Turkish Journal of Electrical Engineering & Computer Sciences

http://journals.tubitak.gov.tr/elektrik/

Research Article

Turk J Elec Eng & Comp Sci
(2019) 27: 37 – 45

doi:10.3906/elk-1803-197

Investigating the occurrence mechanism of cytokine-like formations by the electromagnetic approach

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Received: 30.03.2018 • **Accepted/Published Online:** 01.11.2018 • **Final Version:** 22.01.2019

Abstract: This study aims to elucidate the production mechanism of cytokine-like formations secreted from T cells and similar cells by electromagnetic modeling techniques. Three Hertz dipole antennas polarized in arbitrary directions were placed without touching each other at the center of spherical T-cell models to test the Canbay hypothesis about the formation mechanism of cytokines (CHAFMOC) on T-cell surfaces. A dielectrophoretic force field was created within the gelatin layer of the T-cell model. The prepared control and electromagnetically stimulated T-cell model samples were incubated in water in a glass container in a Faraday cage for the specified period and then photographed. At the end of the experiments, cytokine-like formations were observed in the samples, depending on their contents. The results of these experiments, carried out in accordance with the CHAFMOC, show that a dielectrophoretic force is the main cause of the cytokine formation and secretion mechanism in the outer layers of rough T-cell models. Given these results, new approaches and developments may be expected to better our understanding of the immune system in the subjects of cell science, pharmacology, and bioengineering.

Key words: Cytokine, T cell, dielectrophoretic force, Hertz dipole, immune system

1. Introduction

The mechanism of cytokine secretion is a widely studied process, although little is known regarding the specific factors that regulate cytokine formation and release. Protecting a living system from external influences plays a very important role in the process of formation of complex multicellular living things. The immune system is a natural result of this process. Some cells have begun to interact in different ways because of natural and artificial electromagnetic field effects of foreign cells or structures. Chemical effects can be considered as a subset of these electromagnetic effects. The destruction of harmful and unwanted structures in their bodies is greatly advantageous to living systems. Therefore, T cells and cytokines can be affected easily by electromagnetic fields of both natural and artificial (technological) origin. Living systems also produce their own electromagnetic fields [1]. Cytokines, which have a low molecular weight [2], can be seen as a crucial factor in the immunological system. Their formation and secretion mechanisms are formed with respect to substances taken into the biological structure. This work evidences the contribution of the dielectrophoretic field inside and outside T cells to cytokine formation. T cells, the special immune system cells that fight pathogens and are produced from liver or bone marrow stem cells that mature in the thymus, can secrete cytokines, which play a very important role in the functioning of immune system cells. They can be secreted by a variety of cells and have variable functionality. In the literature, detailed laboratory studies related to cytokine production and

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immune system relation are reported [3–5]. Each study produced new findings about both cytokine functions and origins. In a laboratory study, proinflammatory mediators (TNF-α, IFN-g, IL-12, IL-1) and LPS were obtained when noradrenaline was released nonsynaptically from sympathetic axon terminals, and convincing evidence of increased concentrations near the immunocytes (IL-10) showed that sympathetic innervation under stressful conditions exerted fine tuning control of the production of TNF-α and other cytokines [6]. Knowledge of the mechanisms of action of cytokines on cell division sheds light on methods that could be developed for the treatment of cancer, known as uncontrolled cell division, involving the use of cytokines in cancer immunotherapy [7].

The structure of DNA and RNA in microscopic dimensions consists of different sequences of the basic molecular structures adenine (A), thymine (T), guanine (G), and cytosine (C). These structures are the basic elements of an antenna array formed on a double helix. According to the Canbay hypothesis, electromagnetic interaction between these elements in the double helix is an essential factor in the formation of the final molecular shape, cell shape, and cytokines. A total polarization vector may be found for each element. In such helical arrays, it is mathematically and physically simple to transform the helical geometry into a cylindrical or spherical geometry. The physical equivalent of this property is the production of the molecular structure with different geometries sourced from differences in the arrangement of basic elements, i.e. their dielectrophoretic force. The change in parameters of the resultant antenna array of these vectors is the main cause of the geometric image of the molecular structure. It should not be forgotten that the effect of the dielectrophoretic force is very important in the formation of cytokines on and around the T cells. In a study on the etiology of multiple sclerosis (MS) by Canbay [8], motions and interactions of T and B cells with other cells in a vein were expressed as simultaneously developing events. These behaviors do not originate from previously learned or memorized information. Kinematic quantities of a particle in blood depend on hydrodynamic principles and electromagnetic, especially dielectrophoretic, force conditions. In fact, Canbay defined cytokines on T and similar cells as molecular formations on the outer layer of these cells produced by dielectrophoretic fields. These formations could originate from the dielectrophoretic field of diverse complex arrays with gene antennas of the bases A, T, G, and C inside of a cell. Moreover, the secretion of cytokines from T cells must depend on the surrounding dielectrophoretic conditions and the mass, content, and shapes of cytokines. Two previous studies [9, 10] found a correlation between cytokine release and electrical stimulation, while the physical mechanism of this correlation was not stated.

This study is a product of laboratory results based on the Canbay hypothesis. It is concerned with the different contents such as expired antibiotics and some fruit juices of T cells and similar cell models. In the future, it is expected that much more useful results will be obtained with better equipped facilities. If the comprehensive Canbay hypothesis on the etiology of MS is considered, it will be possible to evaluate this study objectively [8, 11–14]. The results of these experiments, performed in accordance with the Canbay hypothesis, have shown that a dielectrophoretic force is the main cause of the mechanism of cytokine formation on the outer layers and secretion of cytokines from T cells.

1.1. Dielectrophoretic force

Dielectrophoresis is a phenomenon that occurs when suspended neutral particles in medium migrate to a place with graded electric field. The Clausius–Mossoti factor $K(\omega)$ depends on the complex permeability of both the particle and the environment, and it is a measure of the effective polarizability of the particle. When Re $[K(\omega)] > 0$, the particles are attracted towards the stronger electric fields. This is called positive dielectrophoresis

(p-DEP). When Re [K(ω)]$<$ 0, the particles are moved towards the lower electric fields. This case is called negative dielectrophoresis (n-DEP). For spherical particles, the time-averaged DEP in Eq. (1) and Clausius–Mossoti factor, K(ω), in Eq. (2) are as follow [11]:

$$F_{DEP} = 2\,\pi\, r_p^3\, \epsilon_0\, \epsilon_m\, Re[K(\omega)]\, \nabla(E^2), \tag{1}$$

$$K(\omega) = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*}, \tag{2}$$

$$\epsilon_{i(i=p,m)} = \epsilon(\omega)_i - j\frac{\sigma_i}{\epsilon_0\,\omega}, \tag{3}$$

where r_p is the particle radius, ϵ_0 is the permittivity of free space, ϵ_m is the real part of the permittivity of the suspending medium, E is the electric field in the medium, ω is the angular frequency of the applied field ($\omega = 2\pi$f), j=$\sqrt{-1}$, $\epsilon_{i(i=p,m)}$ is the complex permittivity of the particles and surrounding medium, and $\epsilon(\omega)_i$ is the permittivity of the particles and surrounding medium. The indices p and m refer to the particle and the surrounding medium, respectively. The values ϵ and σ are the permittivity and the conductivity of the medium, respectively,

1.2. Materials and methods

In this study, electromagnetic modeling of T and similar cells was performed. Organic animal gelatin, which is a translucent, fragile, colorless material, was used because of the difficulty of modeling individual material in terms of material content. This material is obtained by boiling tendons, ligaments, skin, and bone with water. Gelatin is in powder form under standard conditions (25 °C). Three Hertz dipoles were fixed in a table tennis ball in contradictory positions to form a better dielectrophoretic force field at the center of the T-cell models. The ratios of gel to other materials in the mixture were selected to achieve the most suitable hardness for placing in the spherical mold after many experiments. The components of the mixture were blended to homogeneity. The prepared table tennis ball, which contained the Hertz dipole antennas, was placed in a second spherical mold with a radius of 3 cm, and the gap between the tennis ball and the second mold was filled with the gel–-material mixture. The gel–-material mixture ratios in different experiments are given in Tables 1, 2, and 3. The spherical container was left to dry for 3 h, until the gel mixture solidified. After these steps, models of T cells were constructed and were placed in a water tank. Before each experiment, whether the antennas worked or not and the occurrence of a dielectrophoretic force field were controlled by broadband voltmeter. The anechoic chamber (Faraday cage), which is shown in Figure 1, was designed to prevent incoming electromagnetic fields from the outside; at the same time, this cage was used to provide thermal stability between the internal and external media. By changing the frequency supplied by the function/arbitrary waveform generator (Hewlett Packard 33120A), which is shown in Figure 2, the production of 10 Vp-p was investigated, and the effects of different frequencies on the electromagnetic models were observed. At the end of the experiments, outer layer formations of the models were imaged outside of water to provide higher image quality and they can be seen in Figures 3 and 4. Laboratory experiments were performed at different frequencies, but cytokine-like formations appeared to be more effective at a frequency of 12 MHz. The effects of the additive materials in a cell on cytokine-like formations created by dielectrophoretic force were investigated.

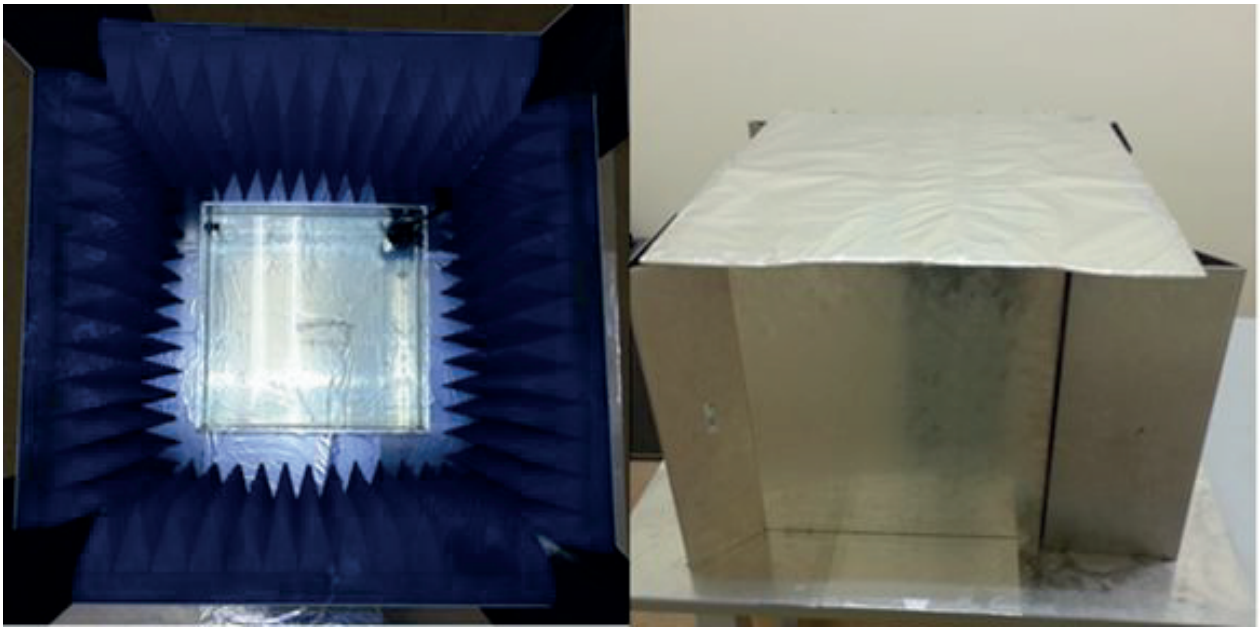

Figure 1. Inside and outside view of the Faraday cage.

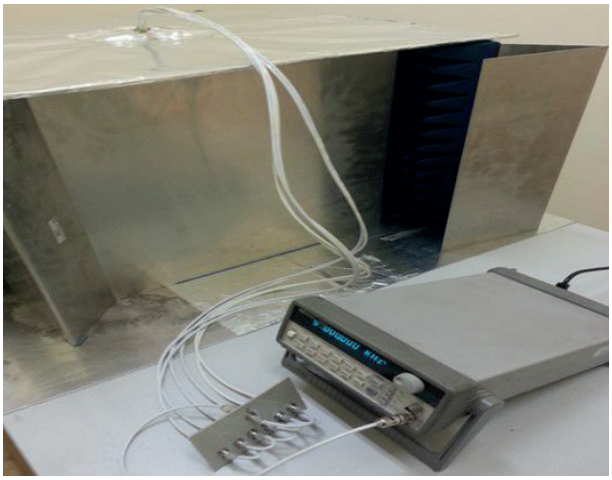

Figure 2. The frequency generator operating at 0.001–15 MHz frequencies and 10 V_{pp} voltage.

2. Results

2.1. Experimental results with pure mixture

The first test series was carried out with antennas in the case of working (active) and passive (control) tests in a water tank in an anechoic chamber under constant temperature and pressure conditions. The experiment conditions and mixture ratios are seen in Table 1. Active and passive T-cell models with identical contents were kept in a water tank in the anechoic chamber for 4 h. Photographs were taken of the samples outside of the anechoic chamber so as to provide high-quality images. As shown in the photographs in Figure 3, no change was observed on the surface of the T-cell model while the antennas were not connected to the function generator. However, at the end of the experiment in which the antennas were connected to the function generator, bubbles were observed on the surface of the T-cell model.

Table 1. Ratios and experimental conditions for the pure mixtures.

Figure no.	Type of added substance	Amount of added substance (L)	Amount of animal gelatin (g)	Type of test medium	Temp. of test medium (°C)	Waiting time in fluid (h)	Mixing time (min)	Operating frequency (Hz)
3a	Water	0.13	23	Water	20	4	3	12 M
3b	Water	0.13	23	Water	20	4	3	—

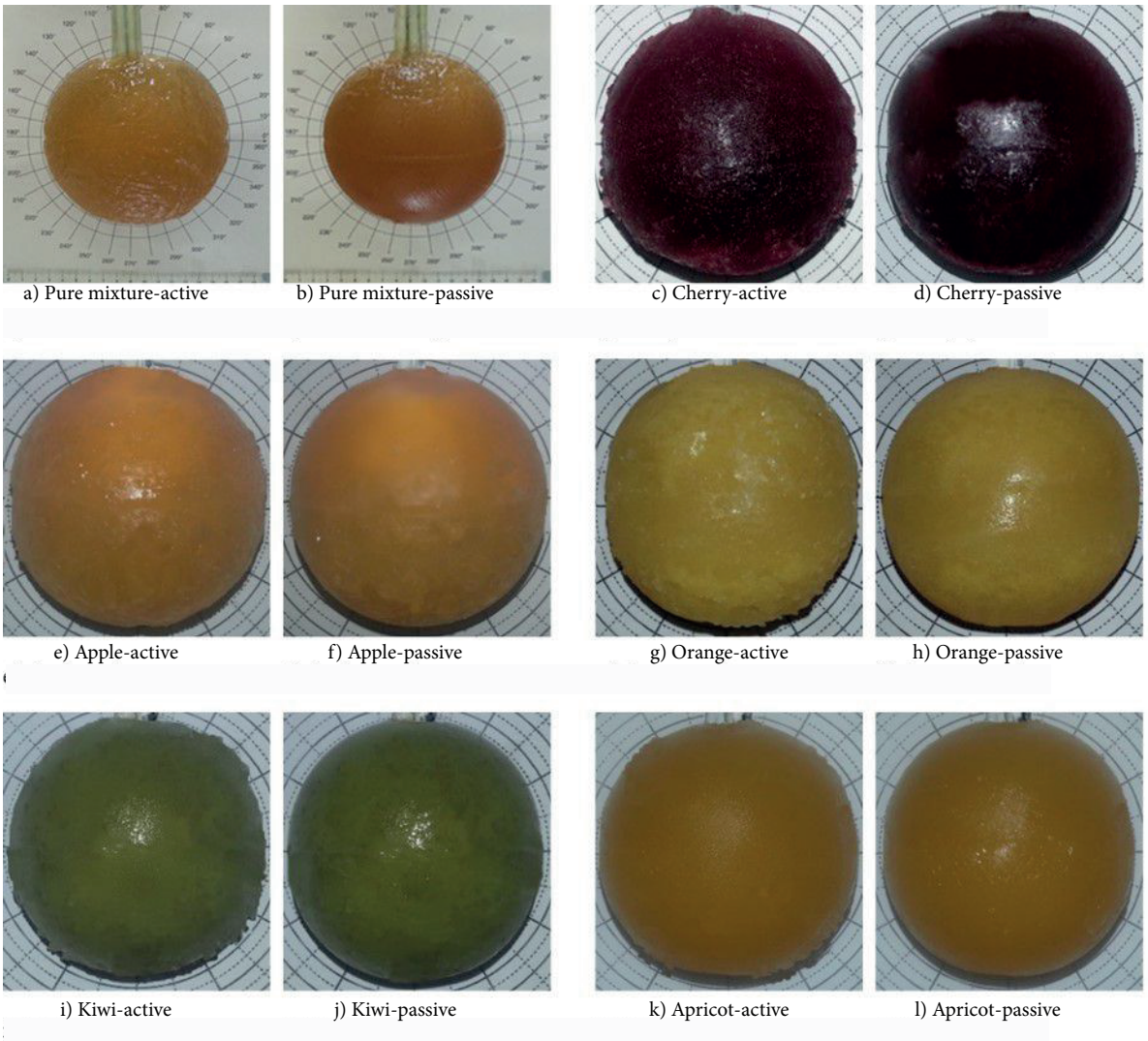

Figure 3. Experimental results for pure mixtures and different juice mixtures.

2.2. Experimental results with fruit juices added to mixtures

In the second series of tests, experiments were carried out by varying the juice–-gelatin mixture ratios, which are seen in Table 2. T-cell models with fruit juices such as cherry, apple, orange, kiwi, and apricot juices were left in the water for 4 h and then their photographs were taken. To observe the effects of dielectrophoretic force

for each experiment, a control test was carried out. In the control tests, while all conditions were kept the same, antennas were disabled. As seen in Figure 3, the cytokine-like formations on the surfaces of the T-cell models in the active state are quite noticeable and distinct from each other.

Table 2. Ratios and experimental conditions for the fruit juices added to mixtures.

Figure no.	Type of added substance (fruit juice)	Amount of added substance (L)	Amount of animal gelatin (g)	Type of test medium	Temp. of test medium (°C)	Waiting time in fluid (h)	Mixing time (min)	Operating frequency (Hz)
3c	Cherry	0.13	23	Water	20	4	3	12 M
3d	Cherry	0.13	23	Water	20	4	3	—
3e	Apple	0.13	23	Water	20	4	3	12 M
3f	Apple	0.13	23	Water	20	4	3	—
3g	Orange	0.13	23	Water	20	4	3	12 M
3h	Orange	0.13	23	Water	20	4	3	—
3i	Kiwi	0.13	23	Water	20	4	3	12 M
3j	Kiwi	0.13	23	Water	20	4	3	—
3k	Water	0.13	23	Water	20	4	3	12 M
3l	Water	0.13	23	Water	20	4	3	—

2.3. Experimental results with expired antibiotics added to mixtures

In the third test situation, the effects of four different expired antibiotics on the outer layers of T-cell models were observed. They were abbreviated as $Antb.1^{exp}_{pas}$, $Antb.1^{exp}_{act}$, $Antb.2^{exp}_{pas}$, $Antb.2^{exp}_{act}$, $Antb.3^{exp}_{pas}$, $Antb.3^{exp}_{act}$, $Antb.4^{exp}_{pas}$, and $Antb.4^{exp}_{act}$, omitting the names of the antibiotics. For example, Antb : antibiotic, $Antb.1^{exp}_{pas}$: first expired antibiotic-passive experiment, $Antb.1^{exp}_{act}$: first expired antibiotic-active experiment. Ratios and experimental conditions for the expired antibiotic mixtures are shown in Table 3. Water (75 g) is used in each experiment for the gel mixture. T-cell models with antibiotics were left in the water for 4 h, and then their photographs were taken. As shown in the photographs in Figure 4, it is observed that cytokine-like formations increased on the surface of the T-cell models in the active state. However, each active and passive T-cell model was the same and expired antibiotic was mixed at the same rate.

As seen from the results for $Antb.1^{exp}_{act}$, $Antb.2^{exp}_{act}$, and $Antb.3^{exp}_{act}$, more cytokine-like formations were observed on the surfaces of active T-cell models. At the end of the experiment realized with an expired antibiotic of 400 mg while the antennas were active, fewer cytokine-like formations on the surface of the T-cell model were observed.

3. Discussion

The concept of dielectrophoretic force has been used to separate cells from cytokines and platelets from blood in medicine and biology [3, 15]. Some studies have also been used to assemble biological particles in a desired location [3, 10, 15]. In some studies, the trapping and accumulation of neuron cells with electromagnetic fields was accomplished [11, 16]. However, no experimental work has provided clear information on the mechanism

Table 3. Ratios and experimental conditions for the expired antibiotics added to mixtures.

Figure no.	Type of added substance (Antb)	Amount of added antb (mg)	Amount of animal gelatin (g)	Type of test medium	Temp. of test medium (°C)	Waiting time in fluid (h)	Mixing time (min)	Operating frequency (Hz)
4a	Antb1	1000	20	Water	20	4	3	12 M
4b	Antb1	1000	20	Water	20	4	3	—
4c	Antb2	4000	20	Water	20	4	3	12 M
4d	Antb2	4000	20	Water	20	4	3	—
4e	Antb3	4000	20	Water	20	4	3	12 M
4f	Antb3	4000	20	Water	20	4	3	—
4g	Antb4	400	20	Water	20	4	3	12 M
4h	Antb4	400	20	Water	20	4	3	—

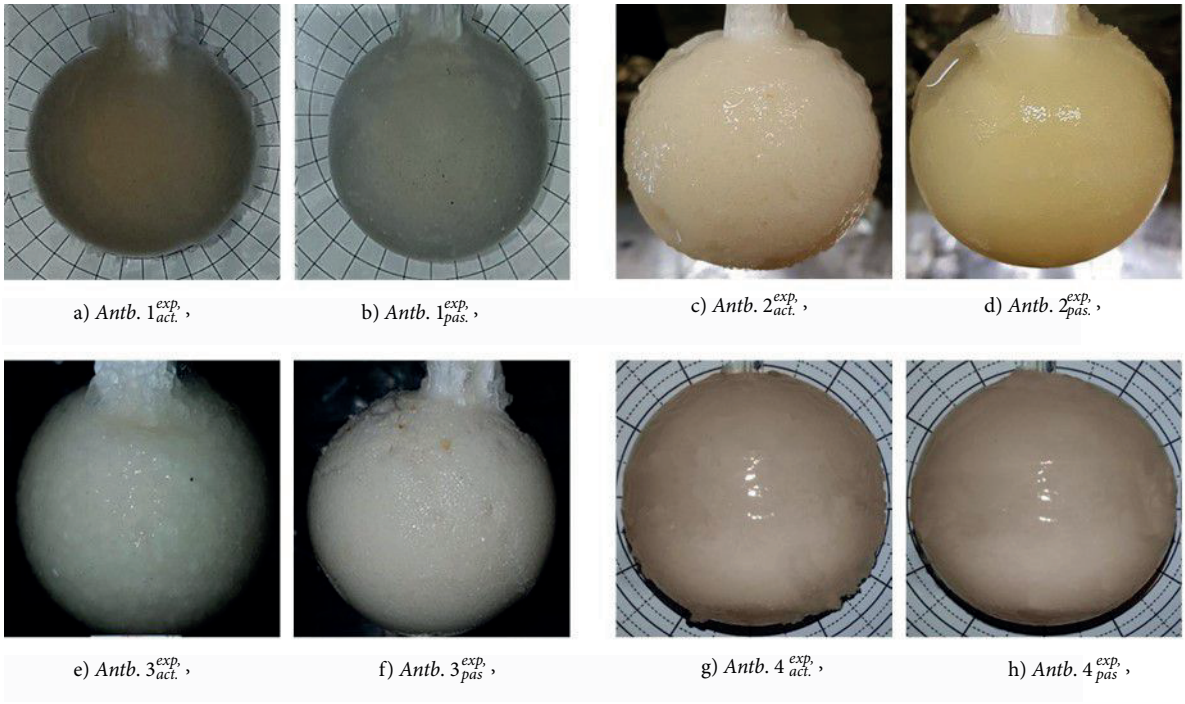

Figure 4. Experimental results for expired antibiotic mixtures.

of formation of cytokines. This study is a candidate to fill this data gap in terms of elucidation of the cytokine formation mechanism on the surface of T cells.

As seen in these experimental results, when the function generator worked, the surfaces of T-cell models were rough, and cytokine-like formations were observed. On the other hand, cytokine-like formations were not observed in the control tests, and the surfaces of T-cell models were very smooth. These results show that a dielectrophoretic force affects molecular formation and distribution. Similar effects to those outlined in the Canbay hypothesis on the etiology of MS [11–14] are reminiscent of the deformation of the myelin sheath and

accumulation of myelin basic proteins in the form of plaques. It is also an experimental demonstration of his hypothesis. The dielectrophoretic force causes the T cells to move in different directions as they travel through the veins.

When the experiments are considered in terms of actual cell sizes and in light of the CHAFMOC, the dielectrophoretic forces created by an antenna array, including elemental antennas such as adenine (A), thymine (T), guanine (G), and cytosine (C) bases and their combinations on the double helix ladder, are mainly responsible for their formations and secretions. Other types of organic materials in the blood give a separate feature to T cells in terms of variation in the formation and secretion of cytokines. Given the results of experiments with pure gel mixtures, the cytokine-like formations on T-cell models in the active state appeared with increasing frequency when compared with passive conditions. In the results of the experiments on the T-cell models under the same dielectrophoretic force effect, excessive amounts of cytokine-like formations were observed when the expired antibiotic-gel mixtures were used. These results clearly imply that the use of expired antibiotics may have objectionable consequences. It is easy to understand why antibiotics of low molecular weight can increase the production of cytokines via their contribution to the dielectrophoretic force and thus contribute to protecting living organisms from harmful particles in the blood. The characteristics of the cytokines produced due to the action of antibiotics are easily separated from T cells and adherence to other undesirable foreign particles in the blood. Obviously, an expired antibiotic can harm all cells in the blood, regardless of whether they are beneficial to the body. At the same time, experimental results with some fruit juices such as kiwi and cherry showed that these fruits produce more cytokine-like formations. Naturally, it is necessary to perform such experiments for all ingredients in order to systematically evaluate T cells in terms of cytokine production.

The concept of exocytosis involves numerous interactions, stages, and components among the ingredients, from the smallest part of a cell to the whole cell. Here, electrical forces are effective in the formation of molecular shapes. The initiation and acceleration of a molecule's movement within a cell depend on the intensity of the electromagnetic forces, such as the dielectrophoretic force. All these events can be explained by exocytosis. They have the same biological and physical conditions. For this reason, the interpretation of the experimental results is not contrary to the concept of exocytosis.

4. Conclusion

These results are crucial to understanding how much organic materials contribute to cytokine production and how biological structures in the immune system communicate with each other. The CHAFMOC and the understanding of these experimental results realized by the electromagnetic approach may open new doors in medicine, pharmacology, and bioengineering.

Acknowledgment

The authors would like to thank Oğuz Türker Akpınar, Can Henrik Schmidt, and Oğuzhan Karaoğlu for their contributions in the laboratory experiments.

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