Effect of Faradarmani Consciousness Field on Cell Culture, Bacterial Contamination of Cell Culture, and SARS-CoV-2 Replication *In Vitro*

Mohammad Ali Taheri[1], Laleh Amani[2], Simin Sharifi[3], Ahmad Khalili[4], Ali Zaman Vaziri[4], Hossein Keyvani[5*]

1. Sciencefact R&D Department, Cosmointel Inc. Research Center, Ontario, Canada
2. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
3. Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
4. Cancer Biomedical Center (CBC) Research Institute, Tehran, Iran
5. Department of Virology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

* Corresponding author: Hossein Keyvani, Department of Virology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.
Email: Keyvanlab@yahoo.com

DOI: https://doi.org/10.61450/joci.v1i1.10

Abstract

The highly contagious and life-threatening coronavirus SARS-CoV-2, which has caused a global outbreak since December 2019 (COVID-19), is a beta coronavirus that has spread rapidly and become a worldwide pandemic. So far, there is no definitive treatment for this disease. The Faradarmani Consciousness Field, as one of many T-Consciousness Fields (TCFs) introduced by Mohammad Ali Taheri, is a novel field that is neither matter nor energy. Therefore, they are non-quantifiable and cannot be directly observed or measured. However, it is possible to demonstrate and measure the effects of these fields through standard scientific experiments. The present work aimed to study the effect of Faradarmani CF on SARS-CoV-2 replication and on contaminated cell culture flasks inoculated with the bacteria. According to the results, Faradarmani CF induced virus proliferation in the virus culture. In contaminated cell culture flasks inoculated with bacteria, significant differences in color, turbidity, and Vero cell viability were observed between the Faradarmani CF treatment and control groups, demonstrating the effect of Faradarmani CF. Further studies are highly recommended to investigate the effect of TCFs *in vivo*.

Keywords: COVID-19, SARS-CoV-2, Faradarmani, Taheri Consciousness Fields, T-Consciousness, Replication

Introduction

Over the last 20 years, the world has seen three emerging coronavirus pathogens: severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic (Al-Tawfiq, 2020). SARS-CoV-2 is a new member of the beta coronavirus genus, first reported in China in mid-December 2019 and rapidly spreading worldwide, causing the COVID-19 pandemic. The spread of coronavirus pathogens has become a global concern for the World Health Organization (WHO) over the last decade. Therefore, it is imperative to consider effective treatments to prevent the spread and mortality from coronavirus disease (Liu et al., 2020).

According to Taheri's theory, T-Consciousness is the main component of the universe and the origine of matter, energy, and information. Also, various T-Consciousness Fields (TCFs) of a non-frequency nature have been introduced. These fields cannot be directly measured by science, but their effects can be investigated across various subjects through reproducible laboratory experiments. The effect of TCFs begins with the brief and immediate attention of the *Faradarmanagar* (trained individual) to the subject of study (Taheri, 2013).

In previous research, the effects of the TCFs on the MCF7 cancer cell line (Taheri et al., 2020a), spatial memory and avoidance behavior of a rat model of Alzheimer's disease (Taheri et al., 2021a), wheat plant (Torabi et al., 2020), bacterial population growth (Taheri et al., 2021b), and the electrical activity of the brain during Faradarmani Connection in the Faradarmangars population (Taheri et al., 2020b) have been shown. The present work aimed to study the effect of the Faradarmani CF on SARS-CoV-2 replication and cell culture flasks contaminated by the bacteria.

Material and Methods

Application of Faradarmani Consciousness Field

Faradarmani CF was applied to the samples according to the protocols regulated by the COSMOintel research center (www.cosmointel.com). More details are provided in the Common Considerations section of this issue. In the present study, Faradarmani CF was used simultaneously with the inoculation of the virus in cell culture flasks.

Cell and virus preparation

The effects of Faradarmani CF on cultured Vero cells inoculated with SARS-CoV-2 were studied, and the propagation and growth of SARS-CoV-2 were evaluated.

Collection and transportation of positive COVID-19 samples

Three samples were collected using nasopharyngeal and/or oropharyngeal swabs from COVID-19-positive patients. Patients were diagnosed by Real-Time PCR analysis with low Cycle threshold (Ct < 20). Swabs were placed into 3 mL Viral Transportation Medium (VTM) and transferred to the BSL-3 facility laboratory units at 4 °C on the same day for culture and propagation. VTM contained DMEM (High glucose), 2% Penicillin-Streptomycin solution, and 5 µg/mL Amphotericin.

Vero cell culture

Vero cells (from the same T-75 flask) were seeded in eighteen T-25 flasks in culture media that were composed of high-glucose DMEM (Gibco) with 10% fetal bovine serum (Gibco) and incubated in 5% CO2 at 37°C until 80% confluency. The eighteen T-25 flasks were divided into three groups of six flasks.

In the first group, three flasks were under the influence of Faradarmani CF, and the other three flasks were considered as controls without

applying Faradarmani CF. This group of flasks was visually monitored every day for three days to reach full confluency. In the second group, three cell culture flasks were marked as treatment flasks to evaluate the effect of Faradarmani CF on virus replication, TCID50, and cytopathic effect (CPE). The other three cell culture flasks were considered the control group. In the third group, three cell culture flasks were utilized for the investigation of the effect of Faradarmani CF on cell contamination with bacteria, and the remaining three flasks were considered as the control group.

Preparation of virus samples, inoculation, and isolation in cell culture

The suspension of 12 samples was diluted and mixed with phosphate-buffered saline (PBS) and then centrifuged at 4000 rpm for about 25 minutes. Then, the suspension was filtered twice through 0.45 and then 0.22 µm pore filter membranes under a Laminar hood in sterile and safe conditions. An isolation procedure was followed with the T-25 flask with a Vero cell line. Initially, the medium of the flask was discarded, and following inoculation (1000µl of virus sample) and the virus adsorption period (1.5h, at 37°C and 5% CO2), the medium was removed, and fresh medium (DMEM high glucose with 2% serum) was added and incubated at 37°C, 5% CO2. Each day, CPE was recorded under an inverted microscope for six days. On day six, samples were directed for RT real-time PCR for verification of virus isolation. Then, isolated viruses were titrated in a 96-well plate from dilution 10^3 to 10^8 using the Reed–Muench method (Reed et al., 1938). The observed CPE flask with confirmation of RT real-time PCR, and the titrated virus was transferred for storage at -20 °C freezer until use for the next sub-culturing.

Virus preparation and titration

A virus sample with 1×10^6 TCID50/mL, and 4×10^6 virus RNA copy number was selected, and 1 mL of it was added to flasks of treatment by Faradarmani CF and control groups.

Effect of Faradarmani CF on contaminated culture medium with bacteria

In parallel with virus culture, six cell cultures were prepared for inoculation with the contaminant (a cell culture diagnosed as contaminated with Bacillus spp.). In the Faradarmani CF treatment group, simultaneous with initiation of the Faradarmani CF effect, 20 µL (1×10^5 CFU/ mL) supernatant from a flask was inoculated directly into three cell culture flasks. Also, three flasks were inoculated as a control. Cultures were visually observed daily for 3 days to monitor changes in color, turbidity, cell death, and other visible characteristics.

Statistical analysis

Data were analyzed in SPSS version 21 using t-test and normal analysis, and p-value <0.05 was considered statistically significant.

Results

Cell culture results

The difference between cell count results and growth rate of Vero cells in the Faradarmani CF treatment compared with the control group was not significant ($p \geq 0.05$).

Virus replication in cell culture

Figure 1 shows CPEs observation after six days on non-infected Vero cells (A), culture SARS-CoV-2 without Faradarmani CF treatment (B), and with Faradarmani CF treatment (C). In the TCID50 assay for virus titration in infected Vero cells, in the Faradarmani CF treatment and control groups, there was an average of TCID50/mL $4 \times 10^{7.5}$ and TCID50/mL $2 \times 10^{6.4}$ for cultured flasks, respectively. This result showed that Faradarmani CF has significantly increased TCID50/mL virus infectivity in treatment flasks ($p \leq 0.05$) (Figure 1). In addition, in microscopic observation, more CPE regions were seen in the Faradarmani CF treatment group (Figure 1C).

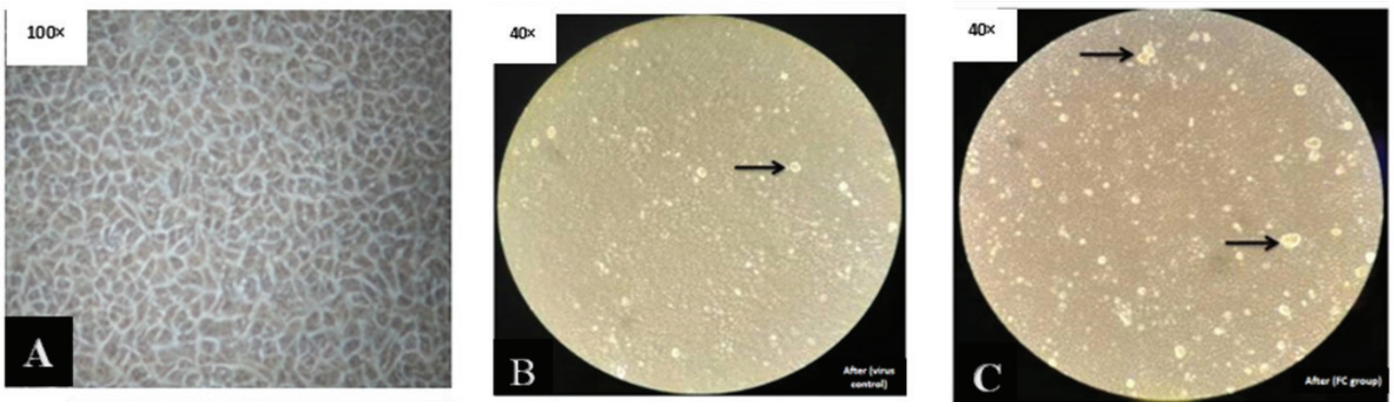

Figure 1. (A) Non-infected Vero cells, (B) SARS-CoV-2 infected Vero cells, and (C) SARS-CoV-2 infected Vero cells with Faradarmani CF treatment demonstrated by inverted light microscopy with 100× and 40× magnification. Some CPEs (rounded, detached, and aggregated bright spots) are shown by black arrows.

Cell contamination results

The phenol red is used as a pH indicator in cell culture media (phenol red has a yellow color at a pH of 6.4 or below and a red color at a pH of 8.2 and above). Gradual acidification (turning yellow) is a sign of the use of media glucose in the uninfected cell. Especially, in bacterial contamination, the color of the environment quickly turns yellow, and the media becomes turbid, without any cell growth in the flask.

The contaminated flask under the influence of Faradarmani CF treatment (A) and the contaminated flask without Faradarmani CF treatment (B) are shown in Figure 2. Significant differences were found in the color, turbidity, and viability of the cultured Vero cells. Although contamination was clearly seen in both cultures, and cells were completely detached from the bottom of the flasks after 18h for flask B and 48h for flask A.

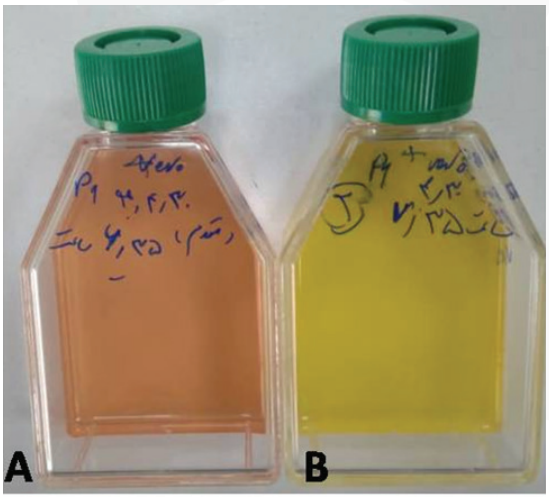

Figure 2. The samples of contaminated cell culture T-25 flasks after 24 h observation. A: contaminated flask with Faradarmani CF treatment, B: contaminated flask without Faradarmani CF treatment (control). The significant differences in the color and turbidity of the cell culture supernatant could be observed in the flasks.

Discussion

The results demonstrated that Faradarmani CF promotes virus growth and proliferation as well as Vero cells' protection against bacterial contamination. In virus cultures, Faradarmani CF treatment increased viral proliferation and growth. However, the cells showed longer survival time, more robustness, and better ability to withstand harsh conditions against the virus.

Most routine virology laboratories add antibiotics to cell culture medium in order to protect the cells from the damaging effects of bacterial contamination (Cruickshank et al., 1952; Leifert et al., 2001). Cell cultures inoculated with samples to isolate the virus fail due to bacterial overgrowth despite the presence of antibiotics (Gray et al., 1991). In this study, the effect of the Faradarmani CF on bacterial contamination of cell culture flasks was investigated. The Faradarmani CF appears to inhibit bacterial growth in the cell culture medium. Further research on the mechanism and function of Faradarmani CF in the laboratory requires experimentation with different cell types and microorganisms. Research on cells, microorganisms, and biological processes raises numerous questions about their behavior and function.

Our knowledge of the nature and operation of the TCFs is at the very early stages, and although the mechanism of the Faradarmani and other TCFs is not yet definable by science, it is important to investigate their effects on various biological (and non-biological) organisms to understand the nature and extent of their beneficial effects and treatment capabilities.

The results of this study demonstrated an observable change in the behavior of the cells contaminated by bacteria. Further studies investigating the effects of the Faradarmani CF on SARS-CoV are needed in order to discover the full range and all aspects of the TCFs treatment method.

Acknowledgments

This study was performed in Keyvan Virology Specialty Laboratory (KVSL) in Tehran, Iran. We thank the members of this laboratory for their assistance.

Conflicts of Interest

The authors declare no conflict of interest.

References

Al-Tawfiq, J. A. (2020). Viral loads of SARS-CoV, MERS-CoV and SARS-CoV-2 in respiratory specimens: What have we learned? *Travel Medicine and Infectious Disease, 34,* 101629. https://doi.org/10.1016/j.tmaid.2020.101629

Cruickshank, C. N. D., & Lowbury, E. J. L. (1952). Effect of antibiotics on tissue cultures of human skin. *British Medical Journal, 2*(4793), 1070–1072. https://doi.org/10.1136/bmj.2.4793.1070

Gray, J. J., & Brenwald, N. P. (1991). The use of antibiotics to control bacterial overgrowth of cell cultures used for diagnostic virology. *Journal of Virological Methods, 32*(2–3), 163–170. https://doi.org/10.1016/0166-0934(91)90047-4

Leifert, C., & Cassells, A. C. (2001). Microbial hazards in plant tissue and cell cultures. *In Vitro Cellular & Developmental Biology – Plant, 37*(2), 133–138. https://doi.org/10.1007/s11627-001-0025-y

Liu, S.-L., & Saif, L. (2020). Emerging viruses without borders: The Wuhan coronavirus. *Viruses, 12*(2), 130. https://doi.org/10.3390/v12020130

Reed, L. J., & Muench, H. (1938). A simple method of estimating fifty percent endpoints. *American Journal of Epidemiology, 27*(3), 493–497. https://doi.org/10.1093/oxfordjournals.aje.a118408

Taheri, M. A. (2013). *Human from another outlook* (2nd ed.). Interuniversal Press. ISBN: 9781939507006

Taheri, M. A., Semsarha, F., Mahdavi, M., Afsartala, Z., & Amani, L. (2020a). The influence of the Faradarmani consciousness field on the survival and death of MCF-7 breast cancer cells: An optimization perspective. *SSRN Electronic Journal.* https://doi.org/10.2139/ssrn.3705537

Taheri, M. A., Semsarha, F., & Modarresi-Asem, F. (2020b). An investigation on the electrical activity of the brain during Fara-Darmani connection in the Fara-therapist population. *SSRN Electronic Journal.* https://doi.org/10.20944/preprints202009.0679.v1

Taheri, M. A., Torabi, S., Nabavi, N., & Semsarha, F. (2021a). Influence of Faradarmani consciousness field (FCF) on spatial memory and passive avoidance behavior of scopolamine model of Alzheimer disease in male Wistar rats. *SSRN Electronic Journal.* https://doi.org/10.2139/ssrn.3761188

Taheri, M. A., Zarrini, G., Torabi, S., Nabavi, N., & Semsarha, F. (2021b). Influence of Faradarmani consciousness field on bacterial population growth. *bioRxiv.* https://doi.org/10.1101/2021.01.08.426007

Torabi, S., Taheri, M. A., & Semsarha, F. (2020). Alleviative effects of Faradarmani consciousness field on *Triticum aestivum* L. under salinity stress. *F1000Research, 9,* 1089. https://doi.org/10.12688/

f1000research.25247.4