Membrane active peptides and their interactions with REVs protein corona

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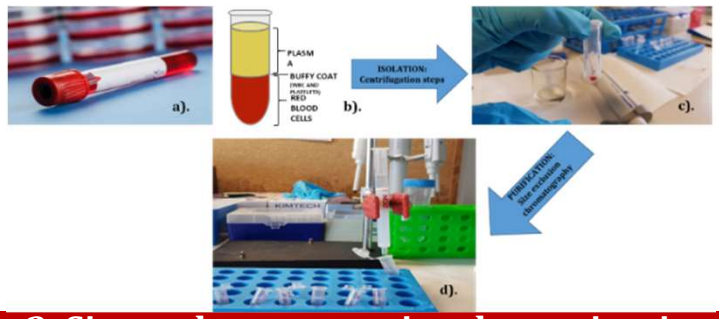
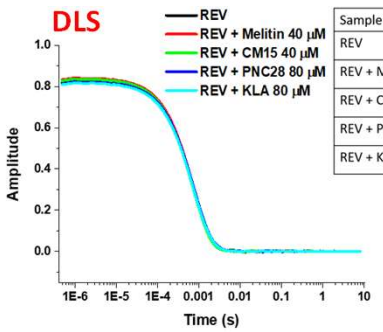
1. Introduction

Extracellular vesicles are lipid bilayer enclosed nanoparticles released by most cells and found in body fluids. They have the advantage of displaying native membrane protein on their surface and are involved in several biological processes like cellular uptake, immune response, and cancer progression. We used red blood cell derived vesicles (REVs) as improved model membrane to investigate action mechanism of membrane active peptides (MAPs) using several biophysical techniques such as polarized light spectroscopy[1], microfluidic resistive pulse sensing and freeze-fracture transmission electron microscopy. Several MAPs were selected to study their interaction with REV membrane such as Melittin (GIGAVLKVLTTGLPALISWIKRKRQQ), CM15 (KWKLFKKIGAVLKVL), KLA (KLAKLAKKLAKLAK) and PNC28 (ETFSDLWKLL) which exert not only antimicrobial but also anticancer effect.

2. REV preparation

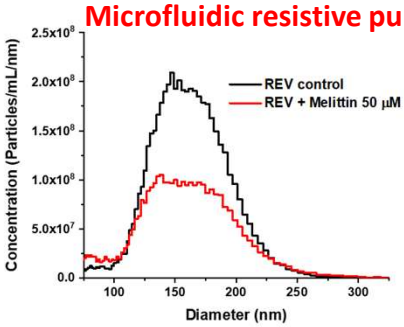


3. Size and concentration determination



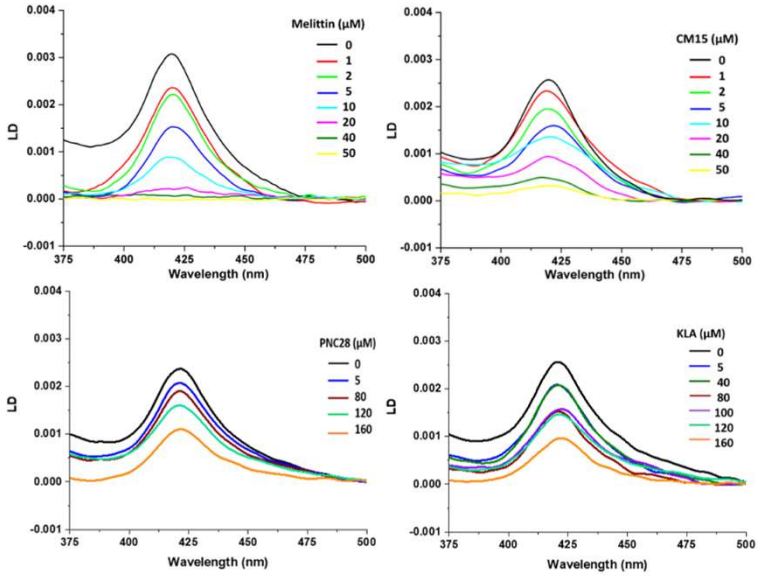
Sample	Mean diameter (nm)	Polydispersity (%)
REV	215±25	12
REV + Melittin 40 μM	225±50	22
REV + CM15 40 μM	218±28	13
REV + PNC28 80 μM	208±29	23
REV + KLA 80 μM	199±37	18

Microfluidic resistive pulse sensing



Top: Correlation function of particle size and hydrodynamic diameter table. Bottom: Size distributions of the REV and REV + 50 μM Melittin samples

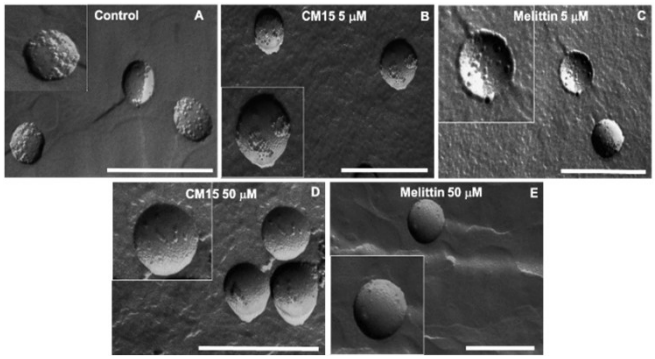
4. Polarized light spectroscopy



LD signal changes of REV sample admixed with increasing concentrations of MAPs

- A positive peak at ~420 nm corresponds to *Soret* band, arises from oriented membrane associated hemoglobin molecules of REV.
- This disappearance of the Soret band was concentration and peptide dependent indicating detachment of membrane-bound hemoglobin[1].

5. "Wiping-off" membrane surface proteins



6. Conclusion

- REV used as improved model membrane provides excellent platform to understand better interaction of membrane active compounds with protein corona of REV and **reveals new functions**[2] for MAPs.
- This further highlights therapeutic potential of EV focused in nanoparticle and surface engineering.

7. Acknowledgements

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8. References

1. Szigyártó, I.C., et al., 2018. ChemBioChem 19, 545–551. https://doi.org/10.1002/cbic.201700378
2. Singh, P., et al., 2020. Frontiers in Chemistry 8. https://doi.org/10.3389/fchem.2020.00703