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Differential viability response of prokaryotes and eukaryotes to high strength pulsed magnetic stimuli

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ABSTRACT

The present study examines the efficacy of a high strength pulsed magnetic field (PMF) towards bacterial inactivation in vitro, without compromising eukaryotic cell viability. The differential response of prokaryotes [*Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, and *Escherichia coli*], and eukaryotes [C2C12 mouse myoblasts and human mesenchymal stem cells, hMSCs] upon exposure to varying PMF stimuli (1–4 T, 30 pulses, 40 ms pulse duration) is investigated.

Among the prokaryotes, ~60% and ~70% reduction was recorded in the survival of staphylococcal species and *E. coli*, respectively at 4 T PMF as evaluated by colony forming unit (CFU) analysis and flow cytometry. A 2–5 fold increase in intracellular ROS (reactive oxygen species) levels suggests oxidative stress as the key mediator in PMF induced bacterial death/injury. The 4 T PMF treated staphylococci also exhibited longer doubling times. Both TEM and fluorescence microscopy revealed compromised membranes of PMF exposed bacteria. Under similar PMF exposure conditions, no immediate cytotoxicity was recorded in C2C12 mouse myoblasts and hMSCs, which can be attributed to the robust resistance towards oxidative stress. The ion interference of iron containing bacterial proteins is invoked to analytically explain the PMF induced ROS accumulation in prokaryotes. Overall, this study establishes the potential of PMF as a bactericidal method without affecting eukaryotic viability. This non-invasive stimulation protocol coupled with antimicrobial agents can be integrated as a potential methodology for the localized treatment of prosthetic infections.

1. Introduction

The biological response of prokaryotic systems to extremely low frequency electromagnetic fields (ELF-EMFs) has been widely researched [1–3]. However, the influence of magnetic fields on microbial survival has yielded ambiguous and conflicting results. The variation in bacterial response depends on the intensity of the magnetic field strength, bacterial strain and growth conditions involved. Some researchers have reported the bactericidal/bacteriostatic effect of magnetic fields manifested as a 37% decrease in the survival of *Staphylococcus aureus* under a combination of electric and magnetic fields [4]. A drop in metabolic activity of *Escherichia coli*, measured as a decrease in glucose consumption was documented, when cultured at 5×10^{-4} T for 8 h [5]. Some others observed membrane rupture of *E. coli* under a weak sinusoidal 60 or 600 Hz magnetic field strength of 2 mT [6] and DNA damage at 1.2 mT and 50 Hz in transformed *E. coli* [7]. In contrast, some studies did not reveal any effect of magnetic fields towards bacterial growth inactivation even at very high magnetic flux densities of 18 T and 50 pulses of 30 μs duration, when applied after mild stresses such as ultrasound, pulse electric fields, high hydrostatic pressure and antimicrobials as nisin and lysozyme [8]. Furthermore, few investigations demonstrated a bacterial growth phase and field homogeneity dependent response to the applied magnetic field. In the logarithmic phase, *E. coli* growth was hindered at a 7 T homogenous field [9], while accelerated bacterial growth and enhanced survival were observed in the stationary phase under inhomogeneous fields of 5.2–6.1 T [9–11]. A recent study showed that a 4 h exposure to a static magnetic field of 100 mT strength caused 60% and 30% reduction in the viability of *E. coli* and *Staphylococcus epidermidis*, respectively when cultured on biomaterial substrates [12]. The ion interference mechanism was hypothesized to promote the rotation of ion–protein complexes causing their dissociation in static magnetic fields between 0 and 110 μT and hence affecting the transport of essential ions such as Zn^{2+}, Ca^{2+} and Mg^{2+} [13]. Transmission electron microscopic evidence of membrane damage in *E. coli* was reported under static magnetic fields of 450 to 3500 mT [14].

In a manner similar to prokaryotes, the adverse effects of electromagnetic fields towards eukaryotic cells/tissue have also been investigated by researchers. At the cellular level, external magnetic fields have been reported to cause cytotoxicity by affecting cell proliferation

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and cell cycle as well as genotoxic effects including DNA damage and altered gene expression. Further reduced metabolic activity, hindrance to transport of essential ions and cell morphological changes are among the additional manifestations of cytotoxicity resulting from magnetic field exposure. A 0.2 T static magnetic field (SMF) was observed to cause changes in the morphology and retard the growth of human skin fibroblasts [15]. Interestingly, the detrimental effects of magnetic fields were minimal in leukemic and breast cancer cells even at 7 T [16]. On the contrary, DNA damage was reported in rat lymphocytes upon simultaneous treatment of rat lymphocytes with Fe^{2+} (10 μg/ml) and 7 mT (static or 40 Hz) [17]. Such observations reflect on the potential health risks due to magnetic field exposure.

In the present study, the differential viability of prokaryotic and eukaryotic cells upon exposure to high strength pulse magnetic stimuli has been investigated by colony forming units (CFU) assay, fluorescence/electron microscopy and flow cytometry methods. For the study, an in-house fabricated pulse magnet was used. We designed a coil which permits kA of current pulses, in which varying PMFs were generated. For the coil design and current-field parameters, guidelines from an earlier report were followed [18]. We exposed three bacterial strains — *S. aureus* (MRSA, USA 300), *S. epidermidis* (ATCC #35984) and *E. coli* (K12 wild type), and two eukaryotic cell lines — C2C12 mouse myoblast cells and human mesenchymal stem cells (hMSCs) to n = 30 pulses of high strength pulsed magnetic fields (1–4 T) with a pulse duration of 40 ms. The two staphylococcal species selected are pathogenic and are implicated in biomaterial associated infections [19,20]. Antibiotic treatment of staphylococcal infections has been met with meager success due their multidrug resistance including methicillin [21] and vancomycin [22]. In light of this, the present study investigates the differential survival response of prokaryotic and eukaryotic cells to high strength pulse magnetic stimuli.

2. Materials and methods

2.1. Pulsed magnetic field setup

A table top pulse magnet was used to treat bacteria/cells in vitro. The circuit used to generate the coil current and the pulse waveform is shown in Fig. S1(a) of supplementary information. The entire setup can be divided into two parts: the charging circuit [DC power supply (0–500 V, 0–100 mA) and capacitor bank (11 electrolytic capacitors of 5 mF/450 V each in parallel)] and discharging circuit [inductor coil (200 μH), diode and capacitor bank]. The capacitor bank is charged to the required voltage and discharged through the inductor coil using a thyristor as a switch. The current resulting from the discharge leads to the generation of PMF. Fig. S1(b) is a calibration of the peak current (kA) flowing through the coil and the consequent magnetic field (T) that is generated against capacitor bank charging voltage (V) for the pulse magnet.

In our experimental setup, the current through the coil during the discharge generates a magnetic field and the field strength is affected by parameters such as damping co-efficient (d), charging voltage (V_o), Inductance (L) and Capacitance (C) of the circuit [23], the details of which are furnished in Supplementary information. Fig. 1(a) shows a schematic of the experimental setup and analysis used for magnetic field treatment of bacteria/cells in suspension, when placed in the center of the inductor coil. The pulse duration (40 ms) and shape, which is due to the damping nature of the RLC circuit, are shown in Fig. 1(b). The magnitude of the field generated was experimentally verified using a GM08 gaussmeter (Hirst Magnetic Instruments Ltd., USA) and it was found to be homogenous over the length of the biological sample.

2.2. Bacterial strains and cultures

The bacterial strains chosen for the study include *S. aureus* (MRSA, USA 300), *S. epidermidis* (ATCC #35984), and *E. coli* K12 wild type (MG1655) strains. *S. aureus* was procured from the Sir Dorabji Tata Centre for Research in Tropical Diseases, Bangalore, while *E. coli* was obtained from the National Centre for Biological Sciences (NCBS), Bangalore. *S. epidermidis* was procured in freeze dried condition from the American Type Culture Collection (ATCC). The freeze dried cultures were revived on nutrient agar plates. A single colony was inoculated in 5 ml of freshly prepared Tryptone Soya broth (TSB; 30 g/l) for overnight culture at 37 °C and 100 rpm in an incubator shaker (Lab. Companion SI-300R). 50 μl of the overnight cultured broth was sub-cultured in 5 ml of fresh TSB media under the same conditions for 2 h, until the logarithmic phase (0.3–1.0 OD_{595}) of bacteria was reached. The optical density of the bacterial suspensions was adjusted to ~0.1, measured with a microplate reader (Bio-rad) at 595 nm and used for performing the experiments.

2.3. Exposure of bacterial cultures to PMF

For the experiments, ~500 μl of each culture at 0.1 OD_{595} (~10^7 bacterial cells in TSB) in 0.5–0.6 ml microcentrifuge tubes was exposed to 30 pulses of 1, 2, 3 and 4 T magnetic field strength over the duration of 0.5, 1.0, 1.5 and 2 h, respectively. The bacterial growth medium treated without any external magnetic field is taken as the control in this study. Both the control and PMF treated cultures were shaken continuously in an incubator shaker at 37 °C and 100 rpm throughout the experiments. The background magnetic field in the incubator shaker was similar to the earth's geomagnetic field (55×10^{-6} T to 70×10^{-6} T). At 50 Hz (AC frequency), the background low frequency fields in the interior of the incubator shaker ranged between 0.2×10^{-6} and 0.5×10^{-6} T, which is nearly the value for most household electrical appliances. The magnetic field exposure cycles for treatment at different field strengths are represented in Fig. 1(b). When the capacitors were charged to the appropriate voltage, both the control and PMF treated samples were removed from the incubator shaker and the PMF treatment of the bacterial suspensions was performed at room temperature and immediately incubated at 37 °C until the next pulse. In the process, both the control and PMF treated cultures were intermittently shuttled between 37 °C and room temperature. It may be noted that the time interval between successive pulses is greater at higher fields due to increased heating of the coil (see Fig. 1(b)). This necessitated more time for the cooling of the coil during which both the control and treated bacterial suspensions were incubated at 37 °C. During the experiments, the B-field parameters were monitored in real time by connecting the gaussmeter to an oscilloscope. The temperature was monitored intermittently by immersing the bulb of a thermometer into the PMF treated bacterial suspension. The bacteria were minimally exposed to the heat generated at high fields and the measured temperature of the suspension was in the range of 37 ± 1 °C, ruling out any thermal effect on bacterial viability. The influence of PMF on bacterial viability was analyzed by flow cytometry and colony forming units assay.

2.4. Assessment of bacterial viability by colony forming unit (CFU) assay

The bacteria were sampled for CFU analysis in two instances in the study: (i) After exposure to check the immediate effect of PMF (1–4 T) on bacterial viability and (ii) after staining with syto9-PI, the live populations were sorted, cultured and sampled for CFU analysis in order to determine the effect of PMF exposure on the generation/doubling times of surviving bacteria. For the CFU assay, ten-fold serial dilutions were made for both the control and magnetic field treated samples in phosphate-buffered saline (PBS; pH 7.4) and 100 μl of the 10^{-5} dilutions was plated, as per the protocol described elsewhere [24]. The number of CFU/ml was calculated for each of the ten-fold dilution using Eq. (1) and the difference in bacterial viability between the control and MF treated samples was quantified using Eqs. (2) and (3).

$$\text{Number of CFU/ml} = \text{number of colonies}/(\text{dilution factor} \times \text{volume of the dilution}) \quad (1)$$