Tunable Membrane Modification of Milk Exosomes for Mucus Penetration

Chenzhen Zhang[1], Armin Vedadghavami[1], Matthew Warren[1], Ambika Bajpayee[1,2]
Departments of Bioengineering[1] and Mechanical Engineering[2], Northeastern University, Boston, MA, 02115, USA

Statement of Purpose: Oral drug delivery is the most preferred route by the patients. However, the protective mucus layer on the gastrointestinal tract limits the hydrophobic drugs absorption, including drugs, proteins, nucleic acid and so on. Bovine milk-derived exosomes (mExo) are non-irritative and biocompatible cargos having great potential in encapsulation and delivery of macromolecular biotherapeutics[1]. Also, compared to the exosomes collected from culture media, a higher yield of mExo can be achieved from the low cost of bovine milk. However, the hydrophobic domains in crosslinked mucin fibers and lipids may hamper transport of mExo through mucin due to the hydrophobic interaction[2]. Here, we use amphipathic DSPE-PEG(2000)-azide (**DPA**) or 1,2-dilauroyl-sn-glycero-3-phosphocholine (**DLPC**) as an insertion to provide modular surface tunability for mExo surface modification (**Fig. 1B**). The hydrophobic part of insertion will insert into lipid bilayer without disturbing membrane integrity, and the hydrophilic end will shield the membrane to prevent hydrophobic interaction with mucin. By introducing the DBCO-NHS linker, the amine group of peptides can be conjugated to the azide group of PEG. We will conjugate mucin-mimic peptide (MP, ISLPSPT)[3] and zwitterionic peptide with alternating positive and negative charges (AP, $(AEAK)_5$)[4] to further increase the mucus penetration rate.

Methods: The mExo was collected from fat-free bovine milk (Hood) using stepwise ultracentrifugation. The mExo was further purified using size exclusion chromatography (SEC) column (35nm pore size) and the size was confirmed using spectradyne particle analyzer and TEM (**Fig. 1A**). The modification procedures are shown in **Fig. 1B**, MP or AP peptide is firstly conjugated to DBCO-NHS in DMF solvent and then mixed with DPA to acquire DPA-MP/AP insertion. Finally, insertion is mixed with mExo in PBS for 1h at 37℃. To prevent the micelle formation, the concentration of insertion in PBS should be lower than 50μg/mL. ExoGlow, DBCO-cy5 and FITC can fluorescently label the mExo, DPA and peptide, respectively. Binding affinity between mExo and DPA was quantified based on microscale thermophoresis using the Monolith NT.115 (NanoTemper Technologies). As shown in **Fig. 1F**, native porcine intestinal mucus was added to transwell setup for mucus penetration study.

Results: TEM confirmed the diameter of mExo was (35-120nm) (**Fig. 1A**). The insertions shielded the charge of mExo (**Fig. 1C**). Although amphipathic polymer is widely used for Exo surface modification, incorporation of mExo and DPA was firstly confirmed by measuring a dissociation constant (K_d) of 347μM for the binding event (**Fig. 1D**). Confocal imaging confirmed the structure of PEGylated mExo based on overlap of green mExo and DPA-cy5 (**Fig. 1E**). As shown in **Fig. 1F**, PEGylated mExo had 2 times higher apparent permeability coefficient (Papp) than mExo. AP conjugated mExo significantly enhanced the Papp of PEGylated mExo. DLPC-mExo having neutral charge showed highest Papp.

Conclusions: Through the tunable hydrophobic insertion, PEGylation of mExo is achieved and significantly improves the mucus penetration. It is an efficient and modular method to create functionalized mExo via introducing different functional reagents (e.g. peptides and proteins) by click chemistry. Cloaking with zwitterionic peptides further enhanced mExo permeability through the mucin. Mucin like peptide, however, did not result in a significant change. The future work includes uptake study of modified mExo by intestinal epithelial cells and sue them for gene silencing.

References: 1. J. L. Betker et al. J Pharm Sci, 2019, 108, 1496-1505. **2.** A. Vedadghavami et al. Nano Today, 2020, 34. **3.** J. Leal et al. Int J Pharm. 2018, 553, 57-64. **4.** L.D. Li et al. Biophys J, 2013, 105, 1357-1365.

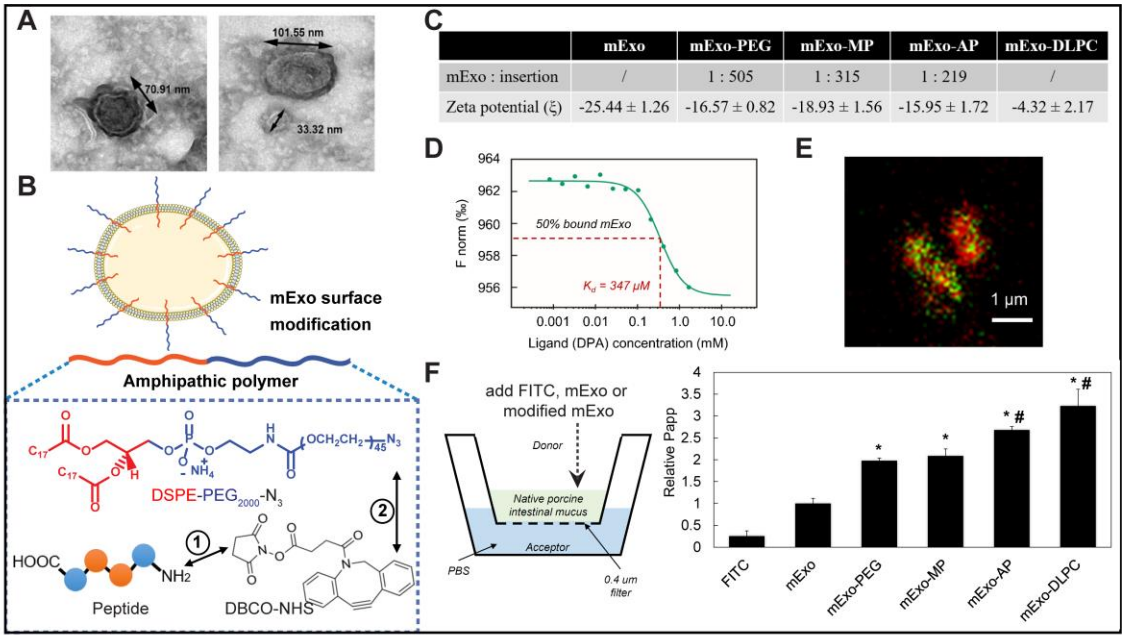

Figure 1A. TEM image of mExo. **B.** Schema of mExo surface modification. **C.** inserted molecules on mExo membrane and zeta potential. D. Binding affinity between mExo and DPA. **E.** Confocal imaging of green mExo and DPA-cy5. F. Mucus penetration study of mExo and modified mExo. * vs mExo, # vs mExo-PEG. $p < 0.05$.