Journal of Microbiology (2017) Vol. 55, No. 10, pp. 809–815
DOI 10.1007/s12275-017-7208-7
eISSN 1976-3794
pISSN 1225-8873

The response of human bacteria to static magnetic field and radiofrequency electromagnetic field[§]

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(Received May 18, 2017 / Revised Aug 18, 2017 / Accepted Aug 21, 2017)

Cell phones and electronic appliances and devices are inseparable from most people in modern society and the electromagnetic field (EMF) from the devices is a potential health threat. Although the direct health effect of a cell phone and its radiofrequency (RF) EMF to human is still elusive, the effect to unicellular organisms is rather apparent. Human microbiota, including skin microbiota, has been linked to a very significant role in the health of a host human body. It is important to understand the response of human skin microbiota to the RF-EMF from cell phones and personal electronic devices, since this may be one of the potential mechanisms of a human health threat brought about by the disruption of the intimate and balanced host-microbiota relationship. Here, we investigated the response of both laboratory culture strains and isolates of skin bacteria under static magnetic field (SMF) and RF-EMF. The growth patterns of laboratory cultures of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis* under SMF were variable per different species. The bacterial isolates of skin microbiota from 4 subjects with different cell phone usage history also showed inconsistent growth responses. These findings led us to hypothesize that cell phone level RF-EMF disrupts human skin microbiota. Thus, the results from the current study lay ground for more comprehensive research on the effect of RF-EMF on human health through the human-microbiota relationship.

Keywords: skin microbiota, bacterial growth, cell phone, SMF, RF-EMF

Introduction

With ever-progressing technological advancements and the fast integration of electronic devices in our everyday lives, sources of low electromagnetic field (EMF) are omnipresent in modern society. Electric devices generate EMF, and cell phone is arguably most significant because cell phones are used directly next to brain and are almost inseparable to an increasing number of people. In fact, the World Health Organization (WHO) placed cell phones in the category of "possibly carcinogenic to human (2B)" based on the expert panel review including the International Agency for Research on Cancer (IARC) working group (Baan *et al.*, 2011; Gaudin, 2011). Some more recent study even argues that RF-EMF should be classified as a Group 2A "probable human carcinogen"(Morgan *et al.*, 2015). A recent preliminary report on the radiofrequency radiation toxicology and carcinogenesis study by the US National Toxicology Program concluded that there was presence of a marginal increase of tumors in male rats (Wyde *et al.*, 2016) (https://www.niehs.nih.gov/health/topics/agents/cellphones/).

EMF frequency ranges between 0 and 300 GHz (Breckenkamp *et al.*, 2003). Radiofrequency (RF) ranges between 30 KHz–300 GHz, and the range for extremely low frequency (ELF) is between 30–300 Hz, which includes 50/60 Hz EMF by power suppliers and lines. RF-EMF is generated by many electronics and household appliances, such as microwave ovens (3–30 MHz) and cell phones (300 MHz–3 GHz). ELF and RF radiation generated by electronic devices have a non-thermal effect on biological systems, while stronger ionizing radiations, such as X-rays or gamma rays (> 30 PHz, > 3×10^{16} Hz), can directly damage DNA structures of biological systems. In addition to the frequency, the dosimetry is another important factor in the effect of EMF. In the range of RF, specific absorption rate (SAR) is often used to estimate the rate of energy deposition per unit mass (Behari, 2010). SAR is a standardized measure for comparing different studies in a biologically meaningful way, as it is the amount of the energy actually absorbed in a biological system. For example, a wide range of SAR can be generated and have a distinctive effect from the same RF-EMF (Koyama *et al.*, 2007).

The direct effect of EMF on humans or others mammals has been studied numerous times with RF-EMF. Mainly by epidemiological studies, the effects on brain tumors, such as glioma and acoustic neuroma (INTERPHONE Study Group, 2010; Hardell *et al.*, 2011; Lerchl *et al.*, 2015) have been the main focus of many studies including in the 2011 WHO panel. Sleep and circadian rhythm (Lustenberger *et al.*, 2015; Danker-Hopfe *et al.*, 2016), and the human thyroid stimulating hormone (TSH) and thyroid hormones (Mortazavi *et al.*, 2009) were significantly affected by RF radiation. A study also showed neuropsychological response to the mice by prolonged RF-EMF exposure (Kim *et al.*, 2017). Additionally, RF radiation rarely showed beneficial effects, such as improved cognitive impairment in Alzheimer's disease

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[§]Supplemental material for this article may be found at http://www.springerlink.com/content/120956.

in mice (Arendash *et al.*, 2010). A more common beneficial effect of EMF is the augmentation of antibiotic activities. Several antibiotics have shown to be more potent in low EMF. There is also a study indicated insignificant direct effect on human health (SCENIHR [Scientific Committee on Emerging and Newly Identified Health Risks], 2015).

The effect of MF on single cell organisms is expected to be more prominent, so there have been numerous studies from earlier years, for example with static magnetic field (SMF) (Jennison, 1937). The effect on the structure and growth of bacteria was often studied with ELF-EMF (Strašák *et al.*, 2002; Fojt *et al.*, 2004; Di Campli *et al.*, 2010; Bayir *et al.*, 2015; Oncul *et al.*, 2016), as well as with RF-EMF (Morozov *et al.*, 1995; Gul Guven *et al.*, 2006; Carlberg and Hardell, 2014; Taheri *et al.*, 2017). Most of the studies were based on the overall response in growth or mortality of bacteria, but several studies focused on the mechanisms of the response, such as the disruption to the cell membrane structure (Shamis *et al.*, 2011; Rougier *et al.*, 2014) and the damage to the DNA replication machinery (Cheng and Zou, 2006). On the other hand, there have been studies showing neutral and even beneficial responses in bacterial growth to RF-EMF (Alexander, 1996; Miyakoshi *et al.*, 2007).

The mutualistic relationship between the human body and human microbiota has ample evidences and has gained tremendous interests in both the scientific community and the general public. The relationship is inter-dependent and well-balanced from long, co-evolutionary processes (Ley *et al.*, 2008; Turnbaugh *et al.*, 2009; Faith *et al.*, 2011; Grice and Segre, 2011; Belkaid and Segre, 2014). Thus, often sickness is attributed to the disrupted microbiota and imbalanced human-microbiota relationship (Moloney *et al.*, 2014; Belizario and Napolitano, 2015). For example, late onset autism has been related with disrupted child microbiota by the infestation of *Clostridium* after vancomycin administration (Finegold *et al.*, 2002). More commonly, prolonged usage of broad-spectrum antibiotics often causes colitis by the *Clostridium difficile* toxin. As discussed before, different bacterial species may respond quite differently to the EMF (Taheri *et al.*, 2017), and thus, those disruptions to the composition of skin microbiota and to the inter-dependency with the human body might be induced. Therefore, we investigated the effect of weak SMF and simulated RF-EMF using standard laboratory cultures and isolates from human subjects' skin. There have been no studies targeted the response of human skin microbiota under EMF yet. Our main hypothesis is that the growth patterns of different species of human skin bacteria will respond differently to both SMF and RF-EMF.

Materials and Methods

Static magnetic field experiment

For the static magnetic field experiment, the laboratory cultures of three bacteria commonly found from human microbiota, (*Escherichia coli* [ATCC 25922], *Pseudomonas aeruginosa* [ATCC 27853], and *Staphylococcus epidermidis* [ATCC 12228]) were obtained from Microbiologics. Laboratory cultures were pre-incubated for 24 h at 37°C, and actual incubation lasted for 72 h with periodic optical density (OD) measurements. Triplicated samples of pre-incubated cultures were inoculated in sterile Difco Nutrient Broth (0.1% v/v) using BD Falcon 48-well plates. Tecan Infinite® M200 plate reader was used for OD measurements. Three incubation conditions were random (inhomogeneous) magnetic field (BR), homogeneous magnetic field (BH), and control (B0) (Supplementary data Fig. S1). Control was background field, and magnetic fields generated by laboratory facilities including incubator were assumed to be same between control and magnetic fields. Magnetic fields prepared with were weak (~50 G) as determined by the IDR- 309 Gaussmeter (Integrity Design & Research) and applied to both pre-incubation and incubation. The data were analyzed by analysis of variance (ANOVA) using R (R Core Team, 2015).

Isolation of human skin bacteria

Four volunteers between the ages of 20 to 22 were selected at random for sampling of skin bacteria from the hand, cheek, and chin (IRB approval, 627393-1, Supplementary data Table S1). Each volunteer completed a cell phone use survey before sampling. The demographics were one Caucasian male with high cell phone usage, one Caucasian female with very high phone usage, and two Asian females with moderate cell phone usage. They all have been using cell phones for the previous 6–7 years. Using aseptic technique, swabs used for sampling skin (palm, chin, and cheek) were each streaked onto Trypticase Soy Agar (TSA) media and incubated at 37°C for 24 h. Using standard isolation procedures and diluting colony density through further streaking, colonies with distinctive morphology were isolated from each sample for a maximum of three bacterial isolates from each sampling skin area. Twenty-four individual colonies were isolated in total. Bacteria were then incubated at 37°C for 48 h in liquid suspension using Difco Nutrient Broth preparation for growth in BD Falcon 48-well plates under RF-EMF.

RF-EMF system setup and growth incubation

An RF-EMF generator was designed to simulate the effect of cell phone use and was connected to an antenna and mounted in an incubator kept at 37°C. Growth was monitored at two distinct power settings, high and low, at 1.563 mW and 0.783 mW, respectively. The power levels were measured by a 50 Ω monopole antenna placed in three different radial positions on a blank plate and averaged over 360° rotations. These power settings were estimated average EMF exposed by typical cell phone use. Two 48-well plates were prepared for each experiment, consisting of two experiments of high and low power, by inoculating each sample from liquid broth in triplicate into sterile Difco Nutrient Broth at 0.1% (v/v) concentration. The samples were randomly replicated on plates to minimize spatial EMF variation. Duplicate blanks were also prepared as controls. Plates were incubated for 48 h and OD was measured regularly using a Promega GloMax®-Multi microplate multimode reader. The control was prepared in identical settings except for the RF-EMF exposure (background level). The data from these readings were then used to plot growth curves and statistical analysis for elucidating the effect of RF-EMF on the growth of isolated skin bacteria by student *t*-test using

R (R Core Team, 2015).

DNA isolation and sequence analysis

Four isolates showed significant growth response (two hand samples from Subject B, and two hand samples from Subject C), and four randomly selected insignificant growth response samples were further processed for 16S rRNA gene sequencing. The Qiagen DNeasy® Blood & Tissue Kit was used for DNA isolation following the protocol for Gram-positive bacteria.

Universal primer set (27F–1492R) for 16S rRNA gene was used in PCR amplification for the sequencing. Each PCR reaction contains 5 µl of Epicentre fail safe buffer E, 0.48 µl (1.2 µm) primers, 1 µl sample of DNA, 2.94 µl of DNA safe water, and 0.1µl of 0.5 U/µl TAQ polymerase. The PCR was run on the Applied Biosystems® Veriti® 96-Well Thermal Cycler, and the cycle started with 96°C hot start for five min followed by 30 cycles of 94°C for thirty sec, 57°C for one min and 72°C for two min. The cycle then finished with a seven min final extension at 72°C and finally a 4°C for storage until the samples were retrieved. These samples were then sent to Macrogen for sequencing and these results were analyzed using NCBI BLAST and Ribosomal Database Project (RDP) database. Sequences are available from GenBank accession number MF077520-MF077527.

Results and Discussion

Laboratory cultures under static magnetic fields

In comparison to control condition (B0), growth of *P. aeruginosa* was suppressed significantly and *S. epidermidis* was marginally suppressed, while *E. coli* growth was significantly increased under SMF (Fig. 1 and Supplementary data Table S2). Between random (BR) and homogenized (BH) magnetic fields, the growth response was marginally different except for *E. coli*, whose growth was significantly increased under BR field over BH field. Overall growth patterns were significantly different among three bacteria under control condition but became virtually indistinguishable under both SMFs (Supplementary data Table S2 and Fig. S2), indicating meaningful effect of static magnetic field on the growth of three bacteria in both a positive and negative direction. Note that both *E. coli* and *P. aeruginosa* are Gram-negative bacteria but produced the opposite trends, which seems to make the hypothesis relating cell envelope structure with magnetic field irrelevant (Shamis *et al.*, 2011). The static magnetic field (homogenized and random magnetic field) clearly differently affected the growth of three bacterial strains of typical human microbiota. However, the bacterial strains used were laboratory cultures, and there are often substantial genotypic and phenotypic differences between laboratory cultures and isolates of same bacteria species (Scherer *et al.*, 2003). Thus we attempted to use isolates of skin microbiota and radio-frequency electromagnetic field (RF-EMF) to investigate further the effects of cell phones and other electronics on human microbiota.

Human skin isolates under RF-EMF

Among all of the initial sampling, half of all the isolated colonies recovered were from hands (12 out of 24), and six were recovered each from chins and cheeks. Fourteen colonies were recovered from two light-use Asian female sub-

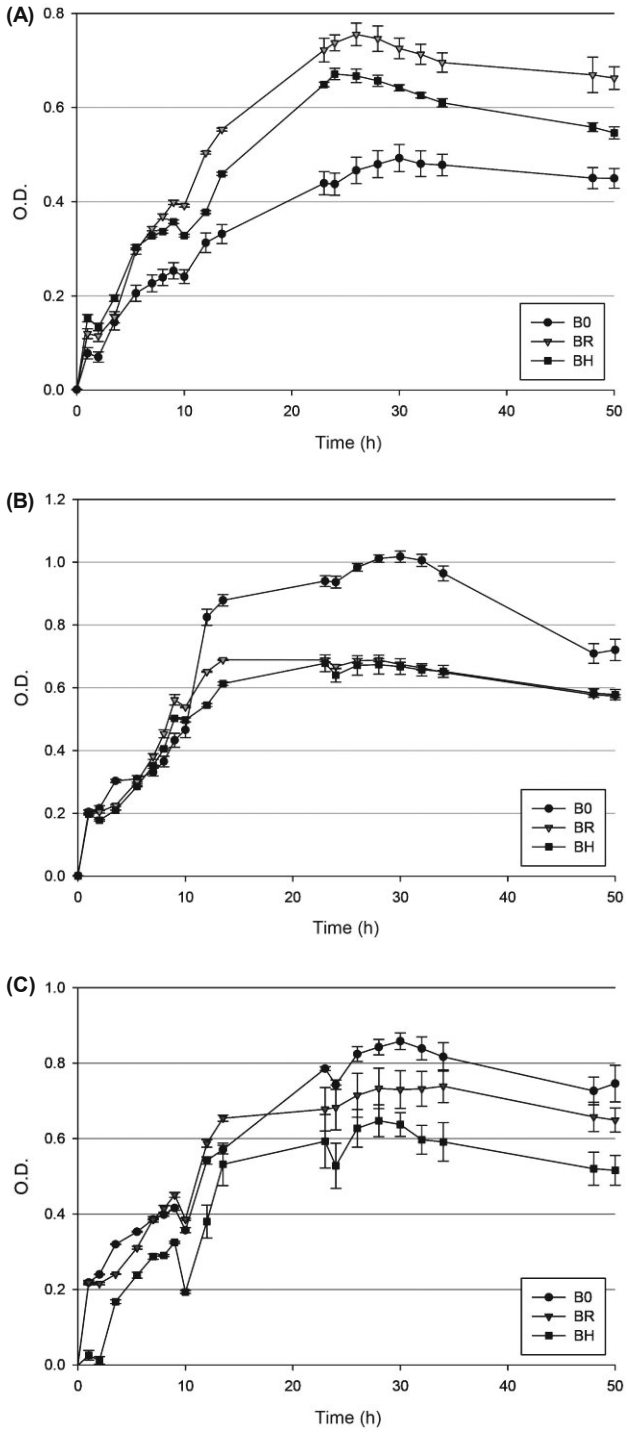

Fig. 1. Growth curves of three laboratory culture bacteria under static magnetic field. (A) *Escherichia coli*, (B) *Pseudomonas aeruginosa*, and (C). *Staphylococcus epidermidis*. Error bars represent 1 standard error with triplicates.

jects (A & B), and ten were recovered from two heavy-use Caucasian subjects (C & D). Of the total of 24 isolated colonies from the four subjects, a total of eight showed altered growth patterns due to exposure to RF-EMFs (Supplementary data Table S3). The growth of three was colonies altered at high power and low power, respectively. Only one (hand 3 sample from subject B) experienced a significant increase in growth at both high and low power. The growth of 5 isolated colonies was increased (Fig. 2A and B and Supplementary data Fig. S3) while growth of two of them was suppressed (Fig. 2C and D). All the isolated colonies identified were the members of genus *Staphylococcus*: *S. pasteuri*, *S. lugdunensis* and *S. epidermidis* from NCBI BLAST and RDP SeqMatch analysis (Supplementary data Table S4). The growth of majority of isolated colonies were not affected, and we suspect this may be due to the RF-EMF generation setting. The power settings were to simulate average exposure in which EMF power is much stronger when cell phone is in use (~25 dBm). The more realistic experimental setting of oscillation of RF-EMF would have produced more obvious responses.

Out of those 12 isolated colonies from hands, growth of six was substantially altered (4 increased and 2 suppressed), while only 1 out of 6 chin colonies' growth was increased under high power. No colony from cheek had altered growth patterns. Subject B had most isolated colonies whose growth patterns were altered by RF-EMF (4 out of 7 colonies tested). Subject B is of Asian descent and considered herself a light cell phone user, meaning she used her phone a few times per day on average and only for phone calls. Subject B, who had multiple isolated colonies affected from the hand, including one at low power, seemed to have the most susceptible bacteria to alteration of growth patterns. The low cell phone usage and lack of exposure to RF-EMF could be linked to a higher chance of affected growth in bacteria. On the other hand, subject A had no colonies with substantial growth change out of seven recovered. Subject A and B were roommates and shared a common life style and culture. However, subject A was under antibiotic treatment at the time of sampling and used face wash regularly, which might have contributed to the difference. The most susceptible component of microbiota may be equally venerable to any antagonistic impacts, in this case for example, antibiotics and RF-EMF. Thus, the remaining bacteria populations might have been quite resistant to environmental perturbations (Chait *et al.*,

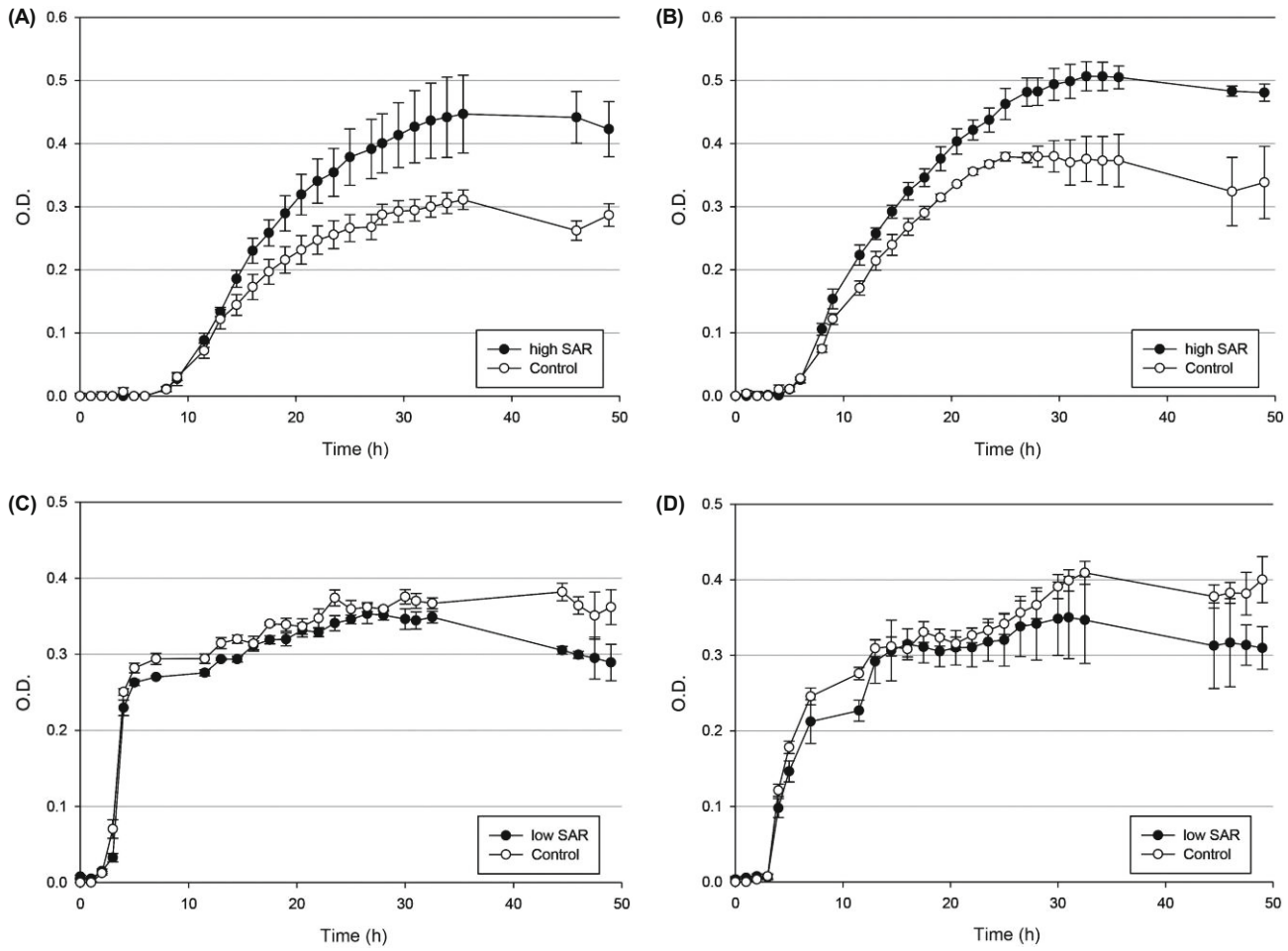

Fig. 2. Growth curves of isolated colonies with substantially altered growth under radiofrequency electromagnetic field compared to the background level (control). (A) Subject B's hand 1 at high power, (B) Subject B's hand 3 at high power, (C) Subject C's hand 1 at low power, (D) Subject C's hand 2 at low power. Error bars represent 1 standard error with triplicates.

2016). Two heavy users (subjects C and D), i.e., texts or other form of phone use at every thirty min or less, had only 3 out of 10 isolated colonies showing altered growth patterns. These habits their skin microbiota might have adapted to a high RF-EMF exposure environment and so showed minimum growth pattern alterations.

Characteristics of bacteria, including high abundance, short reproduction time, and genetic versatility, enable them to quickly evolve and adapt to new environmental challenges (Padfield *et al.*, 2016; Tenaillon *et al.*, 2016). The evolutionary response is a combination of physiological adaptation of existing population and selection of populations with better fitness, resisting traits, and higher phenotypic plasticity (Schaum and Collins, 2014). Our assumption in this study was that there might have been a significant effect of RF-EMF on the growth of skin bacteria from subjects with different cell phone usage histories due to either or both physiological response and competitive selection. Several years of almost constant cell phone use might have been enough to have a substantial effect on the evolution of skin microbiota, so the resulting microbiota may be well adapted to the exposure of RF-EMF by combination of physiological acclimation and more competitive populations being selected. On the contrary, skin microbiota of light users may be still very susceptible to the RF-EMF, thus showing greater response when exposed to the RF-EMF.

Several *Staphylococcus* species are known to cause skin infections: *S. lugdunensis* is the causative bacteria of illnesses, including endocarditis (Schandiz *et al.*, 2015), osteomyelitis (Gahukamble *et al.*, 2014) and peritonitis (Lee *et al.*, 2009). *S. epidermidis* causes infection, too, often in hospital settings or with patients who have medical devices (Sharma *et al.*, 2011; Argudin *et al.*, 2015). These two species of *Staphylococci* are typically commensal to the human skin microbiota, but given uninhibited growth, these coagulase negative *Staphylocci* (CNS) can cause severe complications for any individual. Even if over exposure of skin microbiota to RF-EMFs were not enough on its own to cause an increased growth rate and possible infection, other known or unknown increases may additively affect them and thereby negatively impact human health. The growth of *Staphylococci* from certain individuals were enhanced under RF-EMF, and in some other cases the growth was suppressed, which means the disruption to the balanced skin microbiota make it more vulnerable to infection possibly by those opportunistic pathogens or foreign pathogens (Belkaid and Segre, 2014). These findings add to the ever-growing evidence that even cell phone level RF-EMFs have the potential to negatively impact human health and that human exposure may need to be more strictly regulated.

Conclusion

Both static and radiofrequency electromagnetic fields have significant yet variable effects on the growth of common human bacteria. Under both SMF and RF-EMF, bacterial growth was unaffected, increased, or suppressed per species of bacteria, and the responses seemed to be determined by historic exposure to RF-EMF and life style. Although the current study may not be conclusive with limited number of subjects, it is enough to infer the possibility of indirect effect of SMF and RF-EMF to human health through disruption to the human-microbiome relationship. Future mechanistic studies based on evolutionary response framework using genomics and transcriptomics tools, along with a larger sampling size from distinctive cohort groups consisting of a distinctive demography and cell phone and other personal electronic devices usage history are necessary to further advance the research on the effect of RF-EMF to human health.

Acknowledgements

Authors are grateful for the technical assistance by Dr. Randall Jean and his research team (Department of Electrical and Computer Engineering, Baylor University) for the RE-EMF simulation. We acknowledge the research support by Baylor University Office of Research and College of Arts & Sciences.

Author Contributions

SK and BJH designed the research; DPEC and SK conducted research, analyzed the data and wrote the paper.

Conflict of Interest

The authors declare that they have no conflict of interest.

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