

Effect of static magnetic fields on the peripheral blood mononuclear-like cells

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In this article the role of static magnetic fields (SMF) in the generation of Ca^{2+} currents in peripheral blood mononuclear-like cells (PBMLC) is described. Using the sensitivity of Ca^{2+} channels and pumps to membrane potential and ion concentration we propose a method which uses the conductivity as a dynamical coefficient in the Onsager's reciprocity relations, and the dynamics of the calcium ions described by the electrodiffusion equation deduced by Pelce. The enhanced influx of calcium ion in PBMLC was studied parameterizing the static magnetic fields effects on the conductivity by the coefficients γ, ρ and κ. The parameterization was made according to the symmetry properties of Onsager's reciprocity relations using the most simple expressions. As an example we used available experimental data over chromaffin cell and employing physical considerations concerning to PBMLC, an order of magnitude for the value of $\rho \approx O(-10^{-5}$ mol/(V m^{2}s), $\kappa = 0$, $\gamma \approx O(-10^{-3}$ mol/(V T^{2}m^{2}s))was obtained. The γ parameter was found graphically.
With this parameterization, the time to induce calcium current in the cell was always less than the situation without magnetic field application.

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1. Introduction

It has been established in the past years that living cells subjected to static magnetic fields (SMF) will speed up or slow down certain of their activities. In fact, in many animal and vegetal cells the asymmetric application of an external stimulus such as unilateral light, electric field or ion gradients creates a transcellular calcium ion current whose polarity and direction has been associated with the direction of the cell growth [1]. It is well known that calcium (Ca^{2+}) is a basic intracellular messenger [3,4], which is involved in many cellular phenomena, and study the effects of SMF on calcium-dependent biological processes is very important. For example, it has been reported that magnetic fields interact with cellular ion channels and pumps, and that static magnetic fields effects on degranulation of human leukocytes can be prevented by calcium channel blockers [5]. The purpose of this article is to describe a method that uses the Onsager's reciprocity relations [6] and the electrodiffusion equation for calcium ions deduced in Ref. [2], to study the role of static magnetic fields in the generation of Ca^{2+} currents in PBMLC, considering the sensitivity of Ca^{2+}channels and pumps to membrane potential and ion concentration [1].

2. Method

The method is described as follows: We consider the PBMLC as isolated spheres suspended in blood plasma. This allow us to apply the Pelce's model [2], which is a specific model to study non-uniform steady solutions for the potential and for the ionic concentration in isolated spherical cells, where a transmembrane ionic current exist. The Onsager's reciprocity relations are used when we suppose that the process of enhancement transmembrane calcium ionic current, due to the application of a static magnetic field, is a linear irreversible thermodynamic process, but close enough to the equilibrium. The equilibrium point is taken when there is not magnetic field applied.

The approach with Onsager's reciprocity relations requires to consider this spherical cell inside a “black box”, where we don't need any knowledge concerning to either biochemical or molecular process, that surely are happening in the membrane cell, but that are actually not completely known. The contact point between dynamics of the calcium ion flux, described by the electrodiffusion equation from Ref. [2] and Onsager's reciprocity relations, is the conductivity, that is a dynamical coefficient that measures the sensitivity of the ion channels and pumps to variations and modulations of the membrane potential, whose changes will provide us with the necessary information to evaluate the effect of the static magnetic field application.

In the following sections we describe each part that is used in the method and we present the results in Table 1, Figure 1, and Figure 2.

3. Electrodiffusion

Most cells in the resting state have a steady membrane potential ($\delta\phi$) of approximately 0.1 V [7] between the inside and the outside of the cell whose thickness is typically 50 Å. The membrane potential is usually of the order of 50 to 150 mV, and is the result of a small excess of negative ions over positive ones on the intracellular surface (Φ_I), and a similar opposite ion relationship is conserved on the extracellular surface (Φ_E). The rest membrane potential is maintained by ion channels (in the steady case at least 10 % of total [3] remain opened, we consider that are uniformly distributed), mainly by keeping differential concentrations of K^+ and Na^+ ions in these two cellular sectors. The movements of these ions across the plasma membrane could be analyzed using the electrodiffusion theory. The membrane potential that exists across the extremely thin plasma membrane, permit the generation of an enormous electric gradient of 10^7 Vm^{-1} across the cell membrane. The sensitivity of the ion channels and pumps to variations and modulations of membrane potential is associated with the physical dynamical coefficient called conductivity, which is defined by

$$\sigma = \frac{\partial J}{\partial(\delta\phi)} \qquad (1)$$

where $\delta\phi = \Phi_I - \Phi_E$, and J is the ions flux. Furthermore, since many other mechanisms that participate in the biological processes are not totally known, it is useful to use thermodynamics [8], which treats this systems as “black-boxes” and make no specific demands for a knowledge of either biochemical or molecular details. These approach may reveal missing factors or show interrelations that could not be suspected otherwise.

Therefore, we use the conductivity as a dynamical coefficient in the Onsager's reciprocity relations to connect with the dynamics of calcium ions described by electrodiffusion equation from Ref. [2], to analyze the effect of static magnetic fields in the development of calcium current in PBMLC. The electrodiffusion equation of Pelce come from the determination of nonuniform steady solutions for the potential and ionic concentration in a cell of spherical geometry, and PBMLC can be considered as isolated spheres suspended in blood plasma. Pelce, solve the equations for potential and ionic concentration, in and outside the cell, including boundary conditions at the cell membrane, which is considered as a circle of radius R_{Cell}, determined by the transmembrane ionic currents. He assumes that Ca^{2+} and K^+ ions are relevant for the process, diffusing them in the cytoplasmic and outer media and move under the action of the electric field $\mathbf{E} = -\nabla\Phi$ generated by the ionic disturbances. Furthermore, due to the relatively high permeability of the potassium ion ($P_K \approx 10^{-7}$ cm/s), the potassium active transport is neglected and is used the important property of the calcium that its influx increases when the membrane depolarize. Once the electroneutrality and boundary conditions are applied, and assuming that the term associated to the calcium current is much larger than the term associated to the potassium ion, he finally obtain for the critical wave number equation of the disturbance in the general case:

$$\frac{n}{R_c} = k_c = -2\frac{kT}{e}\frac{\partial J_{Ca^{2+}}}{\partial(\delta\phi)}\left(\frac{1}{D_{Ca_E^{2+}}(c_{10E}+4c_{20E})} + \frac{1}{D_{Ca_I^{2+}}(c_{10I}+4c_{20I})}\right) \qquad (2)$$

where $n/R_c = k_c$ is called the wave number and is defined by an integer n ≥1.If we consider room temperature the factor kT/e will be 25 meV, k is the Boltzmann constant and e is the electron charge. E and I are referring to “extra” and

Table 1. Several values of conductivity for the range of magnetic field 0.1 mT<H<100 mT for the choice $\gamma > \rho(O(-10^{-5}$ mol/(VT^{2}m^{2}s)).

H (mT)	$\sigma(\gamma \approx 10^{-(x=4)}) \cdot 10^{-5}$ (mol/(V m^{2}s))	$\sigma(\gamma \approx 10^{-(x=3)}) \cdot 10^{-5}$ (mol/(V m^{2}s))	$\sigma(\gamma \approx 10^{-(x=2)}) \cdot 10^{-5}$ (mol/(V m^{2}s))
0.1	-1.0	-1.0	-1.0
1	-1.0	-1.0	-1.001
10	-1.001	-1.01	-1.1
20	-1.004	-1.04	-1.4
30	-1.009	-1.09	-1.9
40	-1.016	-1.16	-2.6
50	-1.025	-1.25	-3.5
60	-1.036	-1.36	-4.6
70	-1.049	-1.49	-5.9
80	-1.064	-1.64	-7.4
90	-1.081	-1.81	-9.1
100	-1.10	-2.0	-11.0

"intra" cellular medium, c is the ionic concentration, $D_{Ca^{2+}}$ is the diffusion coefficient for calcium ion, c_{10} is the steady value for potassium (K^+) ion and c_{20} the corresponding steady value for calcium (Ca^{2+}) ion. The equation has been estimated experimentally by using the calcium channel values of the chromaffin cell [9]. According to Pelce's equation the development of ionic calcium currents depends on temperature and others factors, i.e., for a given cellular radius and n=1, room temperature should be high enough to solve Eq. (3). This prediction agrees with the fact that cellular responses, which depend on an increase of intracellular Ca^{2+}, is enhanced by temperature [10, 11].

4. Irreversible thermodynamics

The physical coefficient of conductivity in Eq. (1), has been used as a kinetic coefficient in linear irreversible thermodynamics based on the Onsager's reciprocity relations [6] to study the effect of magnetic fields in the conductivity of electrolyte solutions [12, 13, 14]. Through this coefficient we can consider that all the fluxes are related with all the acting forces. The central assumption of this approach is divide the entropy change in two parts, one due to the internal processes of the cell and the other, to the external processes. The entropy changes, due to the internal processes, is written as

$$\frac{d_I S}{dt} = -\frac{1}{T}\frac{dG}{dt}$$

It can be shown that $T\frac{d_I S}{dt} = \sum_i J_i X_i$ where J is the flux and X is the force. The relations between the fluxes and the forces acting to produce them are

$$J_i = \sum_{j=1}^{N} G_{ij} X_j \qquad (3)$$

That is,

$$J_1 = G_{11}X_1 + G_{12}X_2 + \ldots.$$
$$J_2 = G_{21}X_1 + G_{22}X_2 + \ldots.$$
$$. \qquad \cdots\cdots \qquad + \ldots.$$
$$J_n = G_{n1}X_1 + G_{n2}X_2 + \ldots.$$

where the coefficients G_{ij} relate the fluxes and forces. Thus a particular flux can be considered as due to an appropiate force, called the conjugate force, plus contributions from all other forces acting in the system.In our case we use two fluxes, J_1 and J_2 (the first one for the calcium ions and second one for the electrical flux), and X as the thermodinamic potential. From the Onsager's reciprocity relation [8] with a magnetic field (**H**) as external stimulus

$$G_{ij}(\mathbf{H}) = G_{ji}(-\mathbf{H}) \qquad (4)$$

We obtain the coefficient for the conductivity as follows

$$\sigma = \frac{\partial J_{Ca^{2+}}}{\partial(\delta\phi)} = \frac{\partial J_1}{\partial X_2} = G_{12}$$

under the symmetry property for G_{ij}, we can write, without loss of generality,

$$G_{ij} = S_{ij} + A_{ij}$$

where S_{ij} is a symmetric matrix and A_{ij} is an antisymmetric matrix. In the presence of magnetic field we can find the following relations

$$S_{ij}(\mathbf{H}) = S_{ji}(\mathbf{H}) = S_{ij}(-\mathbf{H})$$

which means that S_{ij} is an even function of **H**. For the term

$$A_{ij}(\mathbf{H}) = -A_{ji}(\mathbf{H}) = -A_{ij}(-\mathbf{H})$$

implying that A_{ij} is an odd function of **H**. In particular $\sigma = G_{12} = S_{12} + A_{12}$. In fact we can choose, due to symmetry properties, the most simple functional way for those terms (σ is in the direction of the magnetic field applied)

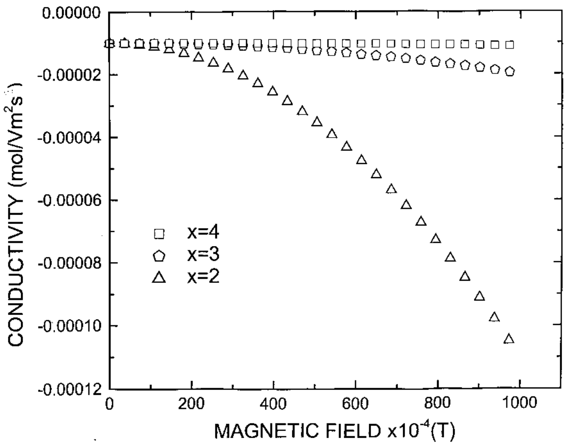

Figure 1. Contribution to conductivity for values of *x*=2,3,4 according Eq. (9). The variable in the horizontal axis should be understood as the given number multiplied by 10^{-4}, this simplification is for the sake of clarity in the figure.

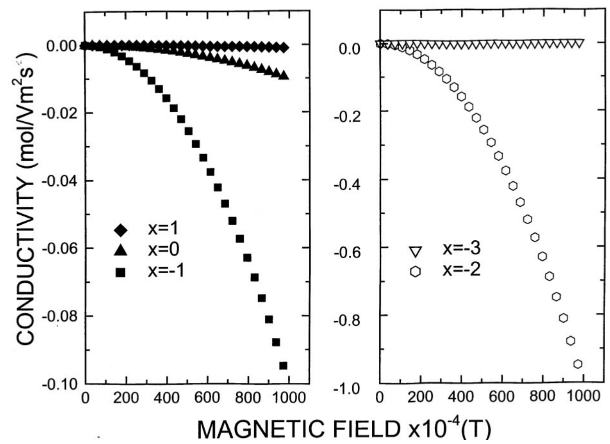

Figure 2. Contribution to conductivity for values of *x*=0,1,-1,-2,-3 according Eq. (9). With this values of *x* we are able to obtain high values of conductivity which are no realistic.

$$S_{12}(H) = \rho + \gamma H^2$$
$$A_{12}(H) = \kappa H \qquad (5)$$

We estimate ρ, γ and κ using the following information: experiments without magnetic fields [1] give $\sigma = G_{12} < 0$ equal to coefficient ρ, the independent magnetic field term, leaving to resolve γ and κ. It is important to measure these values, because if σ is close to solution of Eq. (3), the presence of a constant magnetic field, could increase σ negative value favoring those cells which are in the process of starting the current of ions. Experimental results suggest that γ has a negative value, since static magnetic field intensity of 0.1 T applied to a suspension of human polymorphonuclear leucocytes for 30 minutes is enough to facilitate a transmembrane current of calcium ions [5] and this is possible if σ value is more negative. The contribution to entropy generation from κ is zero because this term is part of the antisymmetric term for σ. The term κH is not important, because in a cell preparation of this kind (PBMLC) there is no preferential direction for the perception of the magnetic field. In steady conditions the promotion of spontaneous calcium currents is carried out through the 10% of the ionic channels, which we consider that are uniformly distributed. Once the magnetic fields are switch on, the corresponding average value to the promotion of spontaneous calcium currents from the antisymmetric part of the conductivity will be null due to this distribution of ionic channels of calcium.

Assuming $\kappa = 0$, we are going to consider a cellular radius $R_{Cell} = R_c + r$ for $\sigma(0) = \rho$ and $R_{Cell} = R_c$ for $\sigma(H) = \rho + \gamma H^2$, to solve Eq. (3). It is possible to estimate the time needed to generate inward calcium current [2] without (τ_1) and with (τ_2) applied magnetic field; r is a small added quantity to R_c used to estimate the intensity of static magnetic field effect

$$\tau_1 = \frac{(R_c + r)^2}{D_{Ca_i^{2+}}}$$
$$\tau_2 = \frac{R_c^2}{D_{Ca_i^{2+}}} \qquad (6)$$

so then,

$$\frac{\tau_1}{\tau_2} = \left(1 + \frac{r}{R_c}\right)^2 = \left(1 + \frac{\gamma}{\rho} H^2\right)^2$$

where we have used Eq. (3) an the corresponding parameterized values for the conductivity without ($\sigma(0) = \rho$) and with ($\sigma(H) = \rho + \gamma H^2$) magnetic field, from whose ratio we can obtain the useful relation

$$\frac{\gamma}{\rho} H^2 = \frac{\sigma(H) - \sigma(0)}{\sigma(0)} \qquad (7)$$

The time needed to induce calcium current when magnetic field is applied will be

$$\tau_2 = \frac{\tau_1}{\left(1 + \frac{\gamma}{\rho} H^2\right)^2} \qquad (8)$$

In the following section we are going to search the conditions over the coefficients γ and ρ to imply that $\tau_2 < \tau_1$.

5. Static magnetic fields

To validate our proposed method, we are going to use the same example from Pelce [2] giving an estimation of the magnitude of the coefficients ρ and γ, using data from chromaffin cell. We start from Eq. (7), in which we can observe that giving an order of magnitude to ρ and γ, we can analyze directly what happen with σ versus H when ρ

and γ varies. To evaluate an order of magnitude of the ρ and γ coefficients, consider from [2] the following information $D_{Ca^{2+}_E} \approx 10^{-6}$ cm^2/s, $D_{Ca^{2+}_I} \approx 10^{-8}$ cm^2/s, $c_{10E} \approx c_{20E} \approx 10^{-3}$ mol C/l, $c_{10I} \approx 10^{-2}$ mol C/l, $c_{20I} \approx 10^{-6}$ mol C/l.

The term $\frac{kT}{e}\frac{\partial J_{Ca^{2+}}}{\partial(\delta\phi)}$, is difficult to estimate because of a lack of data on calcium channels, however to give an order of magnitude of this quantity, it is common practice to consider the I-V curve of the calcium channel of the bovine chromaffin cell [9]. Parameterizing the current density J, by the relation $J = \frac{1}{2F}\frac{I}{d^2}$, F is the Faraday's constant, and $d \approx 10$ μm is the size of the chromaffin cell, it is found that $\frac{kT}{e}\frac{\partial J_{Ca^{2+}}}{\partial(\delta\phi)} \approx 10^{-6}$ mol/(m^2 s) [9]. With this information we estimate the value for ρ ≈ -4 · 10^{-5} mol/(Vm^{2}s). The γ coefficient is estimated graphically by searching for the suitable conditions that satisfy those experimental expectatives, i.e., that σ be more negative to solve Eq. (3) when we apply an external static magnetic field and that coefficients ρ and γ will be negatives. For carry through it, we parameterize the magnitude order of γ by the exponent x, then if we choose numerically γ = ρ in Eq. (7) (the dimension of the ratio γ/ρ will be T^{-2}, so then the quantity in left hand side is without dimensions), then substituting σ (0) = ρ, numerically σ (H) = ρ(1 + H^2). Plotting the graph for the last relation in the range for the magnetic field 0.1 mT<H<100 mT, the choice gives no changes in the value for the conductivity when we apply a magnetic field. Essentially the value without magnetic field dominates, then this choice is not good. If we choose γ >ρ, starting from the value of ρ ≈ -10^{-5} mol/(Vm^{2}s), and assuming that γ ≈ -10^{-x} mol/(VT^{2}m^{2}s), to seek for that value of exponent x implies to satisfy the above conditions and the requirements for the application of the Onsager's reciprocity relations Eq. (4).

For carry out it, we plot the corresponding function for the conductivity σ (H) with magnetic field according the following parameterized equation:

$$\sigma(H) = -10^{-x}(H^2 + 10^{x-5}) \tag{9}$$

where H is the magnetic field expressed in T. The results are shown in Fig. 1 and Fig. 2.

In first instance we have two situations, comparing with respect to the value of conductivity without magnetic field ρ, which we are considering as the mean value for this dynamical coefficient: For the case in which we obtain small values of conductivity compared with ρ and the case in which values of conductivity excessively high exist. Such situations appear because we have no idea how big should be γ compared with ρ. However, once analyzed the restrictions over the applicability of the Onsager's reciprocity relations, i.e., that the fluctuations of the variables that describe the system should be small enough compared with the mean values of the variables [8]; the values of Fig. 2 do not appear to be realistic, therefore the result comes from Fig. 1, where the fluctuations over σ are small close to the equilibrium situation, *i.e*., the one without magnetic field.

In figure 1 we have three possible values for x (see Table 1). We start with the value x=4 and H=10 mT to illustrate, and decrease the x value in steps of one (x=4,3,2,1,0,-1,-2,-3). When x=4, through all range of the magnetic field the variations over σ are very small and therefore suitable to apply Eq. (4), but we can obtain only a 10 % (see Table 1) of increase in the value of conductivity. In x=3, those variations over σ are also small enough. We can obtain increases in conductivity from 1 % with H=10 mT, reaching twice the value of ρ with H=100 mT (see Table 1). For x=2, we can have increases in conductivity for H=10 mT of 10 %, but the fluctuation over the value of σ is abrupt reaching values 11 times ρ for H=100 mT (see Table 1), and Eq. (4) can not be applied. With x=1 we are able to duplicate the negative value of conductivity without magnetic field (ρ whose value was taken as O (-10^{-5})) however, the changes on variable σ are large, avoiding the application of Eq. (4). In general for those values x<3, the contribution to conductivity do not satisfy the conditions of applicability of Onsager's reciprocity relations giving non physical values of conductivity, *i.e*. not realistic. With this option we can say that the optimum value for γ which satisfied all imposed constraints is x=3, although x=4 is also able to be applied for smallest variations in the conductivity. Another possibility is γ/ρ<1. For this choice in Eq. (9) it correspond to the condition x>5 (because 5 is the order of magnitude of ρ), which obviously is useless because for all value of x under this condition, the contribution to conductivity due the magnetic field term (H) will be decreasing each time that we increase the value of x, finishing in the constant value of ρ. In this way and based in this results concerning the coefficients that parameterize the magnetic field effect through conductivity, we return to Eq. (6) and evaluate τ_2.

It is important to estimate the time scale on which static magnetic fields can facilitate the cellular entrance of calcium ions, because in principle, it allows to calculate approximately the exposure time needed to perform successfully such experiments. It could even permit to understand controversial results concerning this kind of experiments when similar times and fields intensities are applied to cells with different cellular radius [5, 15].

Without magnetic field the magnitude of the time scale on which the disturbances develop calcium currents is according to Pelce [2] in which the slowest diffusion time scale is involved, originate from the unsteady diffusion equation for the ions. Using Eq. (6) with $D = D_{Ca^{2+}_I} \approx 10^{-8}$ cm^2/s, for a cell of radius 100 μm the corresponding time without magnetic field is $\tau_1 \approx 10^4$ s. On the other hand, according to Eq. (6), the time, in the presence of a magnetic field, using the same example for H = 10 mT, gives $\tau_2 \approx 9.9$

$\cdot\ 10^3$ s, *i.e.* $\tau_2 < \tau_1$. Other values for τ_2 can be obtained if we use another combination from Table 1 for σ and *H* values. As a final comment we can say that the requirement $\tau_2 < \tau_1$ always will be fulfilled because from Eq. (6), the coefficients ρ and γ, are negatives. For another cell, it will be only necessary taking into account for all the I-V curves of the relevant pumps and channels to evaluate the derivates of the different currents with respect to membrane potential.

6. Conclusions

We have described a simple method in which it is not necessary to include any knowledge about either biochemical or molecular process through the membrane cell, to study the role of the static magnetic fields in the development of calcium ion current for the case of PBMLC. We have used the Onsager's reciprocity relations when we supposed that the enhancement of the calcium ion current due the application of static magnetic fields was a linear irreversible thermodynamic process. The quantitative relationship of temperature, cellular radius, ion concentration and membrane potential expressed by Eq.(3), was deduced in ref [2], and used to describe the dynamical behavior of an isolated spherical cell. The contact point between these two dynamical descriptions was the conductivity. Through their changes we obtained the necessary information to describe the static magnetic field effects over the PBMLC.

In section 4, the static magnetic fields effects through the conductivity was parameterized by three parameters γ, ρ and κ, and an expression to calculate the necessary time to induce calcium current with static magnetic fields was found.

In section 5, using experimental data over the chromaffin cell like an example, we found $\rho \approx O(-10^{-5}\ \mathrm{mol/(V\ m^2s)})$, $\kappa = 0$. In fact, the average value to the promotion of spontaneous calcium currents from the antisymmetric part of the conductivity, was null due to the uniform distribution of ionic channels of calcium. We found graphically the order of magnitude of the value for $\gamma \approx O(-10^{-3}\mathrm{mol/(VT^2m^2s)})$ in this particular case.

This value was found analyzing the changes in conductivity (see Table 1) in the range of static magnetic fields 0.1 mT≤ H≤100 mT, parameterizing the γ coefficient in the form $\gamma \approx -10^{-x}$ and the conductivity as Eq. (9). Applying experimental conditions over the conductivity and the requirements to be valid the Onsager's reciprocity relations, we found using H=10 mT, the values for *x* satisfying the above conditions: *x*=3, and *x*=4. The first case is suitable for small variations in conductivity σ ≈ 10% ρ (ρ is the conductivity without magnetic field). The second one is more suitable for our case because the changes in conductivity are not abrupt and permit us to study a more wide variety of possibilities, we can even reach conductivity values of σ ≈ 2ρ.

A similar study with variant magnetic fields is in process, where the most important effects over living systems have been performed using extremely low frequency electromagnetic fields. As a final comment we can say that we found that the static magnetic fields create conditions to solve Eq. (3) giving origin to cellular Ca^{2+} currents to explain some of the observed effects in literature. The present method is proposed as an alternative to understand the interactions of SMF with living systems [16].

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