (bioMérieux, Marcy-L'Etoile, France), and confirmed by BD Phoenix™ (Becton, Dickinson and Company, Buccinasco, Milan, Italy). Stock cultures were stored at -80°C in a Microbank™ system (Biolife Italiana S.r.l., Milan, Italy). Prior to use, each strain was thawed, subcultured in Trypticase Soya broth (TBS; Oxoid S.p.A., Milan, Italy), then twice on Mueller–Hinton agar (MHA; Oxoid S.p.A), to check purity and restore the original phenotype.

• Magnetic field exposure setup

Experiments on the effects of the ELF-MF were performed by using electronic equipment provided by Prometeo S.r.l (Padova, Italy). The basic experimental setup is shown in **Figure 1A–C**. The setup is composed by a programmable central processing unit that controls an alternating current (AC) generator, a direct current (DC) generator, two coaxial coils (one is driven by the AC generator, the other one by the DC generator). The coaxial coils were placed inside the magnetic shield with a gaussmeter connected to the central processing unit. Along the axis of the coils is placed a cell culture tray. The device is able to generate DC and ELF-AC magnetic fields grace to a pair of coaxial solenoids, aimed at stimulating the motion of selected ionic species (Fe, Ca, Cu, Zn, Mg, K, Na) through the ion resonance effect, as shown in **Table 1**. To obtain a uniform static magnetic field level, the two coaxial coils (A and B) where placed inside a Mu-metal™ (Istituto Nazionale di Fisica Nucleare, INFN, Padova,

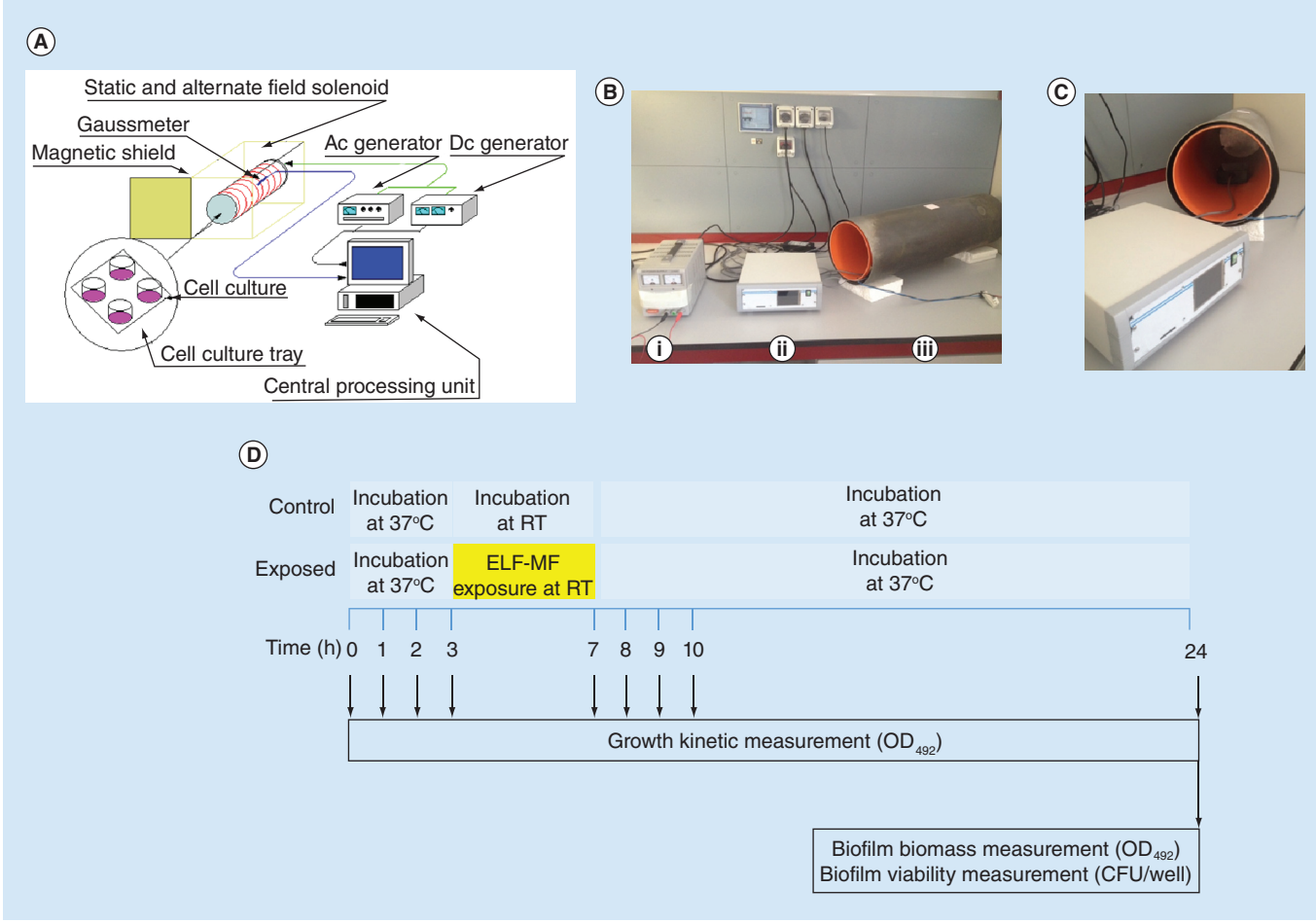

Figure 1. Extremely low-frequency magnetic fields setup and experimental layout. (A) Scheme of experimental setup. **(B)** From left to right: **(i)** the DC generator to power the coil emulating Earth's static magnetic b0; **(ii)** the Prometeo AC frequency generator, for creation of cyclotron resonance fields calculated on the basis of the magnetic field measurements by the gaussmeter (not visible); **(iii)** Mu-metal™ (Istituto Nazionale di Fisica Nucleare, INFN, Padova, Italy) cylinder with two coaxial coils wrapped around a PVC tube containing the gaussmeter (visible in **[C]**). **(C)** A gaussmeter was placed inside the Mu-metal cylinder. **(D)** Experimental layout.
AC: Alternating current; CFU: Colony-forming unit; DC: Direct current; ELF-MF: Extremely low-frequency magnetic field; OD: Optical density; PVC: Polyvinyl chloride; RT: Room temperature.

Italy) a nickel-iron magnetic shield. Coil A (73 × 21 cm, 290 turns) was driven by a DC generator to reproduce a uniform static magnetic field, while coil B (30 × 21 cm, 30 turns, 8-mm space each turns) – placed in the center of the static field coil – was driven by an instrument in AC-mode. The equipment was fitted with a three-axial gaussmeter for the continuous measurement of the artificial static magnetic field inside the Mu-metal shield (77 × 23 cm) to allow the software to calculate the requested ICR frequency (f_c):

$$f_c = \frac{1}{2r}\frac{q}{m}B_0$$

where q and m are respectively the electrical charge and the mass of the ion, and B_0 is a static magnetic field, which is parallel to the ELF-AC magnetic field [16].

Three axial probe was installed in the sample tray inside the Mu-metal casing, 40 cm inside the access and 10 cm equally spaced from the inner wall of the coil. The static-field solenoid did not emit any electric field. An electric component emitted from the AC coil was not revealed by the instrument, the magnetic AC field intensity was in the range of nanotesla, at an extremely low frequency.

In the present work, the geomagnetic field was shielded grace to the Mu-metal. In the

Table 1. Single ion cyclotron frequency of the alternating current magnetic field generated in the exposure system.

Ion	f(ICR) in air (Hz)	Electric charge†	Mass/mol (Da)	B_{DC} || (μT)	f(ICR ||) within the exposure system‡ (Hz)
H_3O+	19.5	1.0	19.0	43.0	35.0
Fe++/Fe+++	14.9	2.3	55.8	43.0	26.8
Ca++	18.5	2.0	40.1	43.0	33.2
Cu++	12.2	2.1	63.5	43.0	22.0
Zn++	11.3	2.0	65.4	43.0	20.4
Mg++	30.5	2.0	24.3	43.0	54.8
K+	9.5	1.0	39.1	43.0	17.0
Na+	16.1	1.0	22.9	43.0	29.0
1/(2π)	0.159	Official geomagnetic field at l'Aquila (μT)			46.5
e/mH	95800000	Geomagnetic measured field at Chieti (μT)			43.0
μT/T	0.000001	Geomagnetic inclination at l'Aquila (°)			58.5
Cos(58°30′)	0.522	Residual geomagnetic field within the coils			0.7

†Electric charge is expressed as a multiple of the electron charge e = 1.6 × 10-19 Coulomb.
‡f(ICR) values were calculated taking into account the geomagnetic field, as suppressed in the cylinder where samples were exposed and considering the supplied direct current magnetic field, B_{DC}.
B_{DC}: Static magnetic field; f(ICR||): Frequency of the alternating current magnetic field tuned with the static magnetic field B_{DC}|| according to the ion cyclotronic resonance definition; e: Electron; f(ICR): Ion cyclotronic resonance frequency; mH: Mass of a proton; mT: 10^{-6} Tesla; T: Tesla.

laboratory, geomagnetic measures provide an average of 43 microTesla (μT). Therefore, the parallel component of the geomagnetic field, G_0, succeeds to be 22.5 μT, since the local geomagnetic field direction forms an angle of about 31.5° with the azimuth line. The Mu-metal shield reduces this component 320 times, thus further reducing the intensity of B_0, inside the coil, down to about 0.7 μT. In order to provide a natural amount of the static magnetic field for the exposure of samples, in the inner coil a DC current was overlapped to the AC current in the outer coil, restoring the outside intensity of the static magnetic field: 43 μT. Therefore, the geomagnetic contribution G_0 to the static magnetic field B_0, within the shielded coils, succeeded to be negligible, if compared with the static magnetic field B_{DC}, provided by the coils:

$$B_0 = G_0 + B_{DC} = (0.7 + 43)nT = 43.7nT$$

and

$$G_0/B_0 = 2/1000$$

All the selected ICR frequencies in the experiment were tuned with the resulting value of B_0.

On the other hand, the natural geomagnetic field outdoors can easily be 43.7 MegaT in many places. In fact, it is variable depending on the coordinates of the place and time.

In our experimental setup, an ELF-MF with the intensity of 265 nT was applied. The samples were placed inside the twin solenoids on the non-conductive stand in the center of the inner coil. Exposures were carried out at room temperature (RT; 20–25°C), as assessed by a calibrated digital thermometer, under aerobic atmosphere. Therefore, the Joule effect – due to the current through the sample inside the twin coils – was negligible and the detected effects could be considered as 'nonthermal' effects, according to the usual classification [34].

• Experimental design

Exposure to ELF-MF was performed as illustrated in **Figure 1D**. Briefly, bacterial suspensions or biofilm samples, prepared in microtiter plates, were incubated at 37°C for 3 h, then exposed to ELF-MF for 4 h at RT. At the end of exposure, samples returned to incubation at 37°C until 24 h. Control unexposed samples were kept in the same conditions as the exposed ones except the sole exposition to the magnetic field. During their incubation, both exposed samples and controls were exposed to ELF (50 Hz), due to the warming resistance inside the incubator, even if at lower intensity, in the order of 0.2 μT. However, this undesired overlap could be neglected because all evaluated parameters of exposed samples have been normalized to those of controls. Both samples and controls were

processed during incubation at prefixed times (for assessing bacterial growth), or at the end of incubation (for measuring biofilm biomass formation, or biofilm viability).

• Inoculum standardization

Each assay was performed starting from a standardized bacterial inoculum. Briefly, for each strain some colonies grew on MHA were resuspended in 10 ml TSB and incubated at 37°C o/night. Broth culture was then corrected at an optical density measured at 550 nm (OD_{550}) of 1.0, corresponding to about 1–3 × 10^8 CFU/ml, then diluted 1:10 in sterile TSB.

• Kinetic of bacterial growth

Two-hundred microliters of the standardized inoculum were dispensed in each well of a microtiter plate (Falcon, Becton Dickinson and Company, Milan, Italy), and cell growth was assessed through OD_{620} measurements by a microplate reader (Sunrise™; Tecan Italia, Milan, Italy) at regular time intervals: hourly in the first 3 h of incubation then, following exposure to ELF-MF for 4 h, at 7, 8, 9, 10 and 24-h incubation. OD_{620} values read from 4 to 24 h incubation were analyzed for significant differences between exposed and unexposed samples.

• Biofilm biomass formation assay

Two-hundred microliters of the standardized inoculum were dispensed to each well of a sterile flat-bottom polystyrene tissue culture 96-well microtiter (Falcon) and incubated at 37°C for 24 h. During incubation, each sample was exposed to ELF-MF as stated above. Following incubation, nonadherent cells were removed by being washed twice with 200 µl sterile phosphate buffered saline (pH 7.3; Sigma-Aldrich Co; Milan, Italy). Biofilm samples were then fixed (1 h at 60°C), stained with 200 µl Hucker-modified crystal violet (5 min at RT), rinsed under tap water and finally air-dried. Biofilm biomass (cells + EPS) was stained with 250 µl of 33% glacial acetic acid for 15 min, and measured as OD_{492} by a spectrophotometric reader (Sunrise™, Tecan Group Ltd, Mannerdorf, Switzerland).

• Biofilm viability assay

Biofilms were allowed to form in each well of a 96-well flat-bottom polystyrene tissue-treated microtiter plate (Falcon). During incubation at 37°C for 24 h, each sample was exposed to ELF-MF as stated above. After incubation, nonadherent bacteria were removed by washing twice with 200 µl sterile phosphate buffered saline (pH 7.3; Sigma-Aldrich Co), and biofilm viability was then assessed by viable count. Briefly, biofilm samples were scraped with a pipette tip following exposure to 100 µl trypsin-EDTA (ethylenediaminetetraacetic acid) 0.25% (5 min, RT) (Sigma-Aldrich S.r.l., Garbagnate Milanese, Italy), then suspension was vortexed (1 min at maximum speed) to disintegrate bacterial clumps. Sample underwent to 10-fold dilutions that were plated MHA plates for bacterial counts.

• Statistical analysis

Each experiment was carried out, as an open trial, in triplicate and repeated on two different occasions. Differences between exposed and unexposed (controls) samples were evaluated for statistical significance using paired t-test (growth rate) or Fisher's exact test (biofilm biomass formation and biofilm viability), considering as statistically significant p-values of <0.05. Statistical analysis was performed by using GraphPad software (ver. 6.02; GraphPad Software, Inc., CA, USA).

Results

• Exposure to ELF-MF significantly improves bacterial growth

The effect of exposure to ELF-MF on bacterial growth, assessed by spectrophotometric method, is summarized in **Table 2**, and graphed in **Supplementary Figures S1–S7** (see supplementary material online at www.futuremedicine.com/doi/suppl/10.2217/fmb.14.96).

Exposure to ELF-MF significantly affected growth of most strains tested, regardless of species and ionic channel considered. Of 408 time points measured following exposure (7, 8, 9, 10 and 24 h incubation), 328 (80.4%) showed a significant difference in growth between ELF-MF exposed and control unexposed samples. Most of the differences observed (312 out of 328; 95.1%) indicated increased growth, appearing already at the end of exposure (7-h incubation) (68 out of 84, 80.9%), and persisting up to 24-h incubation (59 out of 84, 70.2%), without differences among species tested.

Stratifying for ion dynamics, Mg resulted to be the most active (a significant effect was observed in 56 out of 60 time points; 93.3%), while Ca was the weakest one (a significant effect was observed in 40 out of 60 time points;

Table 2. Effect of extremely low-frequency magnetic fields exposure on bacterial growth of cystic fibrosis pathogens: distribution of significant differences compared to controls.

Ionic channels	Significant reductions, n† (%)‡	*Pseudomonas aeruginosa*, n (%§)	*Stenotrophomonas maltophilia*, n (%§)	*Burkholderia cepacia*, n (%§)	*Staphylococcus aureus*, n, (%§)	Significant increases, n† (%)¶	*P. aeruginosa*, n (%§)	*S. maltophilia*, n (%§)	*B. cepacia*, n (%§)	*S. aureus*, n (%§)
Na	1 (6.25)	0	0	0	1 (12.5)	47 (15.1)	12 (14.3)	14 (15)	11 (13.9)	10 (17.8)
Cu	0	0	0	0	0	45 (14.4)	10 (11.9)	12 (12.9)	13 (16.4)	10 (17.8)
Mg	0	0	0	0	0	56 (17.9)	14 (16.6)	15 (16.2)	14 (17.7)	13 (23.2)
K	11 (68.75)	2 (66.6)	3 (100)	2 (100)	4 (50)	42 (13.5)	13 (15.5)	12 (12.9)	10 (12.7)	7 (12.5)
Zn	1 (6.25)	0	0	0	1 (12.5)	39 (12.5)	10 (11.9)	12 (12.9)	9 (11.4)	8 (14.3)
Fe	3 (18.75)	1 (33.3)	0	0	2 (25)	43 (13.8)	12 (14.3)	15 (16.2)	12 (15.2)	4 (7.2)
Ca	0	0	0	0	0	40 (12.8)	13 (15.5)	13 (13.9)	10 (12.7)	4 (7.2)
Total(%)	16 (4.9)#	3 (18.75)‡	3 (18.75)‡	2 (12.5)‡	8 (50)‡	312 (95.1)#	84 (26.9)¶	93 (29.8)¶	79 (25.3)¶	56 (18)¶

A standardized bacterial suspension was first incubated at 37°C for 3 h, exposed to extremely low-frequency magnetic field for 4 h at RT, then re-incubated at 37°C up to 24 h. Growth kinetic was spectrophotometrically (OD_{620}) monitored at prefixed times (0, 1, 2, 3, 7, 8, 9, 10 and 24 h). Only significant differences ($p < 0.05$, vs unexposed control, paired t-test) observed from 7 h throughout 24-h incubation were considered.

†$p < 0.05$ compared to unexposed control (from 7 h throughout 24-h incubation), paired t-test.

‡Percentages referred to the total number of significant reductions (n = 16).

§Percentages referred to the total number of significant reductions/increases observed for this species.

¶Percentages referred to the total number of significant increases (n = 312).

#Percentages referred to the total number of significant differences (n = 328).

OD_{620}: Optical density read at 620 nm.

66.6%). Most of the reductions in bacterial growth observed following exposure to ELF-MF at 24-h incubation (11 out of 16, 68.75%) were caused by stimulating K dynamics.

With regard to species tested, *S. aureus* strains were the less susceptible to ELF-MF, being affected in 60.9% (64 out of 105) of time points measured. Particularly, *S. aureus* Sa4 and *B. cepacia* Bc11 were the less susceptible strains to ELF-MF exposure, since their growth resulted in being significantly reduced in only 37.1% (13 out of 35) and 42.8% (15 out of 35) of time points measured, respectively.

To the contrary, *S. maltophilia* strains were the more susceptible to ELF-MF being affected in almost all exposures (99 out of 105 time points; 94.3%). In particular, *S. maltophilia* Sm139 and *B. cepacia* Bc6 were the most susceptible strains being always affected by ELF-MF exposure.

A characteristic effect was observed in the case of *S. aureus* Sa3 strain following exposure stimulating Cu, K and Fe dynamics. In fact, although exposed bacteria showed lower growth rate than unexposed ones, an inverted trend could be observed from 8-h incubation.

• Exposure to ELF-MF significantly affects biofilm biomass formation

The effect of ELF-MF exposure against biofilm biomass (consisting of cells and EPS) formation is summarized in **Table 3**, and graphed in **Figure 2**.

Overall, most of differences measured (26 out of 42, 61.9%) showed a significant reduction in biofilm biomass formation, compared with unexposed controls, while increased biofilm levels were observed in only 38.1% (16 out of 42). Most of reductions were found for *S. aureus* (12 out of 26, 46.1%) and *B. cepacia* (11 out of 26, 42.3%). *P. aeruginosa* and *S. maltophilia* were significantly less affected, respectively, in 2 (7.7%) and 1 (3.8%) cases only. *S. aureus* Sa3 and *B. cepacia* Bc6 were the most affected strains, producing lower biofilm biomass levels following exposure to 6 out of 7 (85.7%) ions tested.

Stratifying for stimulated ionic dynamics, Fe and Ca provoked the higher number of significant differences (5 out of 26, 19.2%), not statistically different from Cu and Zn (4, 15.4%), and Na and K (3, 11.5%), but significantly higher than Mg (2, 7.7%; $p < 0.05$).

With regard to differences suggestive for a significant increase in biofilm biomass formed, most of these were observed for *S. maltophilia* (8 out

of 16, 50.0%) and *P. aeruginosa* (5, 31.2%). By contrast, *S. aureus* and *B. cepacia* were respectively affected in 2 (12.4%) and 1 (6.2%) cases only. *S. maltophilia* Sm122 and *P. aeruginosa* Pa7 were the most affected strains, producing higher biofilm biomass levels following exposure to 4 (57.1%) and 3 (42.8%) out of 7 ions tested, respectively.

Stimulation of Mg dynamic provoked the higher number of significant differences (5 out of 16, 31.2%), significantly higher than K (2 out of 16, 12.5%; $p < 0.01$) and Na (1 out of 16, 6.2%; $p < 0.0001$). Fe and Ca stimulation produced any statistically significant effect.

• Exposure to ELF-MEF significantly reduces biofilm viability

The effect of ELF-MF exposure against biofilm cell viability is summarized in **Table 4**, and graphed in **Figure 3**.

Overall, 63 significant differences were found. Most of these (46, 73.0%) were suggestive for decreased biofilm viability, while 17 (27.0%) for increased one.

Reductions in biofilm viability were uniformly distributed in each of the species tested: 12 (26.1%) for *P. aeruginosa*, 10 (21.7%) for *S. maltophilia*, 13 (28.3%) for *B. cepacia*, and 11 (23.9) for *S. aureus*. Stratifying for ions stimulated, Mg and K provoked the higher number of significant differences (8 out of 46, 17.4%), although comparable to those observed for other ions tested. *S. aureus* Sa3 was the most affected strain, significantly decreasing biofilm viability following exposure to all of seven ions tested.

With regard to significant increases, most of the significant differences in biofilm biomass formation were mainly found for *S. maltophilia* (8, 47.0%), followed by *P. aeruginosa* (5, 29.4%), while *S. aureus* and *B. cepacia* were each affected in two (11.7%) cases only. Stimulation of Ca and Zn dynamics provoked the higher number of significant differences (4 out of 17, 23.5%), similarly to Fe (3, 17.6%), Mg and Na (2, 11.7%), but significantly higher than Cu and K (1, 5.9%; $p < 0.01$). *P. aeruginosa* Pa1, *S. maltophilia* Sm122 and Sm139 resulted to be the most affected strains, producing biofilms whose viability was significantly increased following exposure to three out of seven (42.8%) ions tested.

Table 3. Effect of extremely low-frequency magnetic fields exposure on biofilm biomass formation by cystic fibrosis pathogens: distribution of significant differences compared to controls.

Ionic channels	Significant reductions, n† (%)‡	*Pseudomonas aeruginosa* strains	*Stenotrophomonas maltophilia* strains	*Burkholderia cepacia* strains	*Staphylococcus aureus* strains	Significant increases, n† (%)§	*P. aeruginosa* strains	*S. maltophilia* strains	*B. cepacia* strains	*S. aureus* strains
Na	3 (11.5)			Bc9, Bc11	Sa3	1 (6.25)		Sm122		
Cu	4 (15.4)			Bc6, Bc9	Sa3, Sa7	4 (25)	Pa7	Sm122, Sm143		Sa4
Mg	2 (7.7)	Pa5		Bc6		5 (31.25)	Pa1, Pa7	Sm122, Sm139, Sm143		
K	3 (11.5)			Bc6	Sa3, Sa7	2 (12.5)	Pa1			Sa4
Zn	4 (15.4)	Pa5		Bc6	Sa3, Sa7	4 (25)	Pa7	Sm122, Sm139	Bc11	
Fe	5 (19.2)			Bc6, Bc9, Bc11	Sa3, Sa7					
Ca	5 (19.2)		Sm122	Bc6	Sa3, Sa4, Sa7					
Total(%)	26 (61.9)¶	2 (7.7)‡	1 (3.8)‡	11 (42.3)‡	12 (46.1)‡	16 (38.1)¶	5 (31.2)§	8 (50)§	1 (6.2)§	2 (12.4)§

Biofilms were allowed to form in each well of a microtiter plate. Following 3-h incubation at 37°C, samples were exposed to extremely low-frequency magnetic field for 4 h at RT, then re-incubated at 37°C up to 24 h. Biofilm amount was then assessed by spectrophotometric technique. Only differences (reductions or increases) of at least 25% compared to control ($p < 0.001$, Fisher's exact test) were considered.

†Differences (reductions or increases) of at least 25% compared to control ($p < 0.001$, Fisher's exact test) were considered.

‡Percentages referred to the total number of significant reductions (n = 26).

§Percentages referred to the total number of significant increases (n = 16).

¶Percentages referred to the total number of significant differences (n = 42).

Discussion

The main objective of the present work was to assess the effects of exposure to ELF-MF on

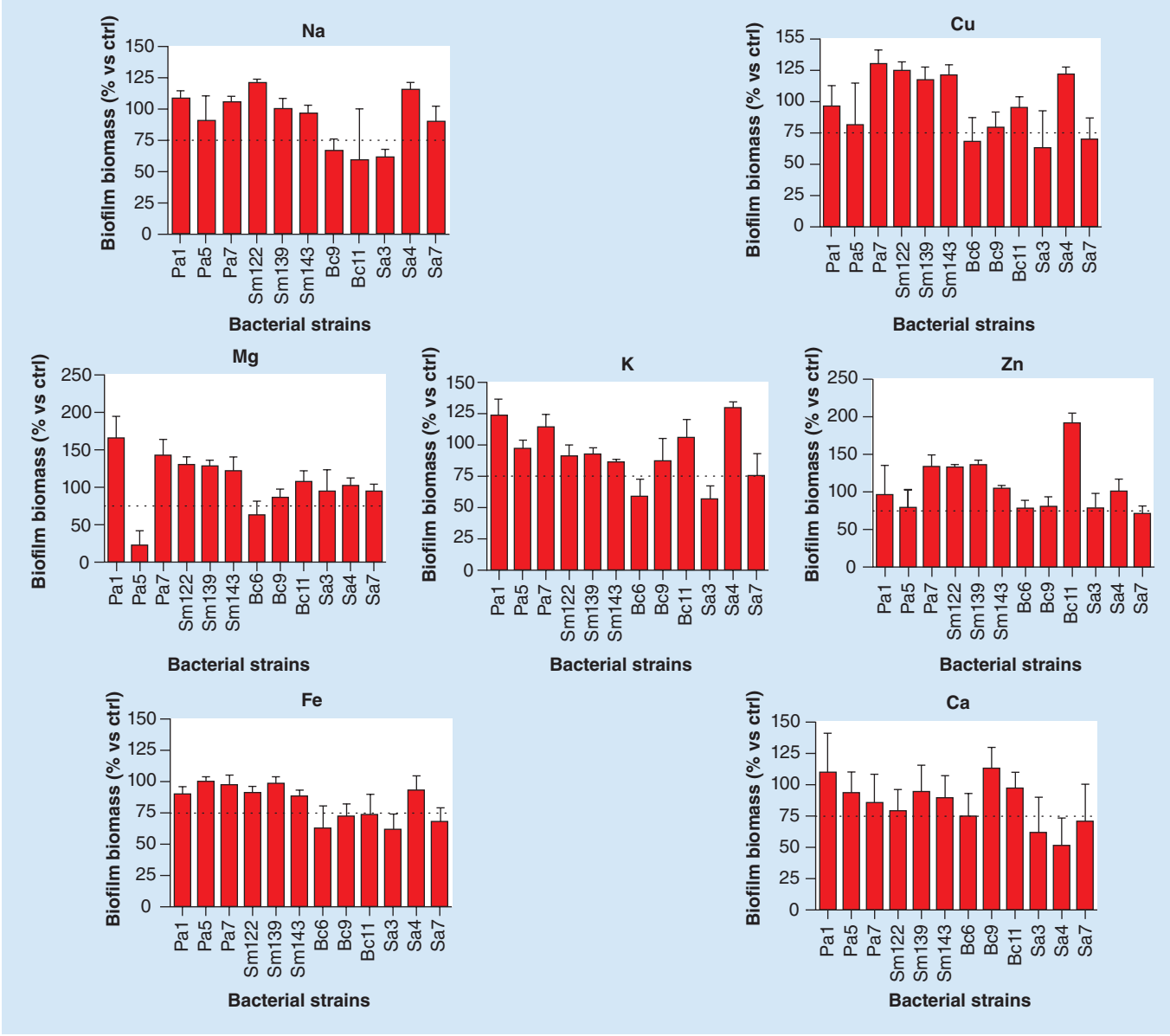

Figure 2. Effect of exposure to extremely low-frequency magnetic fields on biofilm biomass formation by cystic fibrosis pathogens. Standard bacterial inoculum prepared in Trypticase Soy broth was aliquoted in each well of a polystyrene microtiter, incubated at 37°C for 3 h, then exposed to extremely low-frequency magnetic fields for 4 h at room temperature, selecting a specific ionic channel. At the end of exposure, samples were newly incubated at 37°C up to 24 h. Controls consisted of bacteria treated in the same way but not exposed to extremely low-frequency magnetic fields. Biofilm biomass amount formed in each well was then assessed by crystal violet staining measuring optical density at 492 nm (OD_{492}). Results were reported as percentage of biofilm biomass formed, compared with control (100%), and graphed as means + standard deviations (n = 6).
Dotted line indicates a reduction of at least 25% compared with control ($p < 0.001$, Fisher's exact test). *Pseudomonas aeruginosa* strains: Pa1, Pa5, and Pa7; *Stenotrophomonas maltophilia* strains: Sm122, Sm139 and Sm143; *Burkholderia cepacia* strains: Bc6, Bc9 and Bc11; *Staphylococcus aureus* strains: Sa3, Sa4 and Sa7.
ctrl: Control; OD: Optical density.

bacterial growth and biofilm formation by bacterial strains representative both for Gram-positive and Gram-negative CF pathogens. The originality of this work relies on the lack of studies focused on CF pathogens, and also on the assessment of ELF-MF effects by matching the cyclotron frequency corresponding to the charge/mass ratio of each of seven selected metallic ions tested.

Several studies focused on the effect of electromagnetic fields on bacterial growth [28–32,35–36]. Our results showed that exposure to ELF-MF significantly modulates bacterial growth, regardless of species tested. However, in contrast with previous studies [28–31,35], a significant stimulatory effect on bacterial growth was generally observed following ELF-MF exposure, compared with unexposed controls. This effect was rapid, appearing already at the end of exposure, and persistent as it continued up to 24 h of incubation.

The magnitude of this effect resulted to be both species- and ion-specific. *S. maltophilia* was the most susceptible species, especially in the case of Sm139 and Sm143 strains whose exposure to ELF-MF even improved bacterial growth, regardless of stimulated ion. These results are consistent with previous findings suggesting that the impact of magnetic field on bacterial viability could be modulated by cell shape, with rod-shaped bacteria more sensitive compared with spherical ones [31,37].

With regard to stimulated ion dynamics, Mg was the most active ion, in the sense that its ICR frequency seems to be highly effective, while Ca was the worst. In agreement with our results, Obermeier *et al.* [30] found that planktonic *S. aureus* exhibited a faster bacterial growth under the influence of a low-frequency electromagnetic field (5 mT, 20 Hz). Similarly, static and rotating magnetic fields were found to improve *S. aureus* and *Escherichia coli* proliferation [9,32,36].

These controversial results explain why the biological mechanisms underlying the magnetic field effects remain unexplored or not yet understood, even if numerous studies in this regard have recently confirmed that magnetic fields can affect biological functions of organisms by increased rate of enzymatic reactions, changes in gene expression or synthesis of DNA [10,38].

The contrasting nature of results could also be due to the different experimental conditions (i.e., magnitude of magnetic field, duration of exposure; overlapping of DC and AC magnetic fields or of DC magnetic field and of AMRF – amplitude modulated radio frequencies – that are frequently not recognized by the authors). In this regard, Morrow *et al.* [39] found that static magnetic field in the range of 0.05–0.5 T had different impact on *S. pyogenes* growth rate depending on field strength, probably depending on the characteristic patterns of metabolite secretion associated with specific field strengths.

Table 4. Effect of extremely low-frequency magnetic fields exposure on the viability of biofilm formed by cystic fibrosis pathogens: distribution of significant differences compared to controls.

Ionic channels	Significant reductions, n† (%)‡	*Pseudomonas aeruginosa* strains	*Stenotrophomonas maltophilia* strains	*Burkholderia cepacia* strains	*Staphylococcus aureus* strains	Significant increases, n† (%)§	*P. aeruginosa* strains	*S. maltophilia* strains	*B. cepacia* strains	*S.aureus* strains
Na	7 (15.2)	Pa7	Sm122, Sm139, Sm143	Bc9, Bc11	Sa3	2 (11.8)	Pa1			Sa7
Cu	7 (15.2)	Pa1, Pa5, Pa7	Sm139, Sm143	Bc11	Sa3	1 (5.9)		Sm122		
Mg	8 (17.4)	Pa5	Sm122, Sm139, Sm143	Bc6, Bc9	Sa3, Sa4	2 (11.8)	Pa1		Bc11	
K	8 (17.4)	Pa1, Pa5, Pa7	Sm139	Bc9, Bc11	Sa3, Sa7	1 (5.9)		Sm122		
Zn	6 (13)	Pa1, Pa5		Bc6, Bc11	Sa3, Sa7	4 (23.5)	Pa7	Sm139, Sm143	Bc9	
Fe	6 (13)	Pa1	Sm122	Bc6	Sa3, Sa4, Sa7	3 (17.6)	Pa7	Sm139, Sm143		
Ca	4 (8.8)	Pa7		Bc6, Bc9, Bc11		4 (23.5)	Pa1	Sm122, Sm139		Sa7
Total(%)	46 (73)¶	12 (26.1)‡	10 (21.7)‡	13 (28.3)‡	11 (23.9)‡	17 (27)¶	5 (29.4)§	8 (47)§	2 (11.8)§	2 (11.8)§

Biofilms were allowed to form in each well of a microtiter plate. Following 3-h incubation at 37°C, samples were exposed to extremely low-frequency magnetic field for 4 h at RT, then re-incubated at 37°C up to 24 h. Biofilm viability was then assessed by viable count technique. Only differences (reductions or increases) of at least 25% compared to control ($p < 0.001$, Fisher's exact test) were considered.

†Differences (reductions or increases) of at least 25% compared to control ($p < 0.001$, Fisher's exact test) were considered.

‡Percentages referred to the total number of significant reductions (n = 46).

§Percentages referred to the total number of significant increases (n = 17).

¶Percentages referred to the total number of significant differences (n = 63).

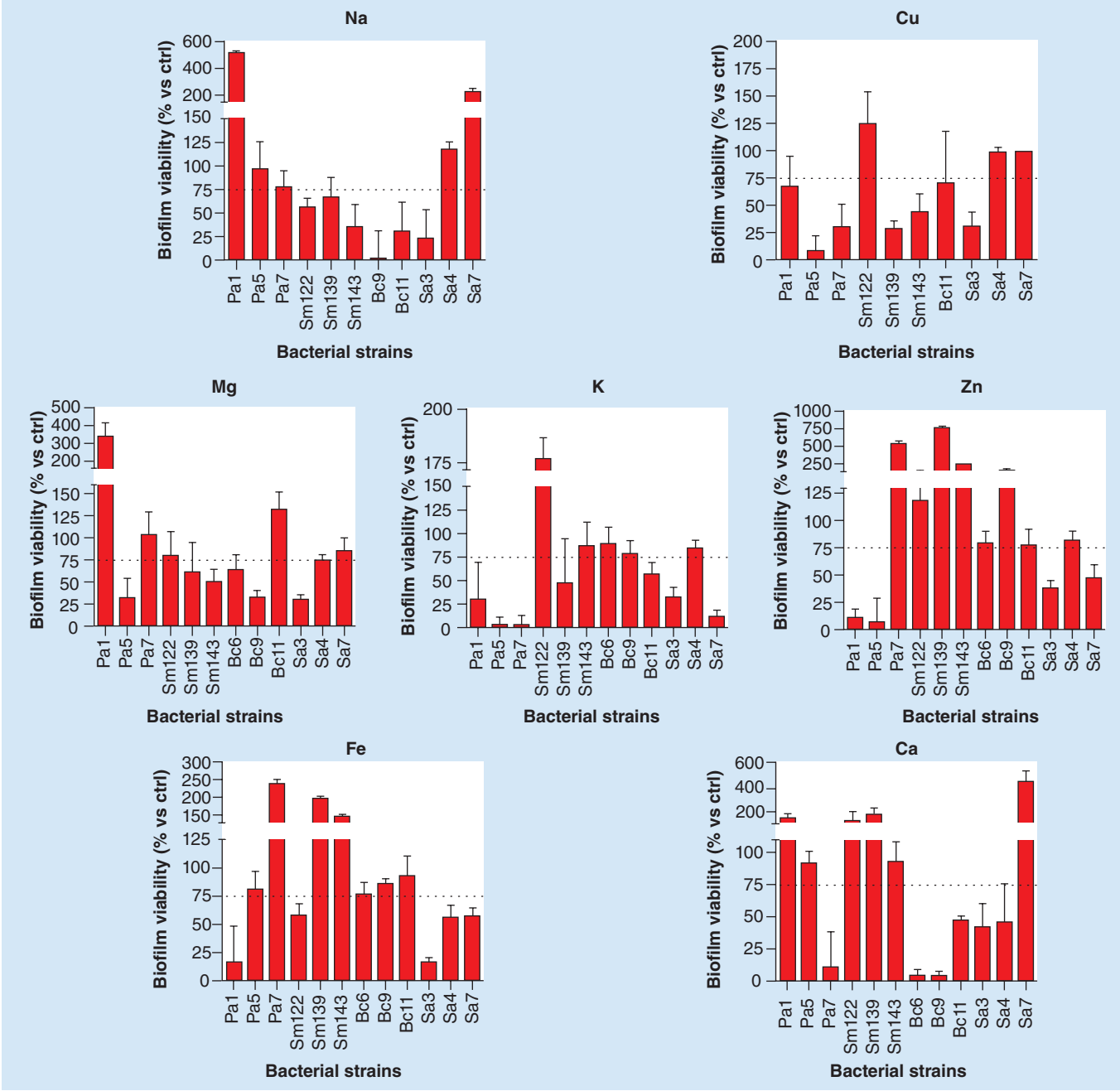

Figure 3. Effect of exposure to extremely low-frequency magnetic fields on the viability of biofilm formed by cystic fibrosis pathogens. Biofilms were allowed to form as stated in **Figure 2**, incubated at 37°C for 3 h, then exposed to extremely low-frequency magnetic fields for 4 h at room temperature, selecting a specific ionic channel. At the end of exposure, samples were newly incubated at 37°C up to 24 h. Controls consisted of bacteria treated in the same way but not exposed to extremely low-frequency magnetic fields. Biofilm viability was then assessed by colony count. Results were reported as percentage of biofilm biomass formed, compared with control (100%), and graphed as means + standard deviations (n = 6).

Dotted line indicates a reduction of at least 25% compared with control ($p < 0.001$, Fisher's exact test). *Pseudomonas aeruginosa* strains: Pa1, Pa5 and Pa7; *Stenotrophomonas maltophilia* strains: Sm122, Sm139 and Sm143; *Burkholderia cepacia* strains: Bc6, Bc9 and Bc11; *Staphylococcus aureus* strains: Sa3, Sa4 and Sa7.

ctrl: Control.

Similarly, Bayir *et al.* [35] and Dunca *et al.* [37] reported that the inhibitory or stimulatory effect of a magnetic field on *E. coli* and *S. aureus* strains is time-exposure dependent. In both cases, due to the room condition of exposures, the presence of AMRF cannot be excluded, even in pulsed microwaves for mobile services. Pulses of global system for mobile communications microwaves, with the frequency of 217 Hz, have the convolution at 8 Hz [40]. That is the ICR of K, when the geomagnetic field is 41 μT with a geomagnetic inclination of 60°. We have therefore considered it to be crucial, in designing the experiments of the present study, that our samples should be protected from outer electromagnetic fields and that the actual intensity of the involved static magnetic field should match the desired value in order to generate the proper frequencies, tuned with ICR of the considered ions.

Contrarily to most of the earlier reported studies, in the present study magnetic fields were generated in controlled conditions, since the geomagnetic field was suppressed and substituted by an artificial DC MF, while the samples were exposed within a Mu-metal cylinder, suppressing the outer electromagnetic signals. Furthermore, in the present study – and it is another pursued plus – the magnetic treatment was applied not only to planktonic cells but also to sessile (biofilm) ones.

On virtually any surface, abiotic or biotic, with which they come into contact, adhered bacteria aggregate to form biofilms, structured and architecturally complex communities encased in a self-produced EPS [41]. Biofilm cells are characteristically in a quiescent metabolic state, exhibiting an altered (or 'biofilm') phenotype whose selective advantage consists of a significantly enhanced tolerance to antimicrobial agents, and immune clearance. The effective therapeutic concentration of some antibiotics to bacteria in biofilm may be even 100- to 1000-fold higher than that of planktonic bacteria, thus rendering biofilm a source of chronic and persistent infection [41].

Several studies aimed at evaluating the inhibiting effect of both electric and electromagnetic fields on biofilm formation [36,42], together with clinical findings, have clearly shown their therapeutic potential as antibiofilm strategies.

Although there is considerable evidence to suggest that *P. aeruginosa, B. cepacia, S. aureus* and *S. maltophilia* grow as polymicrobial biofilms also in the airways of CF patients with chronic pulmonary infections [43–47], to the best of our knowledge there are no published studies on the effects of magnetic field on biofilms formed by CF pathogens.

In the present work we found that exposure to ELF-MF caused different effects on both biofilm biomass amount and biofilm viability depending on species and ions considered. Particularly, stimulation of all ions but Mg caused a significant reduction in biofilm biomass, especially in the case of *S. aureus* and *B. cepacia*, proving to be the most affected species. To the contrary, stimulation of Mg in particular caused significant increase in biofilm biomass, especially for *S. maltophilia* and *P. aeruginosa*. Consistent with these findings, ELF-MF exposure caused a reduced viability of biofilm by *S. aureus*, while increased biofilm viability in the case of *S. maltophilia*.

Although the present study was not aimed at finding mechanisms underlying our results, some speculations can be posed. In the case of *S. maltophilia* exposure to ELF-MF caused improved growth, thus probably explaining increase in biofilm biomass and viability is consistent with improved bacterial growth. To the contrary, in the case of *S. aureus* a different mechanism has to be proposed, involving a modification in the transport of ions by cell membranes [48]. Bacterial adhesion is primarily driven by the electrostatic attraction forces existing between the bacteria cell surface – normally negatively charged because its biological molecules are ionized in aqueous solution [49] – and the positively charged surface of substratum material. The strength of these forces is dependent on the amount of the surface charge, and on mobility and amount of solubilized ions.

In this context, it is plausible to hypothesize that ELF-MF influences the movement of both ions in culture medium and intracellular ones. In this scenario, diamagnetic ions – such as Ca^{2+} – could be attracted by the negatively charged bacterial cell surface, whose mobility seems to be enhanced in the presence of magnetic field. This nullifies the attraction of a positively charged polystyrene surface, thus explaining how magnetically activated Ca^{2+} ions may affect *S. aureus* adhesion to the substratum.

A proof of concept for the use of ELF-MF as a treatment against CF pulmonary infections also comes from Giladi *et al.* [50]. They observed that high-frequency, low-intensity electric fields (10 MHz) significantly inhibited *P. aeruginosa*

growth in a mouse model of lung infection, both as a stand-alone treatment and combined with ceftazidime. However, a potential advantage of using magnetic fields rather than DC to achieve the control is that this method is completely noninvasive since no direct contact to the patient is required. In addition, use of conductive electrodes for the generation of electric currents causes the release, at the electrode surface, of metal ions and free radicals both toxic for living cells [51].

Our results could also have another important implication in CF infection control. Home nebulizers, commonly used to administer aerosolized antibiotics to CF patients, are frequently contaminated by bacteria and fungi, and may be a primary source of airway infection or reinfection [52]. Recommendations for disinfection are often arbitrary and sometimes contradictory. In this frame, our results could represent a rationale for using ELF-MF to prevent or eradicate biofilms formed onto this equipment.

In the light of a possible application of ELF-MF exposure in CF patients to prevent or counteract biofilm-associated lung infections, another point that needs to be investigated is its cytotoxic potential. If any, the magnetic field parameters should be optimized to have a balance between cytotoxicity and antibiofilm effect. In this regard, Gerardi *et al.* [53] recently observed that long-term ELF exposure causes several metabolic alterations in mice (i.e., increased body weight and blood glucose content, reduced lipid metabolism), although at post-mortem examination no major pathological signs related to exposure was observed. In this frame, it is however noteworthy to highlight that the field strength examined in this study is well below those that might be encountered in diagnostic procedures, therapeutic applications and near household devices.

The intrinsic antibiotic resistance of microbial biofilms is a multifactorial phenomenon, and reduced antibiotic penetration is thought to be of relevant importance [41]. The use of electric or electromagnetic fields has been recently proposed to improve antibiotic diffusion and, therefore, therapeutic efficiency against biofilms [54–56]. Benson *et al.* [54] observed enhanced activity of gentamicin against biofilm-forming *P. aeruginosa* adhered on different polymers when exposed to a 0.5 mT magnetic field. Similarly, Pickering *et al.* [55] found that exposure of orthopaedic implants to a pulsed electromagnetic field significantly improved gentamycin activity against mature *Staphylococcus epidermidis* biofilms. Further studies are, therefore, needed to explore this possibility also for biofilms formed by CF pathogens.

Conclusions & future perspective

The present work explored, for the first time in literature, the application of electromagnetic fields in the management of biofilm-related infections caused by CF pathogens. Taken together, our results clearly show that there is high potential in the utilization of electromagnetic fields to combat microbial biofilms. Exposure to ELF-MF in fact significantly decreases biofilm formation, probably not depending on a bactericidal effect but rather to reduced bacterial adherence to substratum secondary to altered permeability of the ionic channels of cell membrane.

The use of ELF-MF could offer new perspectives into both prevention and treatment of biofilm-related difficult-to-treat chronic infections, not only in CF lung but also in wounds or those associated with implants. Further investigations are warranted to confirm our results and to explore mechanisms, potential cytotoxicity and therapeutic efficacy associated to ELF-MF exposure, by means of *in vivo* models and well-designed, evidence-based, randomized clinical studies.

Acknowledgements

The authors would like to thank Prometeo S.r.l (Padova, Italy) for providing us with electronic apparatus necessary to generate ELF-MF.

Financial & competing interests disclosure

This work was supported by the "G. d'Annunzio" University of Chieti-Pescara grant (quota ex-60%, anno 2012). F Barbaro is a consultant of Prometeo S.r.l. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

EXECUTIVE SUMMARY

Key conclusions

- Generally, exposure to extremely low-frequency magnetic field (ELF-MF) increases bacterial growth, while decreases both biofilm formation and viability. However, ion- and species-specific effects were observed.
- Stimulation of Mg or Fe and Ca dynamic results particularly effective against bacterial growth or biofilm biomass formation, respectively.
- *Stenotrophomonas maltophilia* is the most susceptible species with regard to increased growth, while *Staphylococcus aureus* and *Burkholderia cepacia* are the most affected ones in biofilm biomass formation.
- The effect on biofilm viability is not dependent on ionic channel or species considered.

Unresolved issues

- The mechanisms underlying the antibiofilm effect of ELF-MF exposure are not known. Based on our results, it is plausible that the reduced bacterial adherence to substratum is not due to a bactericidal effect, but rather is secondary to altered permeability of the ionic channels of cell membrane.
- Although the field strength used in the present study is well below those that might be encountered in diagnostic/therapeutic applications and near household devices, the cytotoxic potential eventually associated to ELF-MF exposure has to be investigated.
- It is not known whether the antibiofilm effects of ELF-MF could be improved by facilitating antibiotic diffusion throughout biofilm.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Dasenbro EC, Checkley W, Merlo CA, Konstan MW, Lechtzin N, Boyle MP. Association between respiratory tract methicillin-resistant *Staphylococcus aureus* and survival in cystic fibrosis. *JAMA* 303(23), 2386–2392 (2010).

2 Ciofu O, Hansen CR, Høiby N. Respiratory bacterial infections in cystic fibrosis. *Curr. Opin. Pulm. Med.* 19(3), 251–258 (2013).

3 de Vrankrijker AM, Wolfs TF, van der Ent CK. Challenging and emerging pathogens in cystic fibrosis. *Paediatr. Respir. Rev.* 11(4), 246–254 (2010).

4 Millar FA, Simmonds NJ, Hodson ME. Trends in pathogens colonising the respiratory tract of adult patients with cystic fibrosis, 1985–2005. *J. Cyst. Fibros.* 8(6), 386–391 (2009).

5 Peters BM, Jabra-Rizk MA, O'May GA, Costerton JW, Shirtliff ME. Polymicrobial interactions: impact on pathogenesis and human disease. *Clin. Microbiol. Rev.* 25(1), 193–213 (2012).

6 Aziz Z, Cullum N, Flemming K. Electromagnetic therapy for treating venous leg ulcers. *Cochrane Database Syst. Rev.* 2, CD002933 (2013).

7 Mohammed W, Xunning H, Haibin S, Jingzhi M. Clinical applications of susceptibility-weighted imaging in detecting and grading intracranial gliomas: a review. *Cancer Imaging* 13, 186–195 (2013).

8 Teipel SJ, Grothe M, Lista S, Toschi N, Garaci FG, Hampel H. Relevance of magnetic resonance imaging for early detection and diagnosis of Alzheimer disease. *Med. Clin. North Am.* 97(3), 399–424 (2013).

9 Nawrotek P, Fijałkowski K, Struk M, Kordas M, Rakoczy R. Effects of 50 Hz rotating magnetic field on the viability of *Escherichia coli* and *Staphylococcus aureus*. *Electromagn. Biol. Med.* 33(1), 29–34 (2013).

10 Goodman R, Shirley-Henderson A. Transcription and translation in cells exposed to low-frequency electromagnetic fields. *Bioelectrochem. Bioenerg.* 25, 335–337 (1991).

11 Liboff AR, Smith SD, Mc Leod BR. Experimental evidence for cyclotron resonance mediation of membrane transport. In: *Mechanistic Approaches to Interaction of Electric and Electromagnetic Fields with Living Systems.* Blank M, Findl L (Eds). Plenum Press, NY, USA, 102–132 (1987).

12 Seyhan N, Canseven AG, Guler G, Tomruk A, Firlarer A. Cellular enzymatic activity and free radical formation in various tissues under static and ELF electric and magnetic field exposure. In: *Non-thermal Effects and Mechanisms of Interaction between EMFs and Living Matter.* Giuliani L, Soffritti M (Eds). Mattioli 1885, Bologna, Italy, 157–176 (2010).

13 Marinelli F, La Sala D, Cicciotti G *et al.* Exposure to 900 MHz electromagnetic field induces an unbalance between pro-apoptotic and pro-survival signals in T-lymphoblastoid leukemia CCRF-CEM cells. *J. Cell Physiol.* 198(3), 479–480 (2004).

14 Yinnon CA, Yinnon TA. Domains in aqueous solution: theory and experimental evidence. *Mod. Phys. Lett. B* 23, 1959–1973 (2009).

15 Taschin A, Bartolini P, Eramo R, Righini R, Torre R. Evidence of two distinct local structures of water from ambient to supercooled conditions. *Nat. Commun.* 4, 2401–2408 (2013).

16 Liboff AR. Cyclotron resonance in membrane transport. In: *Interactions between Electromagnetic Fields and Cells.* Chiabrera A, Nicolini C *et al.* (Eds). Plenum Press, NY, USA, 281–296 (1985).

•• **First hypothesis that very weak magnetic fields applied to living organisms produce variations of the ion concentration within cells whenever the frequency of applied field matches a characteristic frequency of the involved ion species, called ion cyclotron frequency.**

17 Zhadin MN, Novikov VV, Barnes F, Pergola F. Combined action of static and alternating magnetic fields on ionic current in

aqueous glutamic acid solution. *Bioelectromagnetics* 19(1), 41–45 (1998).

18 Del Giudice E, Fleischmann M, Preparata G, Talpo G. On the 'unreasonable' effects of ELF magnetic fields upon a system of ions. *Bioelectromagnetics* 23(7), 522–530 (2002).

19 Zhadin MN, Giuliani L. Some problems in modern bioelectromagnetics. *Electrom. Biol. Med.* 25(4), 227–243 (2006).

20 Giuliani L, Grimaldi S, Lisi A, D'Emilia E, Bobkova NV, Zhadin MN. Action of combined magnetic fields on aqueous solution of glutamic acid: the further investigations. *Biomagn. Res. Technol.* 6, 1 (2008).

21 Lisi A, Ledda M, De Carlo F *et al.* ICR as a tool in regenerative medicine. *Electrom. Biol. Med.* 27(2), 127–133 (2008).

22 Gaetani R, Ledda M, Barile L *et al.* Differentiation of human adult cardiac stem cells exposed to extremely low-frequency electromagnetic fields. *Cardiovasc. Res.* 82(3), 411–420 (2009).

23 De Carlo F, Ledda M, Pozzi D *et al.* Nonionizing radiation as a noninvasive strategy in regenerative medicine: the effect of Ca^{2+}-ICR on mouse skeletal muscle cell growth and differentiation. *Tissue Eng. Part A* 18(21–22), 2248–2258 (2012).

24 Novikov VV, NoviKov GV, Fesenko EE. Effect of weak combined static and extremely low-frequency alternating magnetic fields on tumor growth in mice inoculated with the Ehrlich ascites carcinoma. *Bioelectromagnetics* 30(5), 343–351 (2009).

25 Ledda M, Megiorni F, Pozzi D *et al.* Non-ionising radiation as a non chemical strategy in regenerative medicine: Ca^{2+}-ICR *in vitro* effect on neuronal differentiation and tumorigenicity modulation in NT2 cells. *PLoS ONE* 8(4), e61535 (2013).

26 Bobkova NV, Novikov VV, Medvinskaia NI, Aleksandrova IY, Nesterova IV, Fesenko EE. The weak combined magnetic fields induce the reduction of brain amyloid-b level in two animal model of Alzheimer's disease. In: *Non-thermal Effects and Mechanisms of Interaction between EMFs and Living Matter.* Giuliani L, Soffritti M (Eds). Mattioli 1885, Bologna, Italy, 235–246 (2010).

27 Saggini R, Bellomo RG, Saggini A, Iodice P, Toniato E. Rehabilitative treatment for low back pain with external pulsed electromagnetic fields. *Int. J. Immunopathol. Pharmacol.* 22(S3), 25–33 (2009).

•• **First work validating clinical use of external pulsed electromagnetic fields.**

28 Matl FD, Obermeier A, Zlotnyk J, Friess W, Stemberger A, Burgkart R. Augmentation of antibiotic activity by low-frequency electric and electromagnetic fields examining *Staphylococcus aureus* in broth media. *Bioelectromagnetics* 32(5), 367–377 (2011).

•• **The combination of electromagnetic fields with the antibiotic gentamicin improves activity against planktonically grown *Staphylococcus aureus*.**

29 Giladi M, Porat Y, Blatt A *et al.* Microbial growth inhibition by alternating electric fields. *Antimicrob. Agents Chemother.* 52(10), 3517–3522 (2008).

30 Obermeier A, Matl FD, Friess W, Stemberger A. Growth inhibition of *Staphylococcus aureus* induced by low-frequency electric and electromagnetic fields. *Bioelectromagnetics* 30(4), 270–279 (2009).

31 Strasak L, Vetterl V, Fojt L. Effects of 50 Hz magnetic fields on the viability of different bacterial strains. *Electromagn. Biol. Med.* 24(3), 293–300 (2005).

32 Potenza L, Ubaldi L, De Sanctis R, De Bellis R, Cucchiarini L, Dachà M. Effects of a static magnetic field on cell growth and gene expression in *Escherichia coli*. *Mut. Res.* 561(1–2), 53–62 (2004).

33 StepanoviĆS, VukoviĆD, Hola V *et al.* Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *APMIS* 115(8), 891–899 (2007).

34 Giuliani L. Why investigate non thermal effects and mechanisms of EMFs on living systems? An introduction. In: *Non-thermal Effects and Mechanisms of Interaction between EMFs and Living Matter.* Giuliani L, Soffritti M (Eds). Mattioli 1885, Bologna, Italy, Vol. 5, IX–XI (2010).

35 Bayır E, Bilgi E, Sendemir-Ürkmez A, HameĠKocabaĆEE. The effects of different intensities, frequencies and exposure times of extremely low-frequency electromagnetic fields on the growth of *Staphylococcus aureus* and *Escherichia coli* O157:H7. *Electromagn. Biol. Med.* (2013) (Epub ahead of print).

36 Fijałkowski K, Nawrotek P, Struk M, Kordas M, Rakoczy R. Effects of rotating magnetic field exposure on the functional parameters of different species of bacteria. *Electromagn. Biol. Med.* (2014) (Epub ahead of print).

37 Dunca S, Creanga DE, Ailiesei O *et al.* Microorganisms growth with magnetic fluids. *J. Magn. Magn. Mater.* 289, 445–447 (2005).

38 Nafziger J, Desjobert H, Benamar B, Guillosson JJ, Adolphe M. DNA mutations and 50 Hz electromagnetic field. *Bioelectrochem. Bioenerg.* 30, 133–141 (1993).

39 Morrow AC, Dunstan RH, King BV, Roberts TK. Metabolic effects of static magnetic fields on *Streptococcus pyogenes*. *Bioelectromagnetics* 28(6), 439–445 (2007).

40 Hyland G. Physics and biology of mobile telephony. *Lancet* 356(9244), 1833–1836 (2000).

41 Mah TF. Biofilm-specific antibiotic resistance. *Future Microbiol.* 7(9), 1061–1072 (2012).

42 Freebairn D, Linton D, Harkin-Jones E, Jones DS, Gilmore BF, Gorman SP. Electrical methods of controlling bacterial adhesion and biofilm on device surfaces. *Expert Rev. Med. Devices* 10(1), 85–103 (2013).

• **Exhaustive review summarizing the significant body of research within the field of electrical methods of controlling microbial adhesion and biofilm formation onto biomedical devices.**

43 Pompilio A, Pomponio S, Crocetta V *et al.* Phenotypic and genotypic characterization of *Stenotrophomonas maltophilia* isolates from patients with cystic fibrosis: genome diversity, biofilm formation, and virulence. *BMC Microbiol.* 11, 159 (2011).

44 Mulcahy LR, Isabella VM, Lewis K. *Pseudomonas aeruginosa* biofilms in disease. *Microb. Ecol.* 68(1), 1–12 (2013).

45 Coutinho CP, de Carvalho CC, Madeira A, Pinto-de-Oliveira A, Sá-Correia I. *Burkholderia cenocepacia* phenotypic clonal variation during a 3.5-year colonization in the lungs of a cystic fibrosis patient. *Infect. Immun.* 79(7), 2950–2960 (2011).

46 Molina A, Del Campo R, Maiz L *et al.* High prevalence in cystic fibrosis patients of multiresistant hospital-acquired methicillin-resistant *Staphylococcus aureus* ST228-SCCmecI capable of biofilm formation. *J. Antimicrob. Chemother.* 62, 961–967 (2008).

47 Pompilio A, Crocetta V, Confalone P *et al.* Adhesion to and biofilm formation on IB3–1 bronchial cells by *Stenotrophomonas maltophilia* isolates from cystic fibrosis patients. *BMC Microbiol.* 10, 102 (2010).

48 D'Inzeo G, Pisa S, Tarricone L. Ionic channel gating under electromagnetic exposure: a stochastic model. *Bioelectrochem. Bioenerg.* 29, 289–304 (1993).

49 Hogt AH, Dankert J, Feijen J. Adhesion of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* to a hydrophobic biomaterials. *J. Gen. Microbiol.* 131(9), 2485–2491 (1985).

50 Giladi M, Porat Y, Blatt A *et al.* Microbial growth inhibition by alternating electric fields

in mice with *Pseudomonas aeruginosa* lung infection. *Antimicrob. Agents Chemother.* 54(8), 3212–3218 (2010).

- **Proof of concept for the use of low-intensity alternating electric fields, applied either as a monotherapy or as an adjuvant to antibiotic therapy, as a treatment against pulmonary infections caused by *Pseudomonas aeruginosa*.**

51 Liu WK, Brown MR, Elliott TS. Mechanisms of the bactericidal activity of low amperage electric current (DC). *J. Antimicrob. Chemother.* 39(6), 687–695 (1997).

52 Blau H, Mussaffi H, Mei Zahav M *et al.* Microbial contamination of nebulizers in the home treatment of cystic fibrosis. *Child Care Health Dev.* 33(4), 491–495 (2007).

53 Gerardi G, De Ninno A, Prosdocimi M *et al.* Effects of electromagnetic fields of low frequency and low intensity on rat metabolism. *Biomagn. Res. Technol.* 6, 3 (2008).

54 Benson DE, Grissom CB, Burns GL, Mohammad SF. Magnetic field enhancement of antibiotic activity in biofilm forming *Pseudomonas aeruginosa*. *Asaio J.* 40(3), 371–376 (1994).

- **Application of a static magnetic field enhances the activity of gentamicin against biofilm forming *P. aeruginosa* adhered to a polymer substrate.**

55 Pickering SA, Bayston R, Scammell BE. Electromagnetic augmentation of antibiotic efficacy in infection of orthopaedic implants. *J. Bone Joint Surg. Br.* 85(4), 588–593 (2003).

- **Exposure to a pulsed electromagnetic field increases the effectiveness of gentamicin against mature biofilms of *Staphylococcus epidermidis* reducing of at least 50% the minimum biofilm inhibitory concentration.**

56 Del Pozo J, Rouse M, Patel R. Bioelectric effect and bacterial biofilms. A systematic review. *Int. J. Artif. Organs* 31(9), 786–795 (2008).

- •• **Exhaustive literature review on the 'bioelectric effect', that is, the enhanced activity of some antimicrobial agents against bacterial biofilms caused by exposure to electric current.**