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Molecular Simulation of Cell Membrane Deformation by Picosecond Intense Electric Pulse

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Abstract The application of pulsed electric field is emerging as a new technique for cancer therapy. The irreversible electroporation is the major bioelectric effect to induce cell death. The pulsed electric field is transferred to target deep tissue non-invasively and precisely when the pulse duration is in picosecond regime. In this proposed work, the intense electric field with 100 ps pulse width is used for irreversible electroporation. If the electric field strength increases, the pore in the cell membrane enlarges, causing a loss of membrane intactness and the direct killing of cancer cells. This phenomenon is explored by molecular dynamics simulation. The electric field in the range of 0.8–5 V/nm is used for membrane dynamics. The membrane deformation occurs at the electric field of 5 V/nm. Picosecond pulsed electric field has a wealth of ultra-band spectrum, with extended time and enhanced spatial resolution and low signal distortion. The ultra-wide band antenna is used as a pulse delivery system for non-invasive skin cancer therapy.

Keywords Irreversible electroporation · Intense picosecond electric pulse · Molecular dynamics simulation · Non-invasive cancer treatment

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Introduction

The research on non-ionizing, non-surgical, and minimally invasive treatments of tumors leads to the development of new local ablation techniques based on pulsed electromagnetic field (Schoenbach et al. 2004, 2007). The aim of developing non-invasive treatments is to limit surgery, reduce the pain, scarring, and mortality of the patients while remaining cost effective and safe. Electric pulses with application to biomedical engineering, drug/gene delivery, and membrane poration have been used for several years (Mir et al. 1995; Neumann et al. 1999; Teissie et al. 1999). Over the past several years, pulsed power technology has developed into a novel and innovative time domain nanotechnology as a new modality to treat cancer. The technology treats cancer in the absence of drugs by generating non-ionizing radiation with subnanosecond pulsed electric fields. A variety of cellular responses can be achieved by the application of high-intensity, ($\sim$100 kV/cm) subnanosecond duration, pulsed electric fields (Schoenbach et al. 2008). The proposed work focuses on the cellular response due to the application of picosecond pulsed electric field.

There are three reasons for using picosecond duration pulse on biological targets. The first one is to be in the transient regime and achieve an enhanced bioresponse through intense electric fields supported by non-equilibrium dielectric parameters. The electric field–cell interactions from the cell membrane to subcellular structures are achieved by reducing the durations of electric pulses into the picosecond range. Under these conditions, direct electric field effects at the molecular level will determine biological outcomes. This dominant bioeffects will happen when the pulse duration is less than the dielectric relaxation time of the cytoplasm. Finally, the practical benefit of using

picosecond pulse is the possibility of using wideband antennas as pulse delivery systems. The traditional pulse delivery system is in therapeutic applications that rely on electroporation (Bockmann et al. 2008) or nanosecond pulsed electric field (Nuccitelli et al. 2006) which requires that electrodes be brought into close contact with the treated tissue or close to the body surface. On the other hand, the use of wideband antennas allows applying such electric pulse to tissue (tumors) that are not easily accessible with needles. Picosecond pulses can be delivered with impulse antennas, offering the potential for non-invasive treatment of subcutaneous tissue. The efficient radiation on targets and narrow spot size can be achieved by focusing pulses with 100 ps duration (Kumar et al. 2010). The high-intensity pulses are required to cause cell death. The intense pulse from the antenna is focused on the target (cancer cells).

During the past several years, there have been a number of advances in the computational and theoretical modeling of lipid bilayer structural and dynamical properties. Both the structure and dynamical properties of biological membranes are enormously complex. For this reason, computer simulation has emerged as a critical tool for modeling the lipid component of membrane. The mixed lipid system and mixed protein systems are also being simulated. There are several research works which provide modeling of different lipid membranes (Tieleman et al. 1997; Jiang et al. 2004; Tieleman et al. 2003; Leontiadou et al. 2004). The modeling and dynamics of lipid bilayers are used to study the spatial and temporal dynamics when the cells are subjected to ultra-short intense electric pulse (Frey et al. 2006). Joshi et al. discussed about the dynamics and shape deformation in biological cells subjected to high voltage ultra-short pulses (Joshi et al. 2001). Their studies are based on a coupled scheme involving the Laplace, Nernst-Plant, and Smoluchowski equations. The physical process is pore generation, drift, and diffusion. Tieleman et al. proposed the molecular dynamics simulation of pore formation and membrane rupture in phospholipid bilayers under mechanical and electrical stress at an atomic level Tieleman et al. (2003). In previous simulation studies, pore formation and membrane rupture have been observed in mathematical and simplified membrane models.

Hu et al. (2006) discussed the dynamics of cell membrane response to nanosecond, high-intensity pulse based on molecular dynamics approach. The molecular dynamics simulation has to include twin layers of water molecules dying on either side of the membrane.

Recent reports by Tekle et al. (2001) show loss of the phospholipid membrane during high voltage pulsing. This phenomenon was shown to occur in addition to a pore formation process. The traditional electroporation happened with relatively low to moderate electric fields 200 V/cm–5 kV/cm. But the application of high fields (5 kV/cm–1 MV/cm) will lead to cell destruction.

The motivation behind this work is to study the intense electric field response on plasma membrane due to application of picosecond electric pulse. The Gaussian pulse of 100 ps is applied on the cell membrane. The strength of the electric field is varied from 0.8 to 5 V/nm and the response of the membrane is presented. Mostly the research on cell-field interaction is based on frequency-dependent electric field. This proposed research work showcases the time-dependent electric field (picosecond regime) response on cell membrane. Some of the important issues are explored such as the minimum field intensity required to create pore formation, increase of water entry, and finally the cell deformation.

Materials and Methods

Numerical Simulation

Molecular dynamics (MD) numerical simulation is used to probe electric field induced effects on a typical membrane. MD simulation relies on the application of classical Newtonian mechanics for the dynamical movement of ions, taking into account the many body interactions within a realistic molecular representation of the biosystem. For example, nanoscale area of lipid membrane or a channel protein is first constructed based on the initial geometric arrangement of all atoms and their bonding angle.

The intense electric field effects are studied by molecular dynamics simulation using GROMACS package. The GROMACS is a software package to simulate motions of the systems with hundreds to millions of particles by solving Newtonian equations (Berendsen et al. 1981). The molecular interaction is divided into bonded and non-bonded interactions. Force parameters are properly defined to describe these interactions within a system. A dipalmitoyl-phosphatidyle-choline (DPPC) membrane is chosen. A protein molecule is placed at the center of the simulation box. A small 2 fs time step has been used for accuracy. Na^+ and Cl^- ions are used in the extracellular water. The water molecules are defined on either side of the membrane to form total simulation space. The SPC water model is used because it has a better chemical potential in mixed systems. The electric field is applied in a direction perpendicular to the bilayer-water interface. The force field parameter is taken from the united atom field of Berger et al. (1997). Nose–Hoover thermostat with a coupling time constant of 0.5 ps is used at constant particle number and temperature (323 K) for DPPC and water. Ziegler and Vernier (2008) demonstrated that the minimum porating

electric field showed no correlation in simulation carried out for the temperature between 280 and 340 K. Semi-isotropic weak pressure coupling with compressibility 4.5×10^{-5} bar^{-1} and time constant 1 ps is applied in the lateral direction with bar in the x–y direction. There is no pressure coupling in the z-direction (normal to the water-DPPC interface).The long-range electrostatics is employed with a cutoff radius of 1 nm to keep the particles within the simulation regions. The Van der Waals interaction cutoff is 1 nm. The linear constraint solver (LINCS) algorithm is used to constrain all the bond lengths. The entire system is coupled to a surface tension pressure coupling along the lateral x & y directions. A heat bath of 323 K is chosen to retain the lipid phase of the membrane Tieleman et al. (1997).

According to a Maxwellian molecules, velocities of water and membrane molecules are generated and rescaled with a coupling constant of 0.1 ps. The algorithms for pressure and temperature control are those discussed by Allen and Tildesly (1987). A 4 fs time step is used with constraints on the bond lengths within the lipids and on the water geometry. A group based twin cutoff scheme was employed for the non-bonded interactions with R cutoff 0.9 nm for Van der waals interaction calculations. Linear constraint solver (LINCS) algorithm (Hess et al. 1997) is used for bond length and bond angle formation. All ions are treated for energy and distance taken from the published literature (van der Spoel et al. 1996; Berendsen et al. 1995; Darden et al. 1993). The Particle mesh Ewald (PME) algorithm (Joshi and Hu 2011) is implemented to calculate long-range electrostatics using Fast Fourier Transforms and conductive boundary conditions. Reciprocal–space interactions are evaluated on a 0.12-nm grid with fourth-order B-spline interpolation. The effects of system size are mitigated by employing periodic boundary conditions.

Systems and Structures

The water-membrane system contained 13,084 atoms. There are 1 protein, 126—DPPC, 2214-SOL, and 4 Cl^- molecules for over all charge neutrality in 6.4 nm × 6.4 nm × 6.5 nm simulation box which results in a system box. Each DPPC molecule consists of 50 atoms in an all-atom model. Molecular modeling images are generated by visual molecular dynamics (VMD) (http://www.ks.uiuc.edu/Research/vmd/).

Electric Field Application

The magnitude of the electric fields used in these simulations is very high relative to the fields encountered in experimental situations. The value of electric field applied to a molecular dynamics simulation is obtained from the non-invasive radiators (ultra-wide band antennas). The PSIRA is excited with higher voltage in the range of 100s of kV in order to obtain output as 1 MV/cm. The electric field of 4 MV/cm with nanosecond pulse duration is applied in order to form the electroporation (Egberts and Berendsen 1988). In this proposed work, a 100 ps intense electric field is used and the electric field of 1 MV/cm is adequate to form the poration to induced cell death since the pulses are in the picosecond range.

The GROMACS molecular simulation is extended to include a pulsed time-dependent electric field (Caleman and van der Spoel 2008).

$$E(t) = E_0 \exp\left[\frac{-(t-t_0)^2}{2\sigma^2}\right] \cos(\omega(t-t_0)) . \quad (1)$$

The angular frequency $\omega=2\pi c/\lambda$ is varied in microwave regime. Different field intensity E_0 is used for MD simulation with pulse width (σ) of 100 ps. t is the time after the pulse maximum at t_0.

Effects of Intense Electric Pulse

Primary Effects

The primary effects of the cell membrane are depicted in Fig. 1. The heads of the lipids are in random motion at time t_0. At t_1, an external electric field is applied and the lipid heads facing the cathode will align with the electric field. The heads of the lipids facing the anode will rotate to try and align, causing repulsion between them. This repulsion causes an opening to allow the water molecules through the membrane as depicted at time t_2.

The DPPC lipid bilayer is used for MD simulation. For each DPPC chain, the head group contains a dipole with positive charge on choline and negative charge centered on the phosphate group. With no electric field present, the dipoles are in random thermal motion with

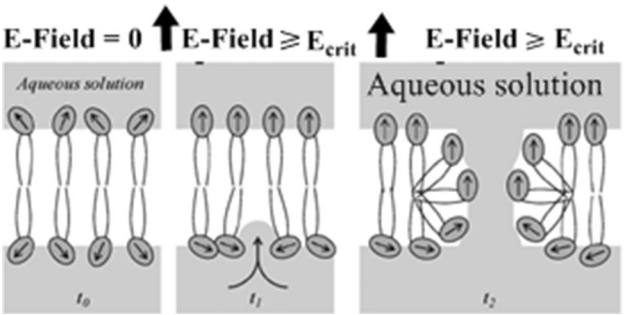

Fig. 1 Primary effects of cell membrane by the application of electric field

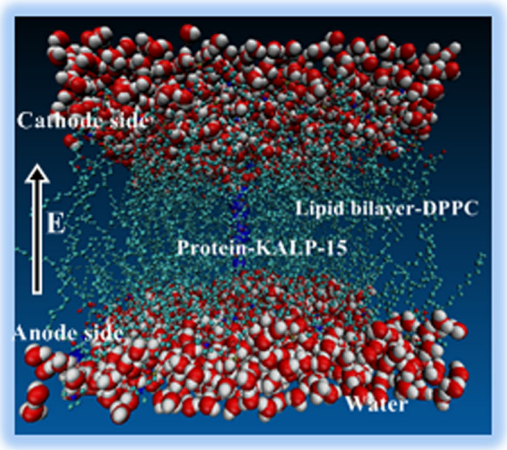

Fig. 2 Snap shot of DPPC lipid bilayer with protein and water model

the positive charges residing on the outermost portions of the lipid. Electric-field-induced defects are initiated by the movement of dipoles on the surface of the membrane. Defects start to form on the anode side of the membrane because positively charged molecules, e.g., choline, on this side are forced to swing around, i.e., reorient in the presence of a strong external electric field and enter the membrane. This same electrical field, however, when acting on the dipoles located at the cathodic surface merely works to stretch the dipoles without any molecular movement into the membrane volume. The alignment of dipolar head groups on the anodic side gradually deviates from the equilibrium orientation, and a defect starts to form.

Irreversible Electroporation

In reversible electroporation, external electric field can permeate the cell membrane temporarily in which, the cell membrane can survive, whereas strong external electric field can cause the cell membrane to permanently permeable by which the cell will die and the process is referred to as irreversible electroporation. The main phenomenon of irreversible electroporation is the mean life time of membrane, which is abruptly decreased with increase of field intensity.

Results and Discussions

The numerical simulation of cell membrane is presented by GROMACS molecular simulation package. The dipalmitoyl-phosphatidyl-choline (DPPC) molecule with protein model is defined. The water molecules are placed on either side of the lipid bilayer as the basis for the lipid membrane.

The set of MD simulation is designed to probe the role of water on the lipid bilayer configurations. Figure 2 shows the DPPC lipid bilayer with protein model. In Fig. 2, the water is represented by the red–white combination (hydrogen–oxygen atoms) and is presented at the top and bottom of the lipid bilayer, and phosphors and Nitrogen atoms are represented by gold and blue spheres within the lipid head groups. The cyan tail denotes the phosphor lipid tails.

Previous MD simulation results in the nanosecond regime and has shown that fields at or above 0.5 V/nm can lead to pore formation over nanosecond time scale

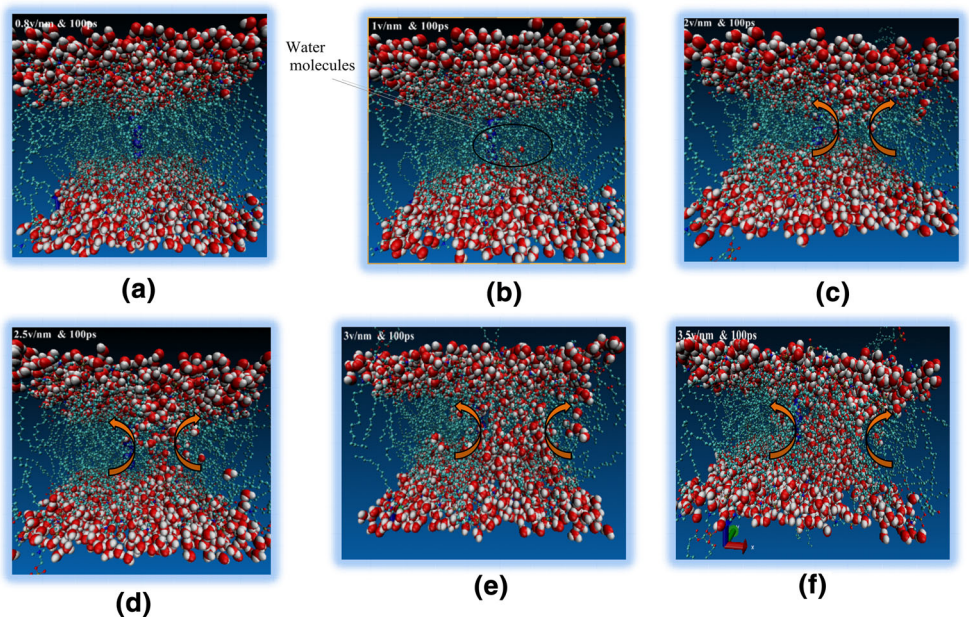

Fig. 3 Sequence of pore dynamics in DPPC lipid bilayer subjected to an electric field at 100 ps pulse duration. Water molecules are represented by the *red–white* combination (hydrogen–oxygen atoms) and are presented at the top and bottom of the lipid bilayer. The *cyan color* denotes the phosphor lipid tails. **a** Bilayer at 0.8 V/nm, **b** bilayer at 1 V/nm, **c** bilayer at 2 V/nm, **d** bilayer at 2.5 V/nm, **e** bilayer at 3 V/nm, **f** bilayer at 3.5 V/nm (Color figure online)

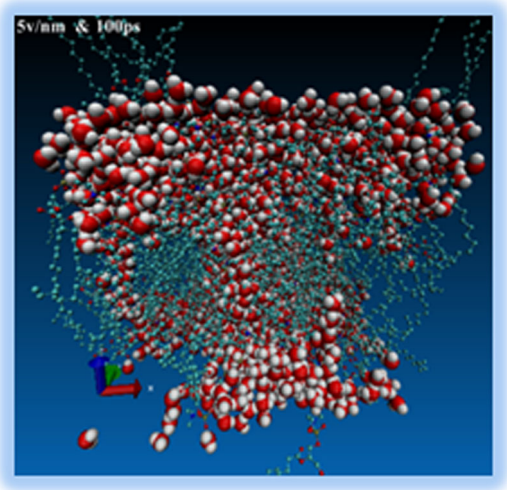

Fig. 4 Cell deformation at the field intensity of 5 V/nm

(Sridhara and Joshi 2014). The molecular mechanism of membrane electroporation and deformation has been elucidated by means of numerical simulations of lipid bilayer subjected to a transverse electric field. The time-dependent electric field is applied along z-direction (perpendicular to the membrane).The reorientation of water molecules at the membrane interface due to the applied electric field causes the structural defect of water molecules that initiates pore formation. Such water rearrangements develop into a water column, penetrating in the region of the hydrophobic. The MD snap shot of membrane under an electric field of 0.8–5 V/nm with 100 ps pulse width is shown in Fig. 3.

The kinetics of the poration and membrane deformation is shown in Figs. 3 and 4. The electric field applied on the water molecules close the membrane interface which is the main factor to promote the initiation of the pore. The pore formation starts at 1 V/nm field intensity which is depicted in Fig. 3b. The size of the pore or water channel between the top and bottom lipid layer is increased at increasing electric field. The pore size variation is depicted in Fig. 3a–f for different electric fields at 100 ps. The cell deformation is shown in Fig. 4. The maximum water molecules are entered into the upper layer after the application of 5 V/nm electric field.

Conclusion

The simulation provides time-dependent cell membrane deformation. It has been carried out by the application of intense electric field with 100 ps pulse width. The goal is to ascertain the potential benefits in pulse duration down to the picosecond regime. The cell membrane response to picosecond pulsing at electric field exceeding 0.5 V/nm has been presented. The membrane deformation occurs at the electric field of 5 V/nm. The use of picosecond pulses not only allows entering into new field of electric field-cell interactions but it also opens the door to a range of non-invasive therapeutic application. The potential benefit of picosecond pulse also includes the use of Impulse Radiating Antenna for allowing access to deeper-lying targets within tissues.

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