Leveraging Hybrid Cell Membrane Cloaking for Minimizing Nanoparticle Phagocytosis

Alexander Egan, Biomedical Engineering
Mentor: Dr. Kuei-Chun (Mark) Wang, Assistant Professor
School of Biological and Health System Engineering

Introduction

Nanoparticles (NP) have emerged as powerful tools to serve as a delivery platform of therapeutic and diagnostic agents, providing a promising approach to target disease sites with precision. One such approach pioneered by the Wang lab uses monocyte (Mo) membrane-cloaked nanoparticles (MoNP) to exploit the natural targeting capabilities of immune cells toward inflammatory sites, such as those found in atherosclerosis [1]. Despite success, MoNPs face the challenge of limited circulation due to clearance from the immune system, resulting in a reduction of therapeutic potential and bioavailability. Red blood cells (RBC) have a significantly longer lifespan and express much greater biostability than Mo due to the expression of CD47, a "don't eat me signal" that helps to evade phagocytosis [2]. This project aims to address the limitations of MoNPs by hybridizing Mo membranes with RBC membranes to create R-Mo hybrid-coated nanoparticles (R-MoNPs), with the goal of increasing bioavailability while preserving the targeting potential of MoNP delivery platforms.

Methods

Cell Collection and membrane Isolation:

Monocytes (Mo) were created by differentiation of mouse bone marrow cells (BMCs). Isolation of monocyte membranes was performed via magnetic activated cell sorting (MACS) followed by the use of a membrane isolation kit. Red blood cells (RBC) were collected from mouse whole blood, and their membranes were harvested by rapid freeze thaw cycles and centrifugation.

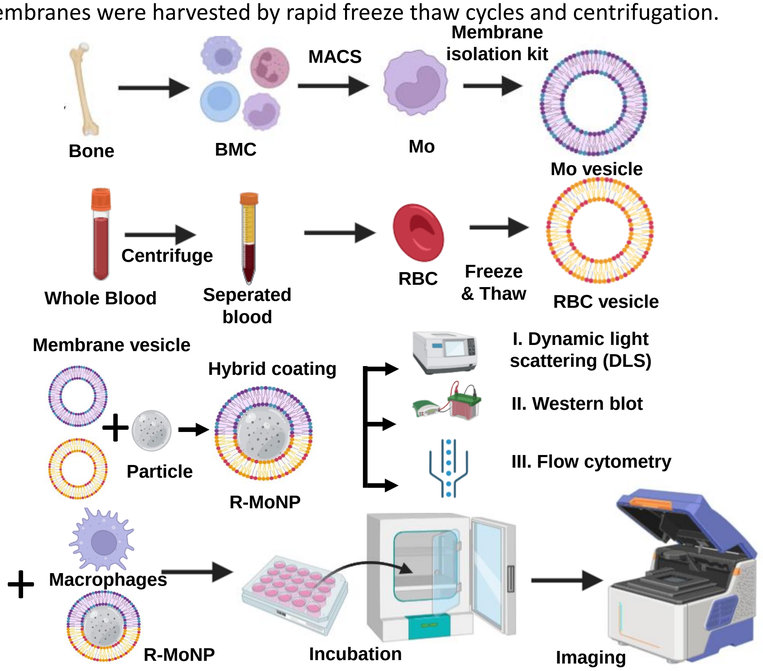


Figure 1: **Illustration of the formulation of RBC-Mo hybrid membrane-cloaked nanoparticles (R-MoNP).** The resulting R-MoNPs were characterized by DLS, Western blot analysis, and flow cytometric assays, to determine their physicochemical properties, surface proteins, and coating of dual membranes. R-MoNP were then incubated in RAW 264.7 cells and imaged. Biorender was used for figure creation

Results

I. R-MoNP characterization through DLS:

The uniform size distribution of R-MoNP was around 290nm. PDI and surface charge measurements show characteristics from each membrane; thus, indicating successful membrane coating. R-MoNP's mean size is closer to that of NP than free bound vesicles signifying successful coating around particle core.

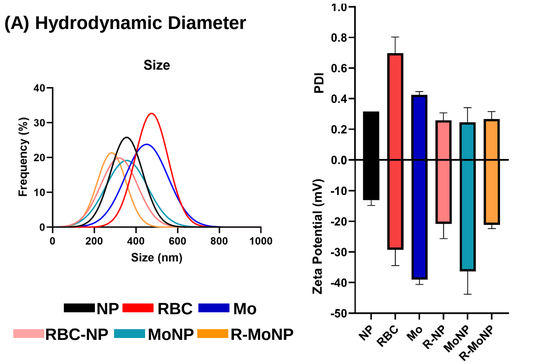


Figure 2: **DLS analysis of nanoparticle cores (NP), RBC, Mo, RBC-NP, MoNP, and RBC-NP.** (A) Hydrodynamic diameter; (B) Polydispersity index (PDI) and Zeta potential.

II. Surface protein characterization:

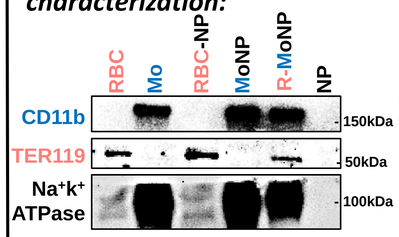
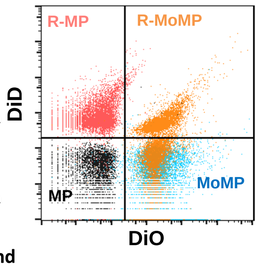


Figure 3: **Western blot analysis of Mo and RBC membrane proteins.** CD11b: Mo membrane protein; TER119: RBC membrane protein; Na^+K^+ ATPase: Plasma membrane protein across all cell types tested.

III. Coating analysis via flow cytometry:



Our data confirm successful coating of dual membranes onto cores, resulting in the formation of hybrid R-MoNP.

Figure 4: **Flow cytometric analysis of membrane cloaking on particles.** RBC membranes: DiD; Mo membranes: DiO; MP: polystyrene microparticles (MP).

IV. Nanoparticle phagocytosis assay:

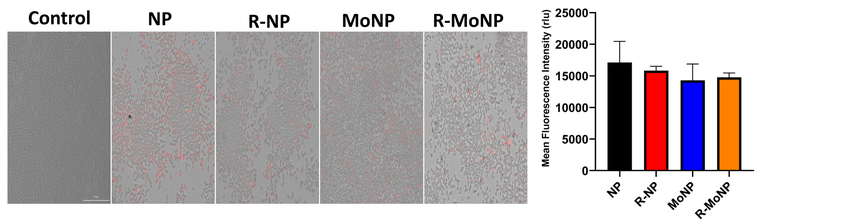


Figure 5: Phagocytic assay of membrane-cloaked NPs. (A) Fluorescently labeled R-MoNP, MoNP, R-NP, and uncoated NP were incubated with RAW 264.7 macrophages. The uptake of these particles by macrophages were measured by fluorescence microscopy. Lower uptake (i.e. fluorescence) indicates greater phagocytotic evasion. (B) Quantification was performed using ImageJ.

Conclusion and Future Work

We have successfully isolated RBC and Mo membrane vesicles from mouse whole blood and bone marrow cells, respectively. Characterization results confirm that dual membranes have been effectively cloaked onto the polymeric cores, creating R-MoNP. We are currently comparing the phagocytic properties of R-MoNP with nanoparticles coated with only RBC or Mo membranes, using an *in vitro* macrophage uptake model. Future directions include:

- Optimizing the ratio of RBC to Mo membranes to further enhance phagocytic properties.
- Incubating R-MoNP with phagocytic cells in mouse whole blood and with human vascular endothelial cells for further assessment.

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References

[1] Huang HC, Wang TY, Rousseau J, et al. Biomimetic nanodrug targets inflammation and suppresses YAP/TAZ to ameliorate atherosclerosis. Biomaterials. 2024;306:122505. doi:10.1016/j.biomaterials.2024.122505
[2] Patel et al. (2017). The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. The Journal of experimental medicine, 214(7), 1913–1923. https://doi.org/10.1084/jem.20170355

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Ira A. Fulton Schools of Engineering
Arizona State University