COMPUTATIONAL STUDY OF HYBRID PLA-PEG NANOPARTICLES AS ANTIPLATELET DRUG CARRIERS

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INTRODUCTION

The use of drug delivery systems based on polymeric nanoparticles (NPs) has generated innovative therapeutic strategies for several diseases[1,2]. Polylactic acid (PLA) is one of the most commonly used polymers for the synthesis of NPs[3], PLA-NPs conjugated with hydrophilic molecules like polyethyleneglycol (PEG) presents improved blood circulation, clearance, biocompatibility, and less cytotoxicity[4].

The current high prevalence of cardiovascular diseases (CVD) and the vast application of *PEGylated* nanoparticles propose an excellent opportunity to develop novel therapeutic approaches for CVD. **In this work, we developed a computational plan to understanding the structural and physiochemical properties that establish the association of cilostazol and adenosine 5'-monophosphate (AMP), both antiaggregant compounds, loaded into PLA nanoparticles as novel nanosystem for CVD.**

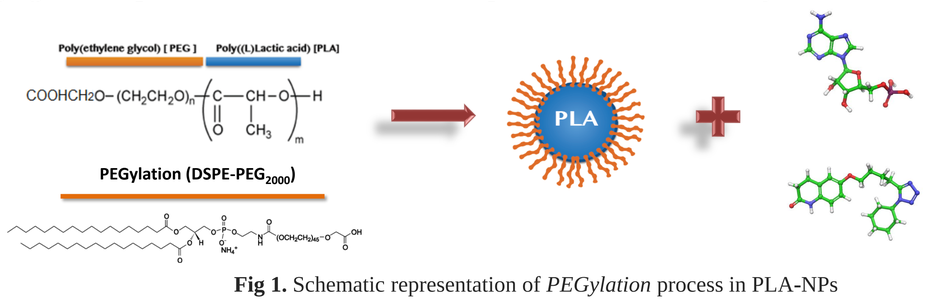

Fig 1. Schematic representation of *PEGylation* process in PLA-NPs

METHODS

A combination of Molecular Dynamics (MD) simulations and Docking techniques were employed to model and predict nanoparticle-drug interactions.

1. Building and optimization of molecules
- Pre-optimization and MD simulations of PLA nanoparticles (ReaxFF (reactive force field)[5] with LAMMPS software[6]).
- 3D structures of drugs

2. *Blind* Docking
- A grid volume enough to cover the entire surface of PLA core was built (126 x 126 x 126 Å^3), using a grid spacing of 0.5 Å.
- Docking with AutoDock4[7]
- Docking with AutoDock Vina[8]

3. Analysis of conformations
- 20 conformations of each drug were generated for each system.
- The docking poses were analyzed by examining their binding energy score and the most visited "hot spots" (putative binding sites). The most energetically favorable conformations were selected as the best poses.

4. Steered Molecular Dynamics
- Simplified system of PLA-DSPE-PEG─drug complexes.
- To determine an appropiate velocity spring constant and velocity of pulling.

4. Preparation of nanoparticles using a nanoprecipitation/self-assembly method

RESULTS

PLA polymer chains were built with the head-to tail connection and syndiotactic (D,L) configuration. The mean length was defined in 20 monomers.

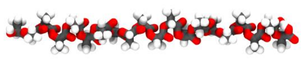

Fig 2. Poly D,L-lactic acid 20 monomer structure

10 PLA chains, each with 20 repeat units, were packed in a 100 x 100 x 100 Å^3 periodic box to assemble the PLA core.

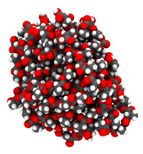

Fig 3. PLA core formation after 2ns of MD simulation

To ensure that the assembling methodology is correct, several MD simulations with more number of PLA chains were performed. Thus, 20, 40 and 80 PLA chains, with same MD conditions were used for this goal. Data collected along the trajectories were used to calculate molecular properties such as radius of gyration and asphericity and thus, to better characterize the shape of PLA core.

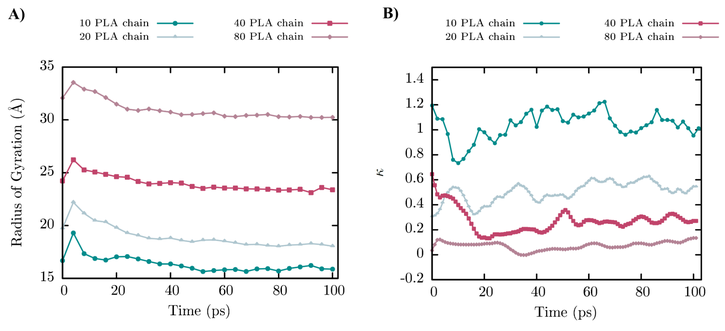

Fig 4. A) Radius of gyration and B) Triaxial parameter as a function of simulated time for PLA cores with different number of PLA chains (10, 20, 40, 80).

DSPE-PEG molecules were arranged around the PLA core randomly to obtain the final PLA-DSPE-PEG coated structure. This starting configuration was minimized followed by an equilibration at 310 K, using a canonical ensemble (NVT) and timestep of 1 fs.

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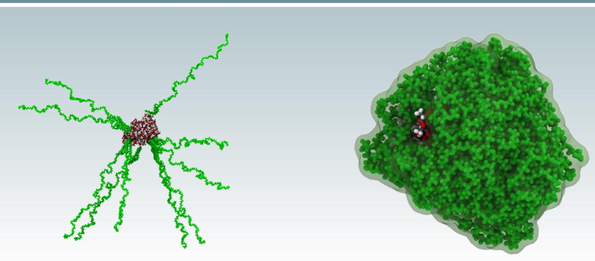


Fig 5. Initial and final conformation of PLA-DSPE-PEG coated structure after 60 ps of simulation

The main goal is to determine the spatial distribution of the drugs into polymeric nanoparticles. Especially, whether drugs would be found predominantly into PLA core or at the interface of PLA and DSPE-PEG.

Antiaggregant compounds were prepared based on their protonation state at pH 7.4. Partial charges were assigned and rotatable bonds were identified.

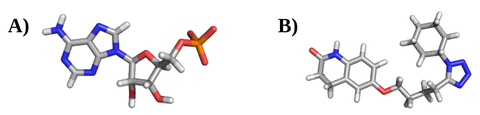
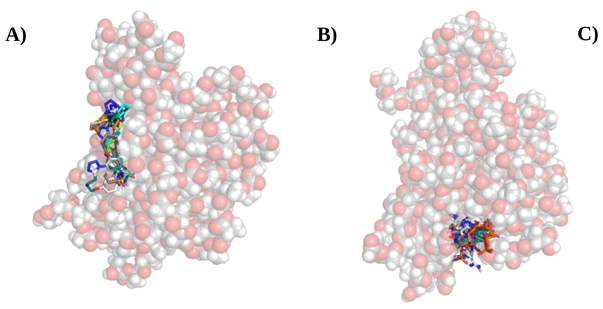


Fig 6. A) AMP and **B)** Cilostazol structures.

The results showed that both drugs are found **at the interface** of PLA and DSPE-PEG.

The most hydrophobic drug, cilostazol, allows a better alignment with the PLA unit than AMP and presented the highest affinity to PLA core, which was consistent with logP values (octanol/water partition coefficient).

AutoDock Vina predicted the strongest drug-polymer affinity in all cases, compared with AutoDock4.



	Cilostazol	AMP
Binding Energies (kcal/mol) of best poses using AutoDock4	-3.95	+5.31
	-3.73	+5.33
	-3.71	+5.35
	-3.62	+5.36
	-2.90	+5.36
	-2.73	+5.37
	-2.48	+5.58
Binding Energies (kcal/mol) of best poses using AutoDock Vina	-6.3	-5.7
	-6.0	-5.3
	-5.8	-5.2
	-5.7	-5.2
	-5.5	-5.1
	-5.2	-5.0
	-4.7	-4.7
AlogP	3.38	-3.13

Fig 7. Most visited putative binding sites of A) Cilostazol and B) AMP obtained in AutoDock Vina. C) ALogP values and computed estimated binding energies for each antiaggregant compound.

Steered molecular dynamics showed a similar behavior of drug release for AMP and Cilostazol, but the different hydrophobic character dominate the formation process of complexes.

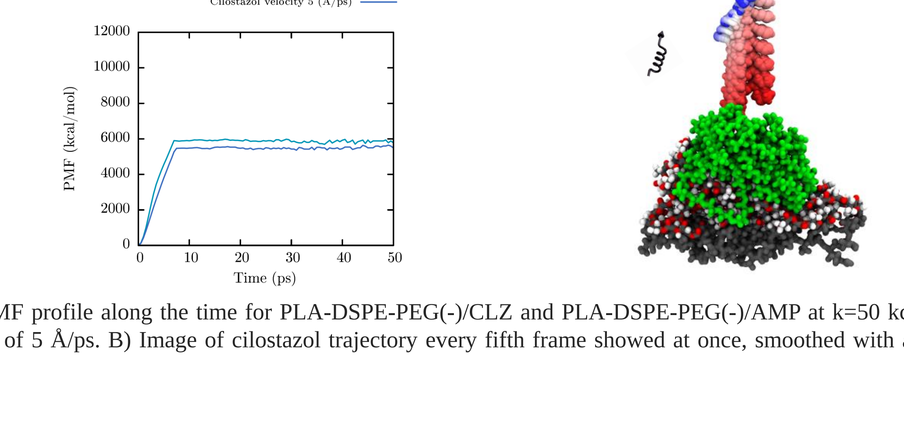


Fig 8. A) PMF profile along the time for PLA-DSPE-PEG(-)/CLZ and PLA-DSPE-PEG(-)/AMP at k=50 kcal/mol*Å^2 and velocity of 5 Å/ps. B) Image of cilostazol trajectory every fifth frame showed at once, smoothed with a 25-frame window.

Nanoprecipitation/self-assembly method allowed to create nanoparticles with a diameter ~ 100 nm and exhibited a suitable stability (data not shown). The analysis of samples by transmission electron microscopy (TEM) exhibited spherical shape nanoparticles, with a uniform diameter, and a high electron density on the Surface of nanoparticles due to the presence of DSPE-PEG groups that absorbs a higher amount of staining agent.

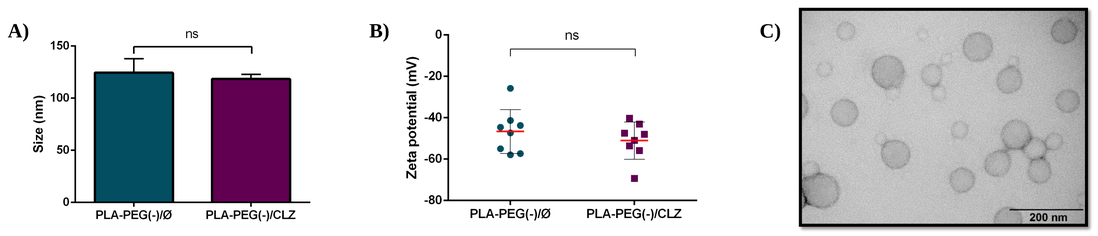


Fig 8. A) Size B) Zeta potential and C) representative TEM image of carboxylic acid-terminated nanoparticles

References

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CONCLUSIONS

- The structural characterization *in silico* of polymers-drugs provides a comprehensive understanding of the factors that contribute to NP formation and drug loading of nanocarriers based on polymeric NPs.
- This is the first time that the reactive force field ReaxFF was used to simulate polymer nanoparticle formation.
- A self-assembling process in which a core-shell structure is observed with PLA in the core and a DSPE-PEG in the shell was detected and this model is consistent with nanoprecipitation synthesis method.
- This approach represents an innovative strategy to evaluate the drug encapsulation of several antiplatelet drugs into PLA-NPs.