Molecular modeling of the PEGylated bilayer as a model for the PEGylated liposome surface in the bloodstream

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ABSTRACT

The criteria for effectiveness of drug delivery liposomes (DDLs) are structural stability, site specific targeting, and lifetime in the bloodstream. Often, to increase the bloodstream lifetime, the DDL is coated with poly(ethylene glycol) (PEG). Although this helps to improve the lifetime, there exists plenty of room for improvement in bloodstream lifetime efficacy. The search for an alternative to PEG is a very active field of research, but to apply rational design to this, a knowledge of the mechanism through which PEG functions in a superior fashion to other superficially similar polymers must be determined, and currently our understanding of this is incomplete. We have used molecular dynamics simulation of a set of PEGylated membranes in varying conditions to gain insight into this. We have also performed MD simulation with the Cholesterol as a formulation component of DDL at its effect on stability of the PEGylated DDL. Lastly we looked at the factors in the targeted delivery of the novel targeting moiety identified with phage display experiments. The moiety couldn't increase the targeting efficacy of the DDL when tested invitro and invivo. By MD we could identify the factors responsible for this by investigating the surface structure of the DDL.

Keywords: drug delivery liposome, molecular dynamics simulation, PEGylated liposome

1 INTRODUCTION

The efficacy of liposomes as a drug delivery platform is severely hampered by their limited lifetime in the bloodstream. In order to increase their lifetime in the blood these drug delivery liposomes are coated with poly(ethylene glycol) (PEG) polymers. PEG is covalently bound to the lipid headgroups to the liposome formulation. The PEG polymer provides the sheilding effect by forming a "stealth sheath" impeding their uptake by the reticuloendothilial system (RES) and thus extending bloodstream lifetime. (from ~1-2 hours to ~72 hours)

While this result is substantial, there remains considerable room for improvement; red blood cells and some antibodies have a bloodstream lifetime that can be measured in months. The search for an alternative to PEG is a very active field of research(2), but to apply rational design to this, a knowledge of the mechanism through which PEG functions in a superior fashion to other superficially similar polymers must be determined, and currently our understanding of this is incomplete. We have used molecular dynamics simulation of a set of PEGylated membranes in varying conditions to gain insight into this.

We have simulated both gel and liquid-crystalline PEGylated membranes, at two separate densities of PEGylated lipid, and in the presence of three separate salts encounted in physiological conditions: NaCl, KCl and $CaCl_2$. The work we discuss represents a continuation of previously published work(2,3) where we determined that (1) Na^+ ions form close interactions with the PEG oxygens, with the PEG chains forming loops around them and (2) at molar density of 1:9 of PEGylated lipids, PEG penetrates the lipid core of the membrane for the liquid-crystalline membrane but is excluded from the tighter structure of the gel membrane (figure 1). In this study, we attempt to understand the effective surface charge on PEG layer due to its interactions with the salts present in the blood stream. The understanding of this phenomena is perticularly important since surface charge is known to play a role in opsonization.

In addition, the reason for failure of the novel (AETP) targeting moiety when attached to the DDL is investigated. The peptide moiety, discovered through phage display experiments, showed no increase in the targeting efficacy in *in vivo* and *in vitro* experiments. In our MD simulations we observed that excessive

coverage of the moiety by PEG polymer is the reason for this failure

To reduce the membrance defects in drug delivery liposome, often cholesterol is included in the membrane structure. The DDL can also be derivatized with targeting moieties to achieve site specific delivery. In the third set of simulations, we are currently investigating the effects of cholesterol on the DDL formulations and its effect on the PEG layer.

2 METHODS

We carried out MD simulations using mixed systems of PEG - phosphatidylethanolamine: phosphatidylcholine (PE-PEG:PC). The Gel system was prepared using distearol (DS) as the lipid tail whereas the liquid crystalline systems were created using dilinolyl (DL) lipid tails. The DSPC, DLPC, DSPE-PEG and DLPE-PEG molecules and the ions present in the solution were parametrized using the all-atom OPLS force field and the implementation of this is described in our publications [1,2,3].

In order to study the PEGylated bilayer interacting with salt ions, the Gel system was simulated with the three separate salts NaCl, KCl and CaCl$_2$. Also the gel and liquid crystalline systems were simulated without the salt.

To investigate the reason for the *invivo* failure of the novel AETP targeting moiety identified by phage display, we simulated the PEGylated gel and liquid crystalline membranes with AETP attached to PEG molecules by maleimide linker. For the control in the present study we also simulated the RGD peptide, which is well known to work in the invivo experiments.

In order to study the effect of the various concentrations of cholesterol in the PEGylated lipid bilyer, 5 different systems with proportion of 5:1, 4:1, 3:1, 2:1 and 1:1 of DSPC:CHOL were simulated.

The simulations were performed using the GROMACS 4.0 software package. For both systems the MD simulations were carried out over 200 ns.

RESULTS

From the MD simulations of PEGylated bilayer with the various salts present in the blood stream, we could observe following:
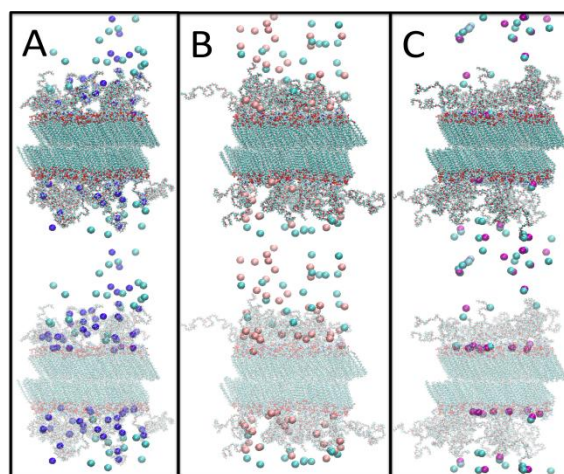

Figure 1: Visualization of the PEGylated mebranes at 1:9 PEGylated lipid formulation density with different salts all at 125 mM concentration: A) NaCl B) KCl and C) CaCl$_2$. Note the absence of Ca^{2+} ions in the PEG layer and their close association with both the lipid headgroups and the Cl$^-$ anions.
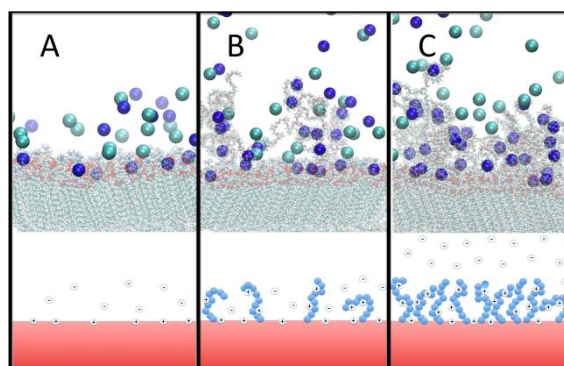

Figure 2: Visualization of gel (DSPC) membrane in physiological salt concentration (125 mM) a) without PEG (result from previous publication [3]) b) at 1:18 molar density PEGylated lipids and c) 1:9 molar density PEGylated lipids. We see the discussed mechanism whereby the Cl$^-$ ions are able to penetrate deeper into the PEG layer for the case of the lower PEG formulation.

1) Na+ ions association with PEG polymer was stronger as compared to the K+ ions and interaction between PEG and the Ca2+ ions is not observed. (Shown in Fig 1)
2) The Cl- ions that we previously observed to be excluded from the PEG region for the case of the gel membrane with a PEGylated lipid formulation density of 1:9, colocated to the PEG layer when the PEG density was reduced to 1:18 (Shown in Fig 2)

From the simulation of the PEGylated liposome with targeting moeity we could observe the following:

1) The novel AETP targeting moeity do not dip into the bilayer.
2) As the AETP moeity is more hydrophobic as compared to the hydrophilic RGD peptide, it interacts with PEG polymer which is known to interact with both hydrophobic as well as hydrophillic groups.
3) The Solvent Accessible Surface Area (as shown in Fig 3) results showed that the amount of area of the targetting novel AETP targetting moeity covered by PEG polymer is more than ~60%, as compared to ~40% for the RGD peptide.
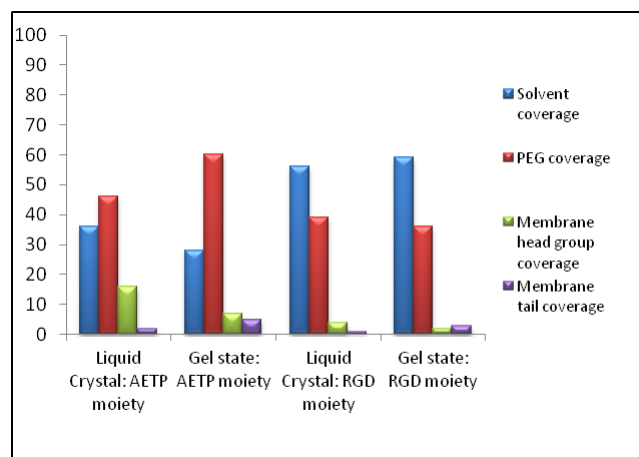

Figure 3: Solvent accessible surface area form MD Simulation, in this analysis percentage coverage of the novel moiety AETP compared to the known moiety (control) RGD peptide with solvent and PEG. The following plots show that the PEG coverage for AETP moiety is considerable higher as compared to the RGD peptide.

The simulations for the PEGylated liposome with cholesterol are currently ongoing.

3   DISCUSSION

In building on our previous work[3,] in the study of the PEGylated liposome interacting with different salt ions and PEG density, we show that, addition of salt slightly expands the PEG layer and expands the region of the PEG layer where the Na+ ions are located. Upon decreasing the molar density of PEG from 1:18 to 1:9, we observe that the Cl- ions excluded from the PEG region are able to collocate to the PEG surrounded by water pockets in the PEG layer large enough to incorporate them. The interaction between K+ ions and PEG was weaker than the case for Na+ ions, and the majority of K+ ions were found to be free in solution. The Ca2+ ions were found to interact very strongly with the membrane headgroups and Cl- anions but exhibit no binding to PEG.

In the study of analysis of cause of failure of the AETP targetting moeity, MD simulation reveals the sheilding of the targeting moeity by the PEG polymer occurs, with a considerably larger portion of the AETP moiety covered by PEG in comparison to the RGD peptide, and the AETP moiety situated deeper within the PEG layer. Hence in comparison with the RGD peptide, the AETP moiety has significantly less availability to target receptors as it has a significantly greater portion of its surface covered by PEG.

4   REFERENCES

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