Chapter 11
The Bioelectric Circuitry of the Cell

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11.1 Introduction

The study of electrical field effects on cells dates back to 1892 when Wilhelm Roux observed pronounced stratification of the cytoplasm of animal eggs when exposed to electric fields. Over the many decades since, a number of electric field effects have been implicated in the functioning of living cells, in particular in the cytoskeletal or cytoplasmic self-organization processes. For example, electrotherapies and wound healing have been hypothesized to involve ionic current flows. At the cell level, cytochrome oxidase enzyme has been linked to electric current action [1] and cell division coherent polarization waves have been proposed as playing a major role in chromosome alignment and subsequent segregation [2]. In addition, endogenous electric currents have been detected in animal cells. In the phase between fertilization and the first cleavage, a steady current enters the animal pole and leaves the vegetal pole. In the silkmoth oocyte-nurse complex, the oocyte cytoplasm is slightly more positive (by 10 mV) than the nurse cell cytoplasm, which allows for the passing of a small electric current on the order of 5×10^{-8} A [3]. A steady current enters the prospective cleavage furrow in both frog and sea urchin eggs during the initial period prior to cleavage formation, but after initiation, this current reverses its direction and leaves the furrow region [3].

Various plant and animal cells have been observed [3] to undergo significant changes when subjected to steady-state weak electric fields, including changes in their regeneration growth rates. A substantial reduction in the mitotic index was found in pea roots exposed to 60-Hz electric fields at a 430 V/m intensity and after

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4 hours of exposure [4]. The effect of 50-Hz electric fields of a 50 kV/m intensity on the mitotic index of cultured human embryo fibroblastoid cells was also found [5]. More recently, AC electric fields in the frequency range between 100 and 300 kHz and an intensity of only 1–2.5 V/cm have been shown to arrest cancer cells in mitosis [6], which is an astonishing effect in view of the weak intensity of the field. This discovery has led to an FDA approved treatment for the deadly brain cancer form, glioblastoma multiforme (GBM) [7]. It has been speculated that these field effects act on microtubules (MTs) as a primary mechanism of action [8]. However, what aspects of MT behavior in the presence of electric fields are involved is still not clear (depolymerization, rotation, electric conduction, etc.). This latter development provides strong motivation to elucidate the response of MTs in cytoplasm or buffer solution to externally applied AC electric fields. Beyond this, the overriding question still remains: if living cells are sensitive to electric fields and even exhibit electric current effects, then which structures within the cell perform the functions of bioelectric circuit elements?

The idea that the building blocks of living cells, especially proteins, may exhibit electric conduction properties should be credited to Albert Szent-Györgyi who viewed them as semiconducting devices [9, 10]. However, they were considered in their monomeric form, which results in a large energy gap between valence and conduction bands, making electronic conductivity of single proteins very challenging. Moreover, protein conductivity is also largely dependent on their hydration state [11]. What was missing in these early studies of biological conductivity was the role of ionic species, which are abundant in living cells, and an examination of polymeric forms of proteins and DNA, which makes a major difference to both electronic and ionic conduction. Significant experimental challenges of measuring electric fields and currents at a subcellular level persist today and studies of cellular components in isolation provide a proxy for intracellular measurements. Specific interest in the electrical properties of microtubules, actin filaments, DNA, and, of course, ion channels, has produced a number of interesting results that merit close examination, especially in terms of frequency dependence for AC conductivity analysis. Since most living cells are composed of 70% water molecules by weight, the role of water in the transmission of electrical pulses [12] is undoubtedly crucial in these processes. In general, electric charge carriers involved in protein and DNA conduction can be electrons and protons, as well as ions of various types surrounding proteins in the cytoplasm. Actin filaments (AFs) and microtubules have been implicated in numerous forms of electrical processes involving mainly positive counterions due to their net negative charge localized largely on their surfaces [13, 14].

The presence of several types of ionic species (especially K^+ at 140 mM, Na^+ at 10 mM, Cl^- at 10 mM, Mg^{2+} at 0.5 mM, and Ca^{2+} at 0.1 μM typical concentrations) as well as positively charged protons at a typical pH of 7 provides the cell with intrinsic ionic conductivity properties, which can be affected by the transmembrane potential and the action of ion channels. These ions can either diffuse freely in the cytoplasm or be directed to move along the electric field lines that can follow well-

defined polymeric pathways in the cell. While cell membranes support strong electric fields on the order of 10^7 V/m, due to Debye screening, these fields decay exponentially away from the membrane. Dielectric studies of biological cells and their constituent macromolecules in solution have been conducted for almost a century [15, 16] and have revealed a wealth of information about transmembrane potentials, macromolecular charges, their dipole moments, and polarizabilities [17].

For example, Lima et al. [18] recently measured the electric impedance Z'(f) of NaCl and KCl solutions. They observed a large plateau between 10 Hz and 400 kHz, increasing in the low frequency range and decreasing at high frequencies. The value of the plateau decreased with increasing salt concentration, yielding the maximum value of resistance R ~ 10^5 Ω at very low frequencies (~10 mHz) that was independent of salt concentration. The imaginary part of impedance, Z"(f), showed anionic relaxation with a precipitous drop in the 100 kHz range. This is important in the context of ionic solutions present in the living cells and their concentration dependence of conductivity as a function of frequency.

The cytoplasm has a high concentration of proteins with actin (2–8 mg/mL) and tubulin (4 mg/mL) being the most abundant cytoplasmic proteins. Both actin and tubulin exist in either polymerized (actin filaments and microtubules, respectively) and unpolymerized states. It is the polymerized state of these proteins that exhibits interesting conducting properties. These properties are due to the fact that AFs and MTs have a very high density of uncompensated electric charges (on the order of 100,000 per micron of polymer length). In an ionic solution, most of these charges are compensated by counterions, but this leads to a large dielectric moment and nonlinear electro-osmotic response [19–22]. As discussed below, AFs and MTs are nonlinear electric conduction transmission lines. These cytoskeletal protein networks propagate signals in the form of ionic solitons [23–25] and traveling conformation transformations [26–28]. Experiments with polarized bundles of AFs and MTs demonstrated propagation of solitary waves with a constant velocity and without attenuation or distortion in the absence of synaptic transmission [25].

While DNA has been shown to also act as a nanowire [29–31], no transformation of signals was observed in experiments with DNA as opposed to MTs, which showed signal amplification [13]. In terms of using these structures as bioelectric wires, there are not only conductive but also mechanical differences, which can lead to different electromechanical arrangements into micro-scale circuits. In contrast to MTs and AFs, which are the most rigid structures in a cell, DNA is mechanically flexible and undergoes coiling transformations including its packaging into chromosomes [32, 33]. Therefore, DNA circuits can be packed and unpacked depending on the ionic environment while MT circuits can be polymerized and depolymerized using magnesium and calcium signals, for example. MTs can be stabilized by microtubule-associated protein (MAP) interconnections, while AFs have the ability to branch out using ARP2/3 constructs. Consequently, each of these bioelectric elements has different abilities to form complex and dynamic circuits.

11.2 Ion Channel Conduction Effects

Each cell has numerous ion channels embedded in its membrane, with specialized roles in terms of their selectivity and the rate of ion flows. Since ions are charged, these ion flows can be viewed as electric conduction events. A single ion takes approximately 5 ps to traverse an ion channel, whose length is on the order of 5 nm, resulting in an average speed of 1000 m/s. In specific ion channels, such as the bacterial KcsA channel, one K^+ ion crosses the channel per 10–20 ns under physiological conductance conditions of roughly 80–100 pS [34]. This allows for a maximum conduction rate of about 10^8 ions/s. Estimating the distances between the center of the channel pore and the membrane surface to scale as 5×10^{-9} m (5 nm), and assuming the most simple watery-hole and continuum electro-diffusion model of channels, this would provide an average speed of 5×10^{-1} m/s per ion (0.5 m/s). All these numbers for KcsA channels are consistent with our generic estimates except for the speed, which is lowered by the inclusion of the refractory period. In fact, while it is known that the ion flow rate per channel is on the order of 10^5 ions/ms, giving a clock time of approximately 10 ns per ion, one must conclude that a 5 ps active event of traversing a channel is separated by a 2000 times longer refractory interval of 10 ns during which there is no electrical signal propagation taking place. Since the value of a typical transmembrane potential is on the order of 100 mV and a flow of singly charged ions like sodium or potassium leads to an electrical current on the order of 10 pA, the Ohmic resistance of an ion channel can be approximated as 10 GΩ. Note that for a given cell, its ion channels can be viewed as resistors in parallel with each other. Liu et al. [35] reported activation of a Na^+ ion channel's pumping mode with an oscillating electric field of 200 V/m, at a frequency of approximately 1 MHz. Channel types and number per cell (densities) strongly vary among different cellular phenotypes. For example, in mammalian medial entorhinal cortex cells (MECs), an average of $5x10^5$ fast-conductance Na^+ and delayed-rectifier K^+ channels per neuron have been estimated to exist [36]. In unmyelinated squid axons, counts can reach up to 10^8 channels per cell. Therefore, these numbers would proportionately reduce the overall electrical resistance of a cell compared to a single ion channel value. In more detailed studies, it has been demonstrated that ion transitions occur through a sequence of stable multi-ion configurations through the filter region of the channels, which allows rapid and ion-selective conduction [37]. The corresponding kinetic energy together with the electrostatic potential energy equals 2×10^{-20} J, which is very similar to an estimate of the ATP energy, hence justifying an active transport requirement as opposed to a thermally activated process.

Finally, in connection with biological relevance of ion channels and ionic currents flowing through them, Levin [38] has extensively investigated ionic signals in regard to such phenomena as morphogenesis and cancer. Ionic currents in cells associated with injury have been shown to be both necessary and sufficient for regeneration [39]. Patterning structural information during embryogenesis and regenerative repair has been shown to be influenced by bioelectric ionic signals

[40]. Moreover, ionic electrical signals, and endogenous voltage gradients affected by ionic flows, have been associated with key cellular processes such as proliferation, cell cycle progression, apoptosis, migration and orientation, and differentiation and de-differentiation [41]. Therefore, it can safely be stated that ion channels and ionic currents are at the center of cellular activities. The question remains whether there is additional electrical activity downstream from ion channels, namely in the cytoplasm. As discussed in the following sections, the complex and well-organized structure of the cytoskeleton lends itself to such interactions, especially since the filaments of the cytoskeleton are now known to be electrically conductive. We next discuss the particular case of actin filaments followed by microtubules.

11.3 Actin Filament Conductivity

Actin filaments, also referred to as F-actin or microfilaments, are approximately 7 nm in diameter and form a helical structure with a pitch of approximately 37 nm. They are highly electrostatically charged [20, 42]. Within an AF, actin monomers arrange themselves head-to-head to form actin dimers, resulting in an alternating distribution of electric dipole moments along the filament [43]. We assume, therefore, that there is a helical distribution of ions winding around the filament at approximately one Bjerrum length. Experimental studies demonstrated that they conduct ionic currents via the surrounding counterion cloud-like layer [20]. The ionic charge distribution along an AF has been modeled as an electrical circuit with the following elementary components representing the functional role of each actin monomer: (a) a nonlinear (saturable) capacitor associated with the spatial charge distribution between the ions located in the outer and inner regions of the polymer, (b) an inductance due to helical nature of the ionic current flow, and (c) a resistor due to the viscosity of the medium opposing the ionic flows. This representation provided the basis for a physical model of F-actin as a conducting polyelectrolyte, where ion flows are expected to occur at a radial distance from the surface of the filament approximately equal to the Bjerrum length and follow a solenoidal geometry due to the actin's double stranded helical structure. Using Kirchhoff's equations and taking the continuum limit for a long transmission line results in nonlinear inhomogeneous partial differential equations for the propagating nonlinear waves of ions along and around the AF. These ionic waves, in the form of elliptic Jacobi functions and solitary waves of the kink-type, have been described as the solutions of the above nonlinear partial differential equations [23].

The objective of this model was to explain the experimental results of Lader et al. [44], who applied an input voltage pulse with amplitude of approximately 200 mV and duration of 800 ms to an AF, and measured electrical signals at the opposite end of the AF. The obtained results showed that AFs support ionic waves in the form of axial nonlinear currents that maintain their amplitude and hence are not dissipative. These data supported an earlier experiment [20] in which the observed wave patterns in electrically stimulated single AFs were remarkably similar to those found in

the recorded solitary waveforms for electrically stimulated nonlinear transmission lines [45]. In view of the fact that the AFs are highly nonlinear complex biophysical structures acting under the influence of thermal fluctuations and supporting the counterionic cloud hypothesis [46], the observation of soliton-like ionic waves is consistent with the idea of AFs functioning as biological transmission lines. Based on the continuum transmission line model, ionic currents along AFs have been estimated to have a velocity of propagation between 1 and 100 m/s [23]. This model has been later updated to include more realistic estimates of model parameters [47, 48]. Interestingly, but not surprisingly, actin filaments can be manipulated by external electric fields [49], which opens the door to electric field manipulations of actin cytoskeleton geometry, resulting in a dynamically flexible electric circuitry within the cell. For a filament with n monomers, the following numbers have been obtained for the electric circuit parameters of each monomer, labeled i, as a fundamental unit of the circuit: an effective resistance (longitudinal and radial, respectively), capacitance, and inductance, where $R_{1,i} = 6.11 \times 10^6\ \Omega$, and $R_{2,i} = 0.9 \times 10^6\ \Omega$, $C_i = 10^{-4}$ pF, and $L_i = 2$ pH. Hence, for a 1 μm length of an actin filament, we find the following corresponding values characterizing it as a conducting bioelectric wire: $R_{eff} = 1.2 \times 10^9\ \Omega$, $L_{eff} = 340 \times 10^{-12}$ H, $C_{eff} = 0.02 \times 10^{-12}$ F.

We can also easily find for a single actin monomer and an AF what characteristic time scales apply to their electrical circuit properties. For a single monomer, the time scale for LC oscillations is very fast, namely $\tau_0 = (LC)^{1/2} = 6 \times 10^{-14}$ s. The decay time for longitudinal ionic waves is also very fast, $\tau_1 = R_1C = 6 \times 10^{-10}$ s, while the corresponding time for radial waves is $\tau_2 = R_2C = 0.9 \times 10^{-10}$ s. As an example of a typical AF, we consider a 1 μm polymer and find the following characteristic time scales in a similar manner to the calculations above: $\tau_0 = 10^{-11}$ s, which is still very short but $\tau_1 = R_1C = 2.4 \times 10^{-5}$ s for longitudinal electric signal propagation is in the range for interactions with AC electric fields in the 100 kHz range.

If actin filaments support ionic conduction, even lossless transmission of electric signals in the cell, it is also natural to expect unusual behavior of microtubules under electric stimulation. This can be inferred from the known structural and electrostatic properties of MTs, which are highly electrostatically charged, even more so than AFs, larger than AFs and they exhibit a cylindrical geometry with a helical pattern of protofilaments wrapping around the cylinder surface.

11.4 Microtubule Conductivity

MTs are a major part of the cell's cytoskeleton. The building block of a MT is a tubulin dimer that contains approximately 900 amino acid residues comprising some 14,000 atoms with an overall mass of 110 kDa (1 Da = 1.7×10^{-27} kg). Each tubulin dimer in an MT has an approximate length of 8 nm, along the MT cylinder axis, a width of 6.5 nm and a thickness along the radial direction of an MT of 4.6 nm. The outer diameter of an MT is 25 nm, while the inner core of the cylinder,

i.e., its lumen, is approximately 15 nm in diameter. A microtubule is a highly asymmetric electrolyte since each tubulin monomer has a charge of −47 elementary charges ($e = 1.6 \times 10^{-19}$ C) and is surrounded by a cloud of neutralizing cations. Based on the physical properties of tubulin, MTs have been theorized to possess intrinsic electronic conductivity as well as ionic conductivity along their length. Their electronic conductivity is envisaged to occur through the macromolecule itself, with mobile (conduction band) electrons hopping through the periodic structure of acceptor sites along the MT [50]. Due to the large electric charges on tubulin, MTs have a highly electronegatively charged outer surface as well as highly flexible C-terminal tails (TTs) whose net charge amounts to 40% of the tubulin's overall charge. This exposed negative charge distribution is predicted to attract a cloud of counterions from the surrounding cytoplasmic environment of the cell. It has been experimentally demonstrated, and later theoretically elucidated, how ionic waves are amplified along MTs [50, 51]. Many diverse experiments were performed to date in order to measure the various conductivities of MTs, with a range of results largely dependent on the experimental method applied. Curiously, Sahu et al. report that intrinsic conductivities along MTs are not length dependent [52], which would indicate at least some of the resistance of this complex system is non-Ohmic, but this conclusion still requires independent confirmation.

MTs have also been implicated in intracellular signaling, communication and even information processing, which would likely be facilitated by the fact that tubulin has a large dipole moment and a large negative charge. Consequently, MTs could be viewed as complex bioelectronic devices with a potential for carrying signal transmission via several independent channels (C-termini states, ionic waves, electronic transitions, conformational changes, etc.). It has also been hypothesized that MTs are involved in information processing, via ionic conductivity effects in neurons, as well as an organism-wide matrix of connected biological wires [28].

Ionic conductivity experiments largely show that MTs are able to increase their ionic conductivity compared to a buffer solution free of tubulin. Minoura and Muto found the conductivity to be increased 15-fold relative to that of the surrounding solution, although the ionic concentration used, at ~1 mM, is much lower than physiological ionic concentrations of just over 0.1 M [53]. Priel et al. demonstrated microtubules' ability to amplify ionic charge conductivity, with current transmission increasing by 69% along MTs [13]. The buffer was close to that of the intracellular ionic concentration, using 135 mM KCl. Ionic current amplification along MTs is explained by the highly negative surface charge density along the outside of the microtubules that creates a counterionic cloud, which allows for amplification of axially transferred signals [13]. From Priel et al.'s conductance data, we approximate the conductivity of their result to be 367 S/m. Next, we quantitatively assess the effect of AC electric fields on MTs in these ionic conductivity experiments, which are expected to be sensitive to the electric field frequencies in the 100 kHz to 1 MHz range.

Measuring intrinsic conductivity of individual MTs has been a major challenge since this requires conducting measurements in solution, which only records the increased ionic conductivity. Fritzsche et al. [54] made electrical contacts to single

microtubules following dry-etching of a substrate containing gold microelectrodes. Their results indicate intrinsic resistance of a 12 μm-long microtubule to be in the range of 500 MΩ, giving a value of resistivity of approximately 40 MΩ/μm in their dry state. The same group [55] later attempted to measure dry protein conductivity, but their setup is far removed from MTs native environment and so any results from these experiments may not be indicative of the intrinsic conductivity of MTs in their biological environment. The major concern is that most of the conductivity contribution measured may come from the microelectrodes and not the protein polymer. Nonetheless, MTs adsorbed onto a glass substrate yielded an intrinsic conductivity of less than 3 S/m, which is very high. The same group performed measurements on microtubules [55] covered with a 30 nm layer of gold. The resistance of these metallized MTs was estimated to be below 50 Ω, i.e., it unfortunately originated entirely due to the metallic coating.

Another attempt to measure MT conductivity involved putting MTs in an ultrapure water solution and bridging gold electrodes that were making contacts with the MTs present [56]. As the setup used only two probes, and the conductivity was estimated from the difference in conductance of buffer solution, MT + buffer, and pure water, using an estimated 50 MT contacts between electrodes, the calculation in effect theorizes ionic conductivity indirectly. More recently, Sahu et al. [52] performed four-probe measurements of DC and AC conductivities (instead of intrinsic conductivity) in an attempt to resolve the problem of measuring ionic conductivity along the periphery of MTs. The DC intrinsic conductivities of MTs, from a 200 nm gap, were found to range between 10^{-1} and 10^{2} S/m. Surprisingly, they found that MTs at specific AC frequencies (in several frequency ranges) become approximately 1000 times more conductive, exhibiting MT conductivities in the range of 10^{3}–10^{5} S/m [52]. These effects were referred to as causing ballistic conductivity along MTs. They further claimed that it is in fact the water channel inside the MT lumen that is responsible for the high conductivity of the MT at specific AC frequencies [52].

Minoura and Muto [53] estimated the conductivity and dielectric constant of MTs using an electro-orientation method applying AC electric fields with frequencies below 10 kHz. The normally resultant convection effect was avoided by applying electric fields with a frequency between 10 kHz and 5 MHz and a sufficient field strength (above 500 V/cm) to successfully orient MTs in solution. For example, MTs aligned within several seconds in a 90 kV/m field at 1 MHz [53]. Based on these experiments, MT ionic conductivity was estimated to be 150 mS/m, which is approximately 15 times greater than that of the buffer solution.

Another attempt to measure the conductivity of MTs used radio frequency reflectance spectroscopy [57]. These investigators concluded that the conductivity of MTs was similar to that of lead or stainless steel, which would be on the order of 10^{6} S/m. This number is unrealistically high and cannot be verified by other independent studies. Furthermore, the authors [57] reported measurements of RF reflectance spectroscopy of samples containing the buffer solution, free tubulin in buffer, microtubules in buffer, and finally, microtubules with MAPs in buffer. The concen-

tration of tubulin was 5 mg/mL and the concentration of MAP 2 and tau proteins was 0.3 mg/mL. The average DC resistance reported by these authors was: (a) 0.999 kΩ (buffer), (b) 0.424 kΩ (tubulin), (c) 0.883 kΩ (microtubules), and (d) 0.836 kΩ (MTs + MAPs). It is virtually impossible to translate these results into an estimate of the resistivity of microtubules without making assumptions about their geometrical arrangement and connectivity as resistor networks. However, assuming that all tubulin has been polymerized in case (c) and formed a uniform distribution of MTs with a combination of parallel and series networks, one can find the resistance of a 10 μm long MT, forming a basic electrical element in such a circuit, to have approximately an 8 MΩ value. This compares reasonably well to an early theoretical estimate of MT conductivity, which used the Hubbard model with electron hopping between tubulin monomers [58]. This model predicted the resistance of a 1 μm microtubule to be in the range of 200 kΩ, hence a 10 μm microtubule would be expected to have an intrinsic resistance of 2 MΩ, which is the same order of magnitude as the result reported by Goddard and Whittier [57].

Very recently, Santelices et al. [59] reported the results of precise measurements of the small-signal AC conductance of electrolytic solutions containing MTs and tubulin dimers, with a number of different concentrations, using a microelectrode system. They found that MTs at a 212 nM tubulin concentration in a 20-fold diluted BRB80 electrolyte increased the overall solution conductance by 23% at 100 kHz. This effect was shown to be directly proportional to the concentration of MTs in solution. The frequency response of the measured electrolytes containing MTs was found to exhibit a concentration-independent peak in the conductance spectrum with a maximum at around 110 kHz that decreased linearly with MT concentration. Conversely, tubulin dimers at a concentration of 42 nM were seen to decrease the overall solution conductance by 5% at 100 kHz under similar conditions. When interpreted in terms of the numbers of MTs polymerized in the sample, and assuming their action as a parallel resistor network with a lower resistance than the surrounding solution, we can estimate the conductance of individual MTs as 20 S/m compared to 10 mS/m measured for the buffer itself. This indicates that indeed MTs have electric conductivities which are three orders of magnitude higher than those of the solution. Additional measurements were made of the system's capacitance and it translated into a value of $C = 600$ pF per average 10 μm MT, which is very similar to the earlier theoretical estimates presented in this chapter.

Finally, it is interesting to address the issue of the power dissipated due to a current flowing along a microtubule. Taking a 10 μm long MT as an example, we estimate the average power drain as

$$\langle P \rangle = (1/2) V_0^2 \left[R / \left(R^2 + X_c^2 \right) \right]$$

where $X_c = 1/\omega C$ is the capacitive resistance. Substituting the relevant numbers as per the discussion above, we obtain the dissipated power to be in the 10^{-11} W range, which is comparable to the power generated by a cell in metabolic processes. To

elaborate on this conclusion, consider that an average metabolic energy production in the human body is 100 W and there are approximately 3×10^{13} cells in the body. Therefore, the power generation per cell is found to be $P_{cell} = 3 \times 10^{-12}$ W. Neurons are the most energy demanding cells, since the brain consumes 25 W of power, we can estimate the power generation per glial or neuronal cell to be $P_{glia} = 10^{-10}$ W. Consequently, additional heat generated by the processes related to MT conduction caused by externally applied electric field in the range of the peak frequency of 100 kHz may be disruptive to living cells, which could provide a mechanistic explanation of the action of TTFields.

The multiple mechanisms of MT conductance provide ample possibility to explain the varied published reports on MT conductivity. Ionic conductivity along the outer rim of the MT, intrinsic conductivity through the MT itself, and possible proton jump conduction and conductivity through the inner MT lumen has been theorized. The experimental challenge is to simulate in vivo conditions, and the possible significance of structured water, ionic, pH, and temperature conditions, over different time scales and at different frequencies. It is possible that the ionic currents generated by externally applied AC fields in the TTField mechanism may overwhelm the intrinsically generated ionic currents in cells undergoing mitosis where electric current densities, j, were measured to be in the range $0.002 < j < 0.6$ A/m^2 [60]. Since $j = \sigma E$, where $E = 100$ V/m for TTFields, and σ had a large range of values reported between 0.1 and 100 A/m^2, even taking the lower limit of 0.1 would result in ionic currents along MTs that could overwhelm the intrinsic ion flows in a dividing cell. It is entirely possible that these externally stimulated currents cause a major disruption of the process of mitosis.

11.5 Conclusions

An important aspect of the impact of external electric fields on a cell is that their penetration into the cell significantly depends on the cell's shape. Theoretical calculations on the electric field strength in a spherical cell indicate that, assuming the conductivities of the extracellular and intracellular fluids of the cell are the same, due to the small conductivity of the membrane versus these fluids, the electric field strength inside a typical cell is approximately five orders of magnitude lower than that outside the cell [61]. Recently, a COMSOL-based computational model has been developed [62] to better understand the application of TTFields to isolated cells during mitosis. The distribution of the scalar electric potential V for frequencies ranging between 60 Hz and 10 GHz was computed, taking into account the variation in cell shape during mitosis, from perfectly spherical through three stages of cytokinesis. The model demonstrated that the intracellular electric field intensity distribution is nonuniform, peaking at the cleavage plane. It also clearly showed that this effect strongly depends on the applied frequency, with the highest rate of field penetration into the cell occurring for frequencies between 100 and 500 kHz depending on the stage of cytokinesis.

In the presence of either endogenous or externally applied fields (e.g., TTFields), the cytoskeleton and, especially, both actin filaments and microtubules become bioelectric wires conducting ionic currents throughout the cell. It is also possible that proton gradients due to uneven pH distributions within cells, e.g., cancer cells, may also contribute to electrical conduction processes in living cells. This chapter discussed how these processes are critically related to the presence of large net electrostatic charges on tubulin and actin, which are largely but not completely screened by counterions. Both of these proteins are abundant in all eukaryotic cells and form long, rigid polymeric filaments. Actin filaments have been shown to provide conduits for lossless ionic transport, while microtubules have been shown to amplify ionic current flows and be orders of magnitude more conductive than the cytoplasmic medium in which they are bathed. The longer the microtubule, the more pronounced the ionic conduction effect under AC electric field influence. Additionally, it is possible that ionic currents can flow not only along the MT axis but also in the direction perpendicular (i.e., radial with respect to the MT axis) to the MT surfaces (this is also true for actin filaments). With proper initial conditions in place, solenoidal flows of ions and protons can also be induced, leading to the generation of the system's inductance. The resultant complex functional dependence of impedance on frequency is also strongly dependent upon the length of each filament and solution pH.

Moreover, in MTs some of the charges are localized on the highly flexible C-termini, leading to the propensity for oscillating charge configurations. In addition, the presence of large dipole moments on tubulin and MTs can lead to a variety of frequency- and amplitude-dependent responses of these structures to both endogenous and external electric (and electromagnetic) fields. Finally, there can be induced dipole moment contributions to the response of these structures to electric and electromagnetic stimulation, making the problem very complex and simultaneously offering a rich spectrum of possibilities for the cell to utilize in terms of communication within its confines and with other cells. Disentangling the relative importance of the various effects under different conditions is nontrivial and requires careful computational and experimental investigations under controlled conditions.

To summarize, depending on the orientation of the electric fields to the microtubule (or AF) axis, there could in general be three types of ionic waves generated: (a) Longitudinal waves propagating along the protein polymer's surface, the polymer acts like a conduction electrical cable with its inherent resistance R but also capacitance C. (b) Helical waves propagating around and along each protein polymer, for MTs there could be three or five such waves propagating simultaneously corresponding to the 3-start or 5-start geometry of a microtubule. (The effective resistance of such cables would be the individual resistance divided by the number of cables in parallel. Each cable has its own capacitance and inductance.) (c) Radial waves propagating perpendicularly to the protein polymer surface. If an electric field is oriented at an angle to the polymer axis, it is expected that all these wave types may be generated simultaneously.

It is also important to note that elongation of dividing cells facilitates penetration of these fields into cells while spherical cells would largely shield the fields and

prevent them from entering into their interiors. Once AC fields generate oscillating ionic flows, these can in turn not only cause electrical currents for the purpose of signaling or communication but also lead to detrimental effects such as: (a) interference with ion flows in the cleavage area of dividing cells, (b) interference with motor protein motion and MAP-MT interactions, (c) perturbations of ion channel dynamics, and (d) changes in the net charge of the cytoplasm. In addition to the above possible subcellular effects of TTFields, there may also arise measurable heating effects in the cytoplasm of the exposed cells due to Ohmic resistance arising from ionic and protonic flows.

Identification of the strength, cause, and function of intracellular electric fields has only recently been experimentally accessible, although speculations in this area have existed for a long time. These insights may assist in devising and optimizing ways and means of affecting cells, especially cancer cells, by the application of external electric or electromagnetic fields. With the advent of nanoprobe technology, which has shown promise in measuring these fields, it is very timely to explore the various physical properties of the cytoplasmic environment including the cytoskeleton and the ionic contents of the cytoplasm.

The research outlined in this chapter promises to contribute to our general understanding of the electroconductive properties of the cytoplasm in living cells and especially the role of microtubules and actin filaments in creating dynamic and structural order in healthy functioning cells. This dynamic order may also involve electrical signal communication within and between cells. Once we are able to properly map the bioelectric circuitry of cell interiors, it should also be possible to identify biophysical differences between normal and cancer cells, which could also lead to the identification of what causes increased metastatic behavior of some cancer cells. Such an understanding may lead to better therapies and to the discovery of specific targets in order to halt metastatic transformation.

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References

1. Cope, F. W. (1975). *Journal of Biological Physics, 3*, 1.
2. Cooper, M. (1981). *Collective Phenomena, 3*, 273.
3. Jaffe, L. F., & Nuccitelli, R. (1977). *Annual Reviews in Biophysics and Bioengineering, 6*, 446.
4. Robertson, D., Miller, M. W., Cox, C., & Davis, H. T. (1981). *Bioelectromagnetics, 2*, 329.
5. Dyshlovoi, V. D., Panchuk, A. S., & Kachura, V. S. (1981). *Cytology and Genetics, 15*, 9.
6. Kirson, E. D., Gurvich, Z., Schneiderman, R., Dekel, E., Itzhaki, A., Wasserman, Y., Schatzberger, R., & Palti, Y. (2004). *Cancer Research, 64*, 3288.
7. Kirson, E. D., Dbalý, V., Tovarys, F., et al. (2007). *Proceeding of the National Academy of Sciences of USA, 104*, 10152.
8. Davies, A. M., & Weinberg U Palti, Y. (2013). Tumor treating fields: a new frontier in cancer therapy. *Annals of the New York Academy of Sciences, 1291*, 86.
9. Szent-Györgyi, A. (1941). *Nature, 148*, 157.

10. Szent-Györgyi, A. (1957). *Bioenergetics*. New York: Academic Press.
11. Gascoyne, P. R. C., Pethig, R., & Szent-Györgyi, A. (1981). *Proceeding of the National Academy of Sciences of USA, 78*, 261.
12. Zheng, J. M., Chin, W. C., Khijniak, E., Khijniak, E. J., & Pollack, G. H. (2006). *Advances in Colloid Interface Science, 127*, 19.
13. Priel, A., Ramos, A. J., Tuszynski, J. A., & Cantiello, H. F. (2006). *Biophysical Journal, 90*, 4639.
14. Sekulić, D. L., Satarić, B. M., Tuszyński, J. A., & Satarić, M. V. (2011). *European Physical Journal E Soft Matter, 34*, 49.
15. Schwan, H. P. (1958). *Advances in biological and medical physics*. New York: Academic Press.
16. Grant, E. H., Sheppard, R. J., & South, G. P. (1978). *Dielectric be havior of biological molecules in solution*. New York: Oxford University Press.
17. Sanabria, H., & Miller, H., Jr. (2006). *Physical Review E, 74*, 051505.
18. Lima, L. F., Veira, A. L., Mukai, H., Andrade, C. M., & Fernandes, P. R. (2017). *Journal of Molecular Liquids, 241*, 530.
19. Chandra Singh, U., & Kollman, P. A. (1984). *Journal of Computational Chemistry, 5*, 129.
20. Lin, E. C., & Cantiello, H. F. (1993). *Biophysical Journal, 65*, 1371.
21. Angelini, T. E., Golestanian, R., Coridan, R. H., Butler, J. C., Beraud, A., Krisch, M., Sinn, H., Schweizer, K. S., & Wong, G. C. L. (2006). *Proceedings of the National Academy of Sciences of USA, 103*, 7962.
22. Raviv, U., Nguyen, T., Ghafouri, R., Needleman, D. J., Li, Y., Miller, H. P., Wilson, L., Bruinsma, R. F., & Safinya, C. R. (2007). *Biophysical Journal, 92*, 278.
23. Tuszynski, J. A., Portet, S., Dixon, J. M., Luxford, C., & Cantiello, H. F. (2004). *Biophysical Journal, 86*, 1890.
24. Sataric, M. V., Sekulic, D., & Zivanov, M. (2010). *Journal of Computational and Theoretical Nanoscience, 7*, 2281.
25. Poznanski, R. R., Cacha, L. A., Ali, J., Rizvi, Z. H., Yupapin, P., Salleh, S. H., & Bandyopadhyay, A. (2017). *PLoS One, 12*, e0183677.
26. Pokorny, J., Jelinek, F., Trkal, V., Lamprecht, I., & Hölzel, R. (1997). *Journal of Biological Physics, 23*, 171.
27. Pokorny, J. (2004). *Bioelectrochemistry, 63*, 321.
28. Friesen, D. E., Craddock, T. J. A., Kalra, A. P., & Tuszynski, J. A. (2015). *Biosystems, 127*, 14.
29. Warman, J. M., de Haas, M. P., & Rupprecht, A. (1996). DNA: A molecular wire? *Chemical Physics Letters, 249*, 319.
30. Beratan, D. N., Priyadarshy, S., & Risser, S. M. (1997). *Chemistry & Biology, 4*, 3.
31. Berlin, Y. A., Burin, A. L., & Ratner, M. A. (2000). *Super-lattices and Microstructures, 28*, 241.
32. Gittes, F., Mickey, B., Nettleton, J., & Howard, J. (1993). *The Journal of Cell Biology, 120*, 923.
33. Sato, M., Schwarz, W. H., & Pollard, T. D. (1987). *Nature, 325*, 828.
34. Roux, B., & Schulten, K. (2004). *Structure, 12*, 1342.
35. Liu, D. S., Astumian, R. D., & Tsong, T. Y. (1990). *Journal of Biological Chemistry, 265*, 7260.
36. White, J. A., Rubinstein, J. T., & Kay, A. R. (2000). *TINS, 23*, 131.
37. Berneche, S., & Roux, B. (2001). *Nature, 414*, 73.
38. Levin, M., & Stevenson, C. G. (2012). *Annual Reviews in Biomedical Engineering, 14*, 295.
39. Levin, M. (2009). *Semininars in Cell & Developmental Biollogy*. Amsterdam: Elsevier.
40. Levin, M. (2012). *Biosystems, 109*, 243.
41. Adams, D. S., & Levin, M. (2012). *Cell Tissue Research, 352*, 95.
42. Cantiello, H. F., Patenaude, C., & Zaner, K. (1991). *Biophysical Journal, 59*, 1284.
43. Kobayasi, S. H., Asai, H., & Oosawa, F. (1964). *Biochimica et Biophysica Acta, 88*, 528.
44. Lader, A. S., Woodward, H. N., Lin, E. C., & Cantiello, H. F. (2000). In V. Faramaz (Ed.), *MEMTMBS* (p. 77). Las Vegas, NV: CRA.

45. Lonngren, K. E. (1978). In K. E. Lonngren & A. Scott (Eds.), *Solitons in action* (p. 127). New York: Academic Press.
46. Oosawa, F. (1971). *Polyelectrolytes*. New York: Marcel Dekker, Inc..
47. Satarić, M. V., Bednar, N., Satarić, B. M., & Stojanovic, G. (2009). *International Journal of Modern Physics B, 23*, 4697.
48. Sataric, M. V., Ilic, D. I., Ralevic, N., & Tuszynski, J. A. (2009). *European Journal of Biophysics, 38*, 637.
49. Arsenault, M. E., Zhao, H., Purohit, P. K., Goldman, Y. E., & Bau, H. H. (2007). *Biophysical Journal, 93*, L42.
50. Tuszynski, J. A., Priel, A., Brown, J. A., Cantiello, H. F., & Dixon, J. M. (2007). In S. E. Lyshevski (Ed.), *CRC nano and molecular electronics handbook* (Vol. 1). London: Taylor and Francis.
51. Priel, A., & Tuszynski, J. A. (2008). *Europhysics Letters, 83*, 68004.
52. Sahu, S., Ghosh, S., Ghosh, B., Aswani, K., Hirata, K., Fujita, D., & Bandyopadhyay, A. (2013). *Biosensensors & Bioelectronics, 47*, 141.
53. Minoura, I., & Muto, E. (2006). *Biophysical Journal, 90*, 3739.
54. Fritzsche, W., Boehm, K., Unger, E., & Koehler, J. M. (1998). *Nanotechnology, 9*, 177.
55. Fritzsche, W., Böhm, K. J., Unger, E., & Köhler, J. M. (1999). *Applied Physics Letters, 75*, 2854.
56. Umnov, M., Palusinski, O. A., Deymier, P. A., Guzman, R., Hoying, J., Barnaby, H., Yang, Y., & Raghavan, S. (2006). *Journal of Materials Science, 42*, 373.
57. Goddard, G., & Whittier, J. E. (2006). *Proceedings of SPIE–Smart Structures and Materials: Smart electronics, MEMS, BioMEMS, and nanotechnology* (Vol. 6172, p. 43).
58. Tuszynski, J. A., Brown, J. A., & Hawrylak, P. (1998). *Transactions of the Royal Society A, 356*, 1897.
59. Santelices, I. B., Friesen, D. E., Bell Lewis, J., Xiao, J., Hough, C. M., Freedman, H., Rezania, V., Shankar, K., & Tuszynski, J. A. (2017). *Scientific Reports, 7*, 9594.
60. Ussing, H. H., & Thorn, N. A. (1973). *Alfred Benzon symposium* (p. 1972). Copenhagen, Denmark: Munksgaard.
61. Hobbie, R. K., & Roth, B. J. (2007). *Intermediate physics for medicine and biology*. Berlin: Springer.
62. Wenger C, Giladi M, Bomzon ZE, Salvador R, Basser PJ, & Miranda PC 2015 In *Engineering in Medicine and Biology Society (EMBC), 2015 37th Annual International Conference of the IEEE* 6892.