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Polyethylene Glycol Repairs Damaged Membrane; Biophysical Application of Artificial Planar Bilayer to Mimic Biological Membrane

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Abstract

Polyethylene glycol (PEG) is a hydrophilic polymer, known to be capable to fuse numerous single cells *in vitro*, to join the membranes of adjacent neurons and giant invertebrate axons, and to seal damaged neural membranes. The molecular mechanism of the action of PEG is still unknown. It is believed that PEG dehydrates membranes and enables their structural components to resolve and rearrange in a lamellar configuration following rehydration. In this study, the effects of different sized PEGs (400, 1000, and 2000Da) at 10–30% w/w on different physical properties of intact and damaged artificial bilayers, including membrane conductivity (G_m), capacitance (C_m), and breakdown voltage (V_b), were studied by voltage clamp technique to address its resealing capability at the molecular level. The unilamellar artificial planar bilayer was formed from soybean lecithin, based on the Montal and Mueller procedure. Our results show that in disrupted membrane, PEG2000 increased G_m and C_m significantly and decreased V_b. Furthermore, PEG1000 at 30% w/w significantly increased G_m and decreased V_b, but had no effect on C_m. PEG400 had no significant effect on G_m, V_b, or C_m. In addition, at the applied concentrations and molecular weights, the PEGs showed no effects on the stability, conductivity, or breakdown voltage of the intact bilayer. We conclude that PEGs repair membrane in concentration- and size-dependent manner; small PEG (400Da) is capable of repairing membrane and re-stabilizing its integrity at certain concentrations, while larger ones, such as PEG2000, destabilize the membrane and fail to re-establish its integrity. The results of this study might shed light on understanding the mechanism (s) by which PEGs repair damaged membranes of neural fibers, and might be considered in clinical treatment of brain and spinal cord injury in the near future.

Keywords: Polyethylene Glycol (PEG), Artificial Planar Bilayer, Membrane Stability, Membrane Sealing, Voltage Clamp.

Introduction

Disruption of the cellular plasma membrane has been considered to play a pivotal role in initiating the events that lead to cell death. The degree of membrane damage and the ability of cells to repair the damage determine the mode of cell death (McNeil and Terasaki, 2001). Membrane damage has been proposed to be responsible for neural cell death and functional deficits caused after traumatic injury to the brain and spinal cord. Breaches in the nerve membrane produced by damage cause an unregulated exchange of ions between intra- and extracellular environments, leading to "secondary injury" (Tator and Fehlings, 1991). Thus, resealing the damaged membrane in the early stages not only maintains the physicochemical condition of the cytosol, but also obstructs the initiation of secondary injury

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and consequent widespread tissue damage.

It has been shown that polymers such as polyethylene glycol (PEG) (Luo et al., 2002), poloxamer-188 (Hannig et al., 2000), and poloxamine-1107 (Hannig et al., 1999) are effective in sealing damaged membranes, ceasing neural cell death (Luo et al., 2002; Shi and Luo, 2007). PEG has long been used to fuse cells, coupling several single cells into one, joining the membranes of cells, and introducing genetic material from one cell into another (Ahkong et al., 1975; Davidson et al., 1976; Nakajima and Ikada, 1994). Bittner and colleagues used PEG to rejoin giant axons *in vitro* to produce axoplasmic continuity (Bittner et al., 1986). It has also been shown that PEGs are able to seal disrupted and crushed membranes of axons *in vitro* (Lore et al., 1999). Rapid induction of membrane fusion in invertebrate neurons treated with PEG has been reported (Bittner et al., 1986). Spaeth and colleagues demonstrated that membrane sealing mediated by PEG is independent of any known endogenous cell sealing mechanism (Spaeth et al., 2012).

PEG is a polymer of ethylene oxide with the generalized formula HO-(CH2-CH2-O) n-H, whose properties depend on medium temperature and available water molecules (Żwirbla et al., 2005). PEGs are capable of forming strong hydrogen bonds with water molecules. The level of PEG solubility in water depends on its molecular weight-larger PEGs are less soluble. Small PEGs (less than 1000Da) form clear viscous liquids, while larger ones create white, waxy solids at room temperature.

The molecular mechanism, biophysics, and intermediate modes of action of fusogenic polymers such as PEG are not fully known at the molecular level (Georgiev et al., 2006; Lee and Lentz, 1997; Lentz, 1994; Lentz and Lee, 1999). However, it has been proposed that PEG can promote membrane sealing and fusion based on its ability to absorb excess water released at the damaged membrane site (Lentz, 1994). It is believed that accessible charged groups of PEG form hydrogen bonds, by which a great amount of water at the disrupted site is absorbed, causing osmotic pressure on the membrane that leads to lipid rearrangement in a lamellar configuration following rehydration (Lentz and Lee, 1999; Nakajima and Ikada, 1994). The ultimate condition set in the membrane is so stable that elimination of PEG does not alter its positive effects on membrane sealing (Borgens et al., 2002). However, the actual arrangement of the introduced PEG at the disrupted site, as well as the way it is packed and its effect on the resulting membrane capacitance, permeability, and stability against potential differences, is not known.

Lipid bilayers act as non-permeable amphipathic barriers that keep the physicochemical conditions of the cytosol at certain pH and ionic strength required for the activity of cytosolic enzymes. The hydrophobic core of the membrane that is formed by the lipid's acyl chains forms a certain dielectric that is entrapped between two hydrophilic layers of the charged head groups. This structure acts as a capacitor and responds to the applied AC field. The capacitance, leakage, and breakdown voltage of the membrane is governed by the level of packing, integrity, and stability of the membrane. Artificial lipid bilayers are assembled in the forms of vesicle or planar structure (Montal and Mueller, 1972). Artificial liposomes or vesicles, which mimic the geometry and size of cell membrane, are used as model systems to investigate membrane fusion and resealing processes (Marsden et al., 2011). The artificial planar bilayers are another commonly used model of lipid bilayers, where the physical characteristics of a small area (100–1000 μm^2) can be studied by means of its capacitance, leakage, and stability in real time. The long-lasting bilayers make it possible to study the minute changes in the mentioned characteristics when their structures are disrupted, as well as in cases when external agents or conditions are introduced to re-stabilize them. The advantage of this system is that both sides of the membrane are easily accessible and can be probed by electrodes (Kramar et al., 2010). It is noteworthy that most of the experiments conducted on the effects of PEG have been performed on vesicle models, where it has been difficult, if not impossible, to study the molecular characteristics of the membrane in a certain area. In this study, we have used a planar bilayer with an area of about 300 μm^2, that mimicked biological membranes; the capacitance, conductance, and the stability in time and against membrane potential differences (pds) and applied chemicals were continuously

monitored. Data acquisition was carried out at sampling intervals of 500 μs, which provided the ability to record instant effects. As any changes in the integrity of the membrane could lead to sudden fluctuating ion current, we were able to monitor, record, and statistically analyze it afterwards. Furthermore, the effects of any physical or chemical intervention, including the applied PEGs, on the integrity and stability of the membrane could be studied. In addition, as the membrane area was constant, any changes in the dielectric of the bilayer core caused by the insertion of PEG molecules were manifested by means of changes in the membrane capacitance (C_m) that was governed by the ultimate membrane thickness and dielectric. We were also able to monitor the packing status and stability of the membrane, measured at different pds and polarities, by means of the breakdown voltage (V_b). Thus, the model made it possible to monitor the effect of different PEGs of various sizes and concentrations on the characteristics of intact membrane, and to record their sealing effects on the disrupted and leaky membrane.

Contradictory results have been reported that address the effects of size and concentration of PEG on membrane sealing ability when vast number of cells, and thus a large membrane area, were studied. Some authors have shown PEG membrane sealing ability in a size- and concentration-dependent manner (Davidson and Gerald, 1976; Nakajima and Ikada, 1995; Vijayalakshmi et al., 1999), while Shi and colleagues reported that changes in the concentration and molecular weight of applied PEG in their *in vitro* and *in vivo* experiments had no significant effect on neural membrane sealing in spinal cord injury models (Shi and Borgens, 1999; Shi and Borgens, 2001). To our knowledge, this is the first study that addresses the effects of PEG on the stability and resealing of membrane in a very small, limited area, at three different molecular weights (400, 1000 and 2000Da) and in a range of concentrations (10–30% w/w), that is able to record the effects on-time and rather rapidly (at a μs time-scale), with a high level of accuracy (capable of recording ion currents as low as 10 pA), by using an artificial planar model.

Materials and Methods

The effects of PEG were studied by using an artificial planar bilayer system, electrically isolated in a Faraday cage and formed from soybean lecithin type II (Sigma). The lipid bilayer formation was performed based on the Montal and Mueller technique (Montal and Mueller, 1972). Briefly, bilayers were formed across a 30 μm diameter hole in a PTFE septum that separated two compartments from each other, each with a volume of 1 ml in a Teflon chamber. The hole was initially treated with 4 μl of a hydrophobic coating solution, consisting of hexadecane in n-pentane 2% (V/V), which facilitated stable contacts between the PTFE and lipid hydrocarbon chains. Each compartment was initially filled up with buffer ($CaCl_2$ 10 mM, KCl 1 M, HEPES 10 mM at pH 7) up to the hole position. Then, 4 μl of lipid solution (0.5 g/ml in n-hexane) was spread on the surface of the buffer in both compartments and was left for 10 minutes for the n-hexane to evaporate. By injecting more buffer into one compartment (*trans*, non-ground side electronically), the lipid monolayer formed on the surface was raised, passing the hole in the septum and forming the first monolayer on the hole. The same process was repeated on the other compartment (*cis*, connected to the virtual ground of the pre-amplifier) to form the second monolayer, which was zipped to the first one, thus forming the bilayer (Montal and Mueller, 1972). The thickness and area of the formed membrane were manifested by the capacitance of the bilayer, which was monitored continuously through charging and discharging of membrane by the applied square signal, 120 Hz, with an amplitude of 2 V peak to peak, via Ag–AgCl electrodes. All chemicals were obtained from Sigma Chemical (St. Louis, MO).

Data recorded by voltage clamp technique were amplified, as required, using an Adimi Filter Amplifier, digitized by CED 1401Plus (Cambridge Instruments, Somerville, MA), filtered by means of two low pass filters at 9 KHz, and analyzed using a PC with PAT7 software (J. Dempster, University of Strathclyde). The applied voltages (pds) ranged from ±0 to ±350 mV, in 50-mV increments. The chamber and pre-amplifier were located inside a Faraday cage that was positioned on an air table to minimize

interference from both mechanical and electromagnetic noises during the bilayer studies. The PEG solutions were introduced to the *cis* compartment, making a final concentration of 10%–30% (w/w). The PEGs were introduced under two conditions: i) following the preparation of an impermeable membrane, stable at potential differences as high as ±300 mV, to monitor the effect of PEG on intact membrane; and ii) after making intentional disruptions in the membrane. During the course of the experiments, the temperature was maintained at 22°C.

Results

Membrane characteristics of control membranes including, capacitance (C_m), conductivity (G_m), and breakdown voltage (V_b) - at different potential differences (pds) and polarities were recorded prior to each experiment. The results of three sets of independent experiments were analyzed, averaged, and reported as the representatives. The intact planar bilayer showed C_m of 80–100 pF in response to the applied square wave of 120 Hz, 2 V peak to peak (Fig. 3A). Further, the current traces of the membrane were recorded in the control group at different pds in the absence of PEG, and they showed no leaks or conductivity in response to the applied voltage below the breakdown voltage (333.3±28.9 mV) (Fig. 1A). Leaky membranes were destroyed and new, stable membranes were formed.

Effects of Pegs on Intact Membrane

PEG molecules of different sizes (400, 1000, and 2000 Da) were added to the *cis* compartment in different concentrations (10–30% w/w). Compared with the control, no significant changes were observed in C_m, G_m or membrane permeability at applied pds of up to ±250 mV, in 50-mV increments. Furthermore, there were no significant differences identified for V_b before and after application of the PEGs on intact membrane. Thus, introduction of the PEGs in the mentioned concentrations had no effect on stability, conductance, or capacitance of the membrane (Fig. 1B).

Effects of Pegs on Damaged Membrane

Following the intentional disruption and breakage of membrane by means of voltage shock (±350 mV), PEG was added to the *cis* compartment, and new membranes were formed in the presence of PEG. The characteristics of these newly formed membranes were evaluated in the presence of the PEGs of different sizes and concentrations.

Effects of Pegs on the Conductivity of Damaged Membrane

Based on our results, when the damaged membranes were treated with PEG400 at different concentrations (10%, 20%, and 30% w/w), small currents were monitored (Fig. 2A) and no significant conductivity was identified compared with the control group (Fig. 2D).

However, treatment of the damaged membranes with PEG1000 and 2000 did not completely seal the membrane, causing more current fluctuations, with higher amplitudes and at higher frequencies, in the resulting membranes. However, treatment with PEG 1000 at low concentrations of 10% and 20% (w/w) showed results similar to the control, and at higher concentration, a permeable membrane was formed.

Application of PEG 2000Da was not an appropriate treatment for sealing of membrane, as no complete packing was formed, and the resulting membranes were completely leaky, with high conductance, as shown in Figure 2C. All point histograms for all recorded current traces were calculated and fitted with a Gaussian function. The standard deviation (SD) of the histogram was reported as an index of deviation in membrane conductivity. As shown in Figure 2D, the SD of the histogram for the PEG400-treated group is not significantly different from that of the control, which means that the conductance of the resulting membrane is close to that of the intact membranes in the control group, as can be seen clearly in Figure 2A.

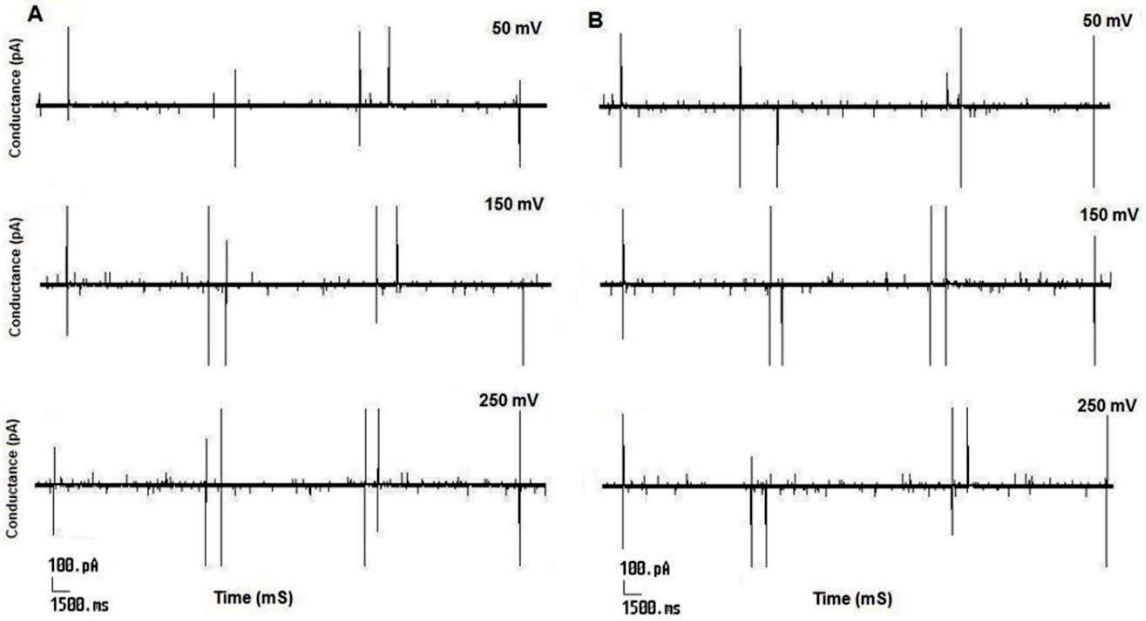


Figure 1. Typical current traces of membrane recorded in the control group with no PEG (A) and treated with PEG400, 10% w/w (B) at 50, 150, and 250 mV. No conductivity was observed before or after PEG addition to the intact bilayer. Baseline represents 0 pA current level. The large vertical lines, from left to right, represent the occasions when the pds were switched off or their polarities were changed. Starting from the left side, 0 ms, they represent when –ve pd, no pd, +ve pd, no pd, and then again –ve pd and no pd were applied, respectively.

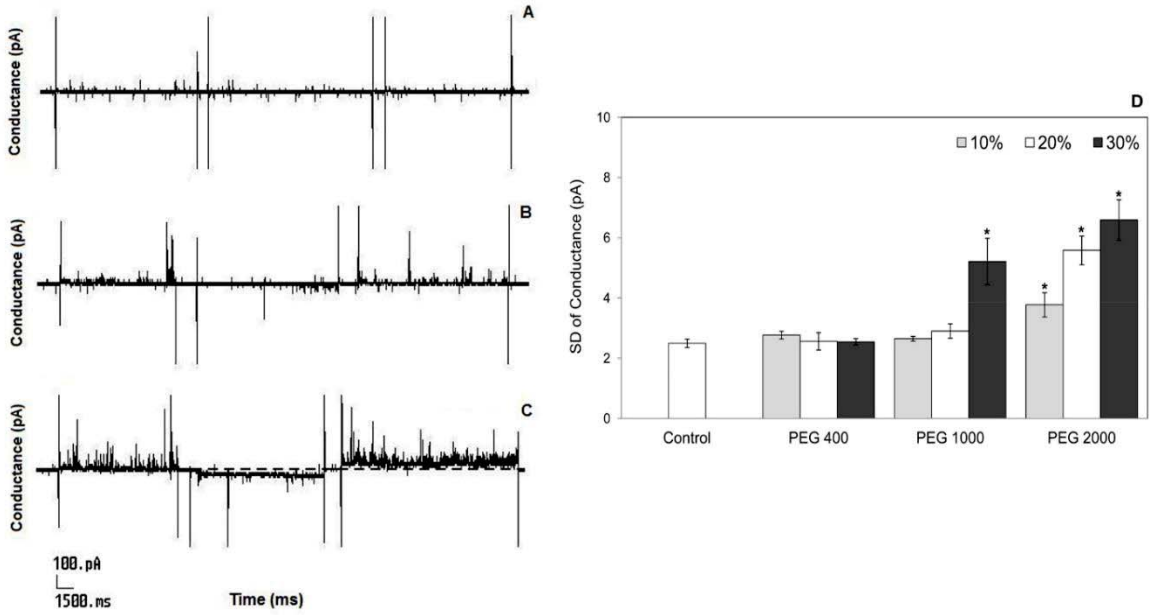


Figure 2. Effects of different PEGs (400, 1000, and 2000 Da) at various concentrations (10, 20, and 30% w/w) on membrane conductivity in damaged membrane. Current traces of membrane recorded in PEG400 (A), PEG1000 (B), and PEG2000 Da (C) (20% w/w) treated groups at 200 mV. Note that application of PEG400 at different concentrations made the membranes less permeable, with a conductance similar to that of the intact membrane represented as the control. The amplitude and frequency of current fluctuation in the damaged membranes treated with PEGs increased as the PEG size increased (baseline represents 0 pA current level). The standard deviation (SD) s of the histograms of all treated groups (D) increased as the size and concentration of PEG increased (*P<0.05).

In the PEG1000 and 2000Da treated groups, increasing PEG concentration resulted in increased conductance. Treatment with PEG1000 (30% w/w) and PEG2000 (10–30% w/w) produced membranes with much higher conductance ($P<0.05$) compared with the control (Fig. 2D). This can occur due to the change in membrane integrity after treatment with these PEGs.

Effects of Pegs on Membrane Capacitance

A normal bilayer in our experiments showed Cm of 80–100 pF (Fig. 3A). The capacitances of the damaged membranes treated with PEG400 were 72.7±6.5 pF, 67.3±7.6 pF, and 76.7±9.5 pF at concentrations of 10%, 20%, and 30% w/w, respectively (Fig. 3D). There were minute marginal differences identified in C_m when the membranes were treated with PEG 400 at concentration of 10% and 20% w/w, and it did not vary when treated at 30% concentration ($P<0.05$).

The capacitances of the damaged membranes treated with PEG1000 were similar to that of the control at all applied concentrations (88.7±10.6, 91.7±6, and 93.3±11 pF for PEG1000 10%, 20%, and 30% w/w, respectively). However, when the damaged membranes were treated with PEG2000, the resulting membrane showed higher capacitance, and the capacitances of the membranes were greater when the membranes were treated with PEG2000 at higher concentrations (147.3±5, 173±13.2 and 177±6.6 pF for PEG2000 at 10%, 20%, and 30% w/w, respectively) (Fig. 3E). Figure 3D shows the capacitance profile of the membrane treated with PEG2000 at 20% w/w.

Effects of Pegs on Stability and Breakdown Voltage of Membrane

The stability of the damaged membranes differed when treated with PEGs of different sizes and concentrations (Fig 4D). Applying membrane pds to the intact membranes, the average stability was found to be 333.3±28.9 mV. The stability of the membranes decreased when larger PEGs at higher concentrations were applied. The most stable membranes were found to be the damaged membranes treated with PEG400, whose V_b did not differ significantly from that of intact membrane (350±50 mV, 366.7± 28.9 mV, and 333.3±28.9 mV at 10%, 20%, and 30% w/w respectively).

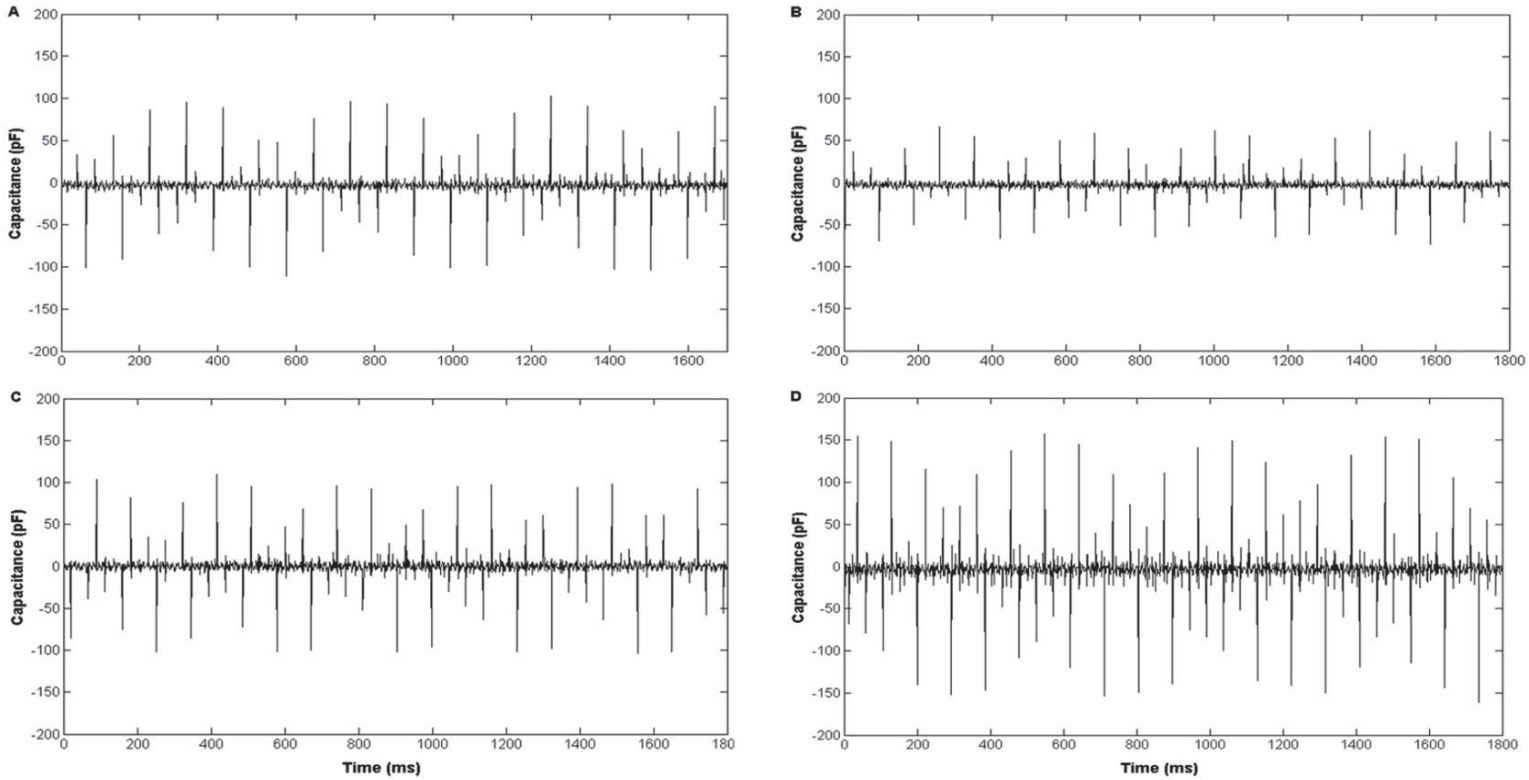
The same stability as that of the intact membranes was observed in the damaged membranes treated with PEG1000 at 10% and 20% concentrations, whose V_b did not differ significantly from those of the control groups (333±28.9 mV and 300±50 mV for 10% and 20% w/w, respectively). However, treating the damaged membranes with PEG1000 at a concentration of 30% significantly decreased the stability of the membrane and showed a breakdown voltage of 267±28.9 mV (*$P<0.05$) (Fig. 4D).

The least stable damaged membranes were those that were treated with PEG2000 at high concentrations. Damaged membranes treated with PEG2000 at concentrations of 20% and 30% w/w were so unstable that they easily broke at pds as low as 267 and 217 mV (267±28.9 mV and 217±28.9 mV for PEG2000 20% and 30% w/w, respectively) ($P<0.05$). However, at the lower concentration, PEG2000 made the damaged membrane as stable as the intact membrane (317±28.9 mV at 10% w/w). The results showed that as the size and concentration of the PEG increased, the stability of the membrane decreased, and membrane breakdown occurred at lower voltages (Fig. 4A-C).

Discussion

Plasma membrane plays a vital role in neural cell function, and damage to this organelle, which is postulated to be an immediate response to the initial traumatic insult, results in uncontrolled ion flux into and out of the cell and leads to delayed cellular injury. An acute increase in membrane permeability due to the injury-induced disruption causes shifts in transmembrane ion concentrations, depleting energy stores, changing membrane potential, and launching deleterious cascades, following brain and spinal cord injury (LaPlaca et al., 2009).

Polyethylene glycol (PEG) is an amphipathic, water-soluble polymer that has been shown to be effective in membrane resealing (Luo et al., 2002; Shi and Luo, 2007), although the exact molecular mechanism of its effect is not fully understood. PEG causes dehydration of the membrane



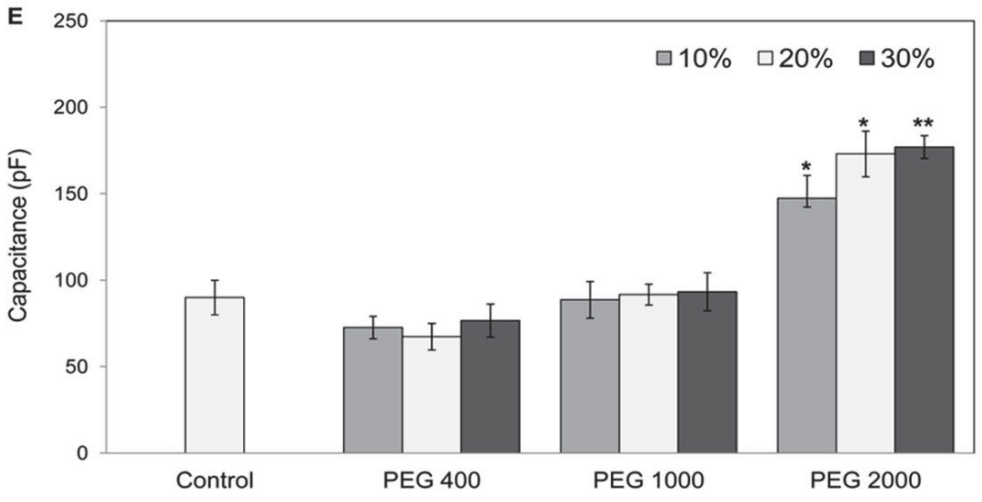


Figure 3. Membrane capacitance (C_m) profile of normal membrane (A), remained significantly unchanged in membrane treated with PEG400 at 20% w/w (B) and PEG1000 at 20% w/w (D), and significantly increased when treated with PEG2000 at 20% w/w (C). The average of changes in C_m after treatment with PEGs of different sizes and concentrations were analyzed statistically (E). Note that C_m increased significantly in the PEG2000-treated group (**P<0.001, *P<0.05).

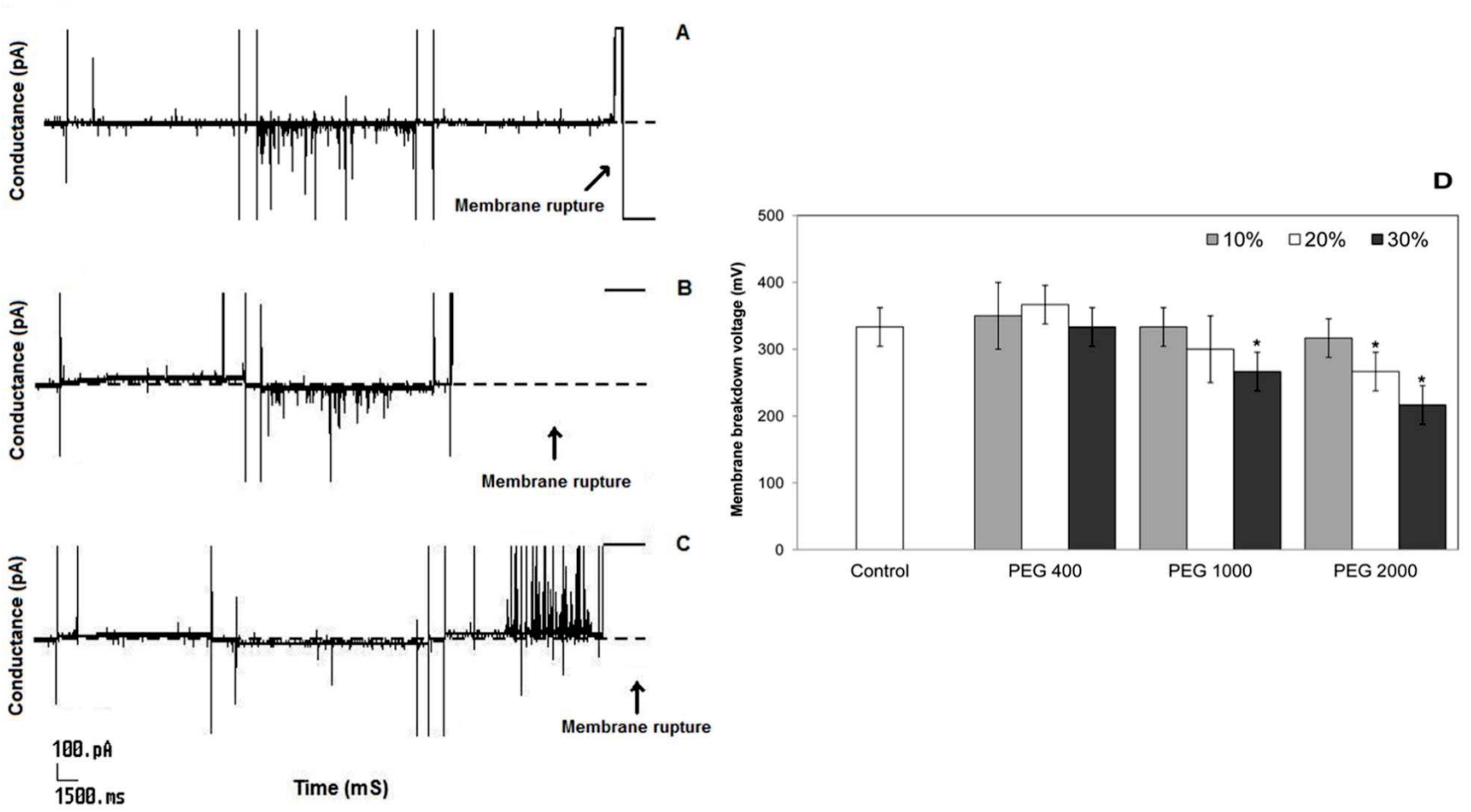


Figure 4. Current traces and the effects of PEGs on the stability of damaged membranes, shown by means of breakdown voltage (V_b). Membrane rupture occurred at 300 mV, 250 mV, and 200 mV in membranes treated with PEG400 (30% w/w) (A), PEG1000 (30% w/w) (B), and PEG2000 (30% w/w) (C), respectively. Membrane stabilities manifested by means of breakdown voltages in different treatment groups (D). Note that treatment with PEG1000 at high concentrations and PEG2000 at all concentrations destabilized the damaged membranes and caused decreases in their corresponding V_b. However, application of PEG400 at all concentrations and PEG1000 at low concentrations re-stabilized the damaged membranes to the level comparable with the intact membranes (*P<0.05).

surfaces, an imbalance of osmolarity between the membrane surface and the bulk aqueous phase, and forces close contact between adjacent membranes (Malinin et al., 2002). Experiments with vesicle models have shown that PEG mediated-aggregation results from dehydration in the water layer and tight packing in the membrane (Mishima et al., 1997). PEGs with different molecular weights possess different physical properties, including surface charges, viscosity, hydrogen bond formation, configuration, and packing in bipolar environments such as lipid bilayers. Due to the effects of PEGs chain length, the viscosity of PEG400, 1000 (50% aq. sol.), and 2000 (50% aq. sol.) are 90, 20, and 40 mPas at 25°C, respectively. Further, NMR studies have shown that small PEGs create a zigzag conformation, whereas larger ones form random coil structures (Winterhalter et al., 1995).

Under our experimental conditions (22°C, KCl 1 M), PEG takes a linear structure, but the viscosity and solubility of this polymer are dependent on its concentration and molecular weight. The solubility of PEG in water decreases as its molecular weight increases. Based on a reported hypothesis, at lower molecular weights, PEG may have detergent-like properties, similar to those of amphipathic polymers, and it may cover or be absorbed into regions of the damaged membrane, where its hydrophobic cores interact with the lipid portion of the bilayer, while its hydrophilic tails extend into the aqueous domains of the membrane, covering breaches formed in the bilayer (Lee and Lentz, 1997; Nakajima and Ikada, 1994). Following the introduction of PEG into the damaged sites of the membrane, it exerts its effect in restoring the integrity of the membrane. on its perfusion, the accessible sites of the membrane are rehydrated, and spontaneous reassembly of lipids and localized PEGs takes place, leading to the restoration of membrane integrity (Donaldson et al., 2002).

Our results showed that treatment of the intact bilayer with PEGs of different sizes (400, 1000 and 2000 Da) and concentrations (10, 20, and 30% w/w) had no effect on the physical properties-capacitance, permeability or conductivity, and stability or breakdown voltage- of intact membrane. Thus, PEG cannot be inserted into intact membranes.

Capacitance (C_m) of planar lipid bilayer is an index that shows the thickness, area, and dielectric of the membrane. This parameter is considered as one of the best tools available for probing the stability and integrity of planar lipid bilayers (Kramar et al., 2010). In addition, the area of the planar lipid bilayer usually affects its capacitance. In our experiments, the mean area of the control membrane, formed by the Montal and Mueller folding technique, was about 300 μm^2.

Rearrangement of the phospholipids in bilayer can be induced by an external electric field, which leads to transitional formation of water-filled pores. If the electric field does not exceed a critical strength and duration threshold, the membrane returns to its normal state at the end of the exposure. Above a critical voltage (breakdown voltage, V_b), defects that are created in the planar bilayer cause an increase in the transmembrane current, and the membrane usually ruptures (Putvinsky et al., 1979). The level of membrane sensitivity to the applied potential difference depends on various factors, including lipid composition, temperature, and electrolyte composition. It has been reported that the presence of ions such as Na^+ and K^+ in the electrolyte bathing the membranes does not change V_b. In addition, it has been shown that increasing temperature is concurrent with a decrease in the V_b of planar lipid bilayer (Benz et al., 1979).

In this study, we used artificial planar bilayer to study the repair of a membrane whose integrity was disrupted intentionally. The integrity and stability of the re-formed membrane, as a capacitor, were evaluated in response to a strong electric field. The resistance of this treated damaged membrane against the applied field showed the packing order and the extent of the hydrophobic molecule arrangements, as well as the homogeneity of the barrier, in analogy to a capacitance that was monitored by means of breakdown voltage strength, V_b. Thus, through our approach, we were able to evaluate the permeability, integrity, and stability of both intact and repaired membranes, by means of their physical characteristics: i) capacitance, as a means of determining the thickness, packing, and dielectric formed by the hydrophobic groups in a constant area of about 300 μm^2 ; ii) conductance, to show how much, how often, and for how long ion current is passed through the bilayer, as well

as to determine the process that seals the transitional water-filled conducting pores formed in disrupted membrane; and iii) breakdown voltage, to determine how stable the newly repaired membrane is and how much it resists against the ionic applied field.

Our results showed that the conductance of the bilayers formed in the presence of PEG400 and PEG1000 (10% and 20% w/w) was not significantly different from that of intact membranes, which means that membrane integrity at the damaged site was reestablished in stable form following the introduction of PEG400 and PEG1000. However, PEG2000 distorted the membrane significantly and caused great ionic current flow, such that bilayer conductivity increased by about 100%. This is consistent with the high osmotic pressure reported to be caused by PEG2000.

In the groups treated with PEG400 and PEG1000, there were no differences in membrane capacitance compared with the intact membrane, which reflected the restoration of membrane with the same thickness, packing, and possibly, area.

The concentrations of the applied PEGs did not show significant effects on membrane capacitance that could be due to partition coefficient values of PEGs in the bilayer at the concentration level implemented here. Thus, based on the equation $C= \varepsilon_0 A/d$ (where C is the capacitance, ε_0 is the dielectric constant, A is the area, and d is the thickness), as the area has remained constant, the effects of changes in thickness have been the same as changes in dielectric in the treated membrane. Based on the partition coefficient of the PEGs used here, it can be inferred that PEG400 is accommodated in the water in the vicinity of the membrane surface, and thus, it does not affect the dielectric of the hydrocarbon core of the membrane. However, reports have shown that PEG1000 can be solubilized in organic solvents; thus, it might be expected to be accommodated in the membrane core, and thus affecting membrane capacitance to an extent.

The capacitance of the membrane in the presence of PEG2000 increased by about 50%, which could be due to its enforcement on thinning the membrane. Alternatively, it might have increased the area by lowering the extension of the Gibs border, which acts as a hydrophobic interface between the inner edge of the hole in PTFE and the bilayer. PEG2000 can be accommodated on the surface of the membrane in two forms, mushroom and brush shapes, forming a rough surface of extrusions 2–3 nm in size (Gregoriadis and McCormack, 1998). This might explain the increase in the capacitance of the membrane, due to the increased area size.

The stability of the re-formed membrane was also affected mainly by the larger PEGs applied in this study, and stable membranes were formed in the presence of PEG400. The application of PEG1000 at low concentrations resulted in membranes as stable, statistically, as the intact bilayer; however, there was great variation in the stability of the individual membranes formed.

In the presence of PEG1000 at 30% w/w, as well as PEG 2000 at concentrations of 20% and 30% w/w, the membranes were stable at low applied potential differences. However, in the presence of PEG2000 (10% w/w), stability was not significantly less than that of intact membrane. According to the results obtained with respect to membrane breakdown voltage, it is obvious that the packing of the membrane was disrupted in the presence of larger PEGs, and that the integrity of the membrane was impaired. These results are consistent with previously reported data, which showed that the sealing effect of PEG decreased in high concentrations (Spaeth et al., 2012). PEG400 seems to have been incorporated into the membrane, possible at the area of the head groups, and it did not cause leakage or permeability. In addition, much higher potential differences were required to break it down.

We believe that the cytoskeleton remains intact after membrane rupture following brain or spinal cord trauma, and that it can play a backbone role for phospholipids or PEG molecules to cover or be absorbed into the holes. Further investigation is needed to elucidate the effects of PEG on the configuration of the cytoskeleton and its interaction with the adjacent membrane in the vicinity of the injury site.

In conclusion, our results showed that the applied PEGs had no significant effect on the characteristics of the intact artificial membrane. Further, membrane sealing and stability can be promoted by low molecular weight PEG (PEG400) in low concentrations, and high molecular weight PEGs (1000 and 2000 Da)

decrease membrane stability and breakdown voltage in high concentrations. These data can be used in designing new polymer-mediated treatment strategies for central nervous system injuries.

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