41

Enhancing Inflammation Targeting Using Tunable Leukocyte-based Biomimetic Nanoparticles

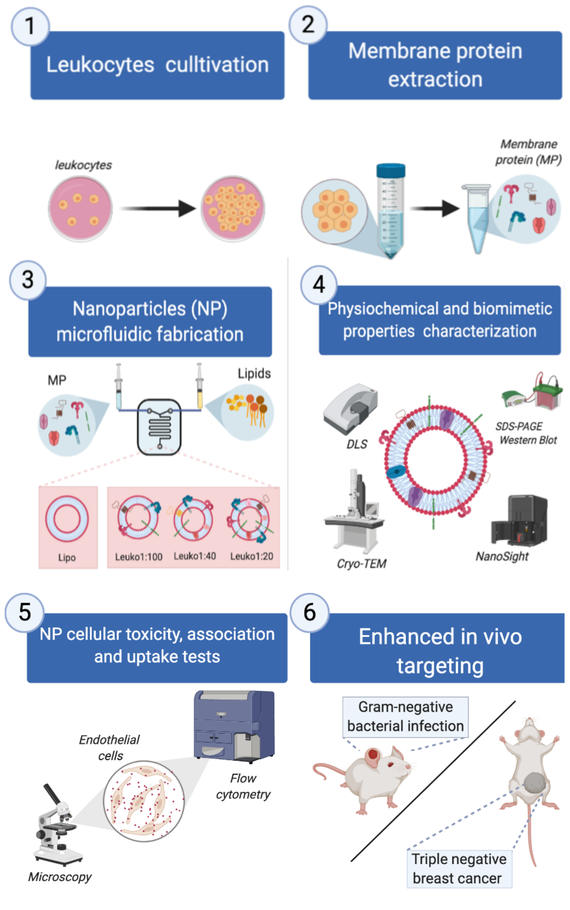
Assaf Zinger[1,2], Manuela Sushnitha[1,2] and Francesca Taraballi[1,2]
ayzinger@houstonmethodist.org

HOUSTON Methodist LEADING MEDICINE

Introduction

1. Biomimetic nanoparticles[1] aim to effectively emulate the behavior of either cells or exosomes. **Leukocyte-based biomimetic nanoparticles**[2], for instance, incorporate cell membrane proteins to transfer the natural tropism of leukocytes to the final delivery platform.
2. **Tuning the protein integration** can affect the *in vivo* behavior of these nanoparticles and alter their efficacy.
3. Here we show that, while increasing the protein:lipid ratio to a maximum of 1:20 (w/w) maintained the nanoparticle's structural properties, increasing protein content resulted in **improved targeting of inflamed endothelium** in two different animal models.
4. Our combined use of a microfluidic, bottom-up approach enabled the synthesis of reproducible biomimetic nanoparticles that have the potential to i**mprove treatment of inflammatory-based diseases** through targeted nano delivery.

Our Approach



Leukosomes Characterization

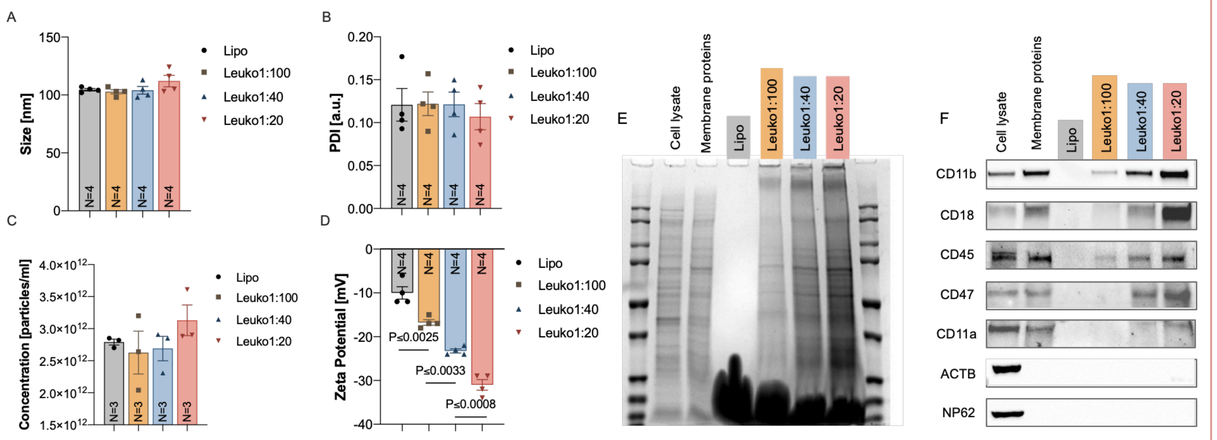


Figure 1. Physicochemical and biological characterization of Leukosomes.
(A) Average size, (B) PDI, (C) Particles number, (D) Zeta potential, (E) SDS-PAGE, (F) Western blot, ** p < 0.005, *** p < 0.001.

In Vitro Studies Using Inflamed Endothelium

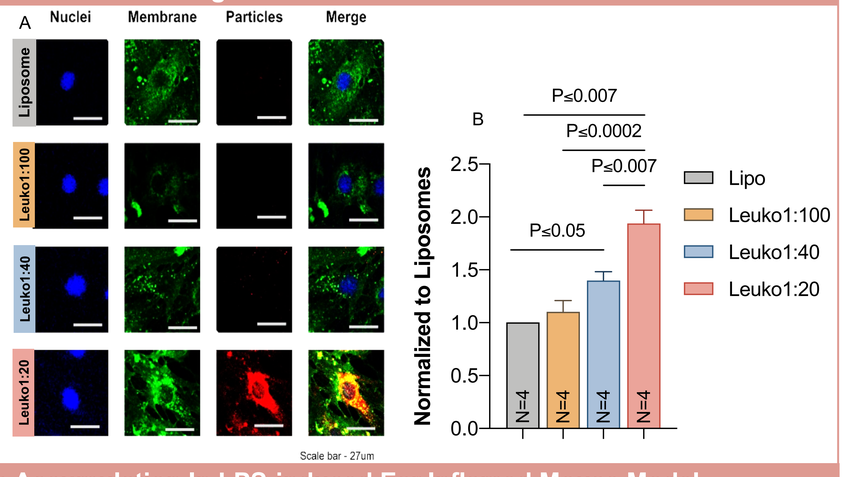


Figure 2. In vitro uptake and association of biomimetic NP by inflamed endothelial cells.
Increasing protein content on NP resulted in increased (A) uptake and association (B) evaluated by confocal microscopy and flow cytometry respectively, by inflamed endothelial cells.

In Vivo Leukosomes Accumulation In LPS-induced Ear Inflamed Mouse Model

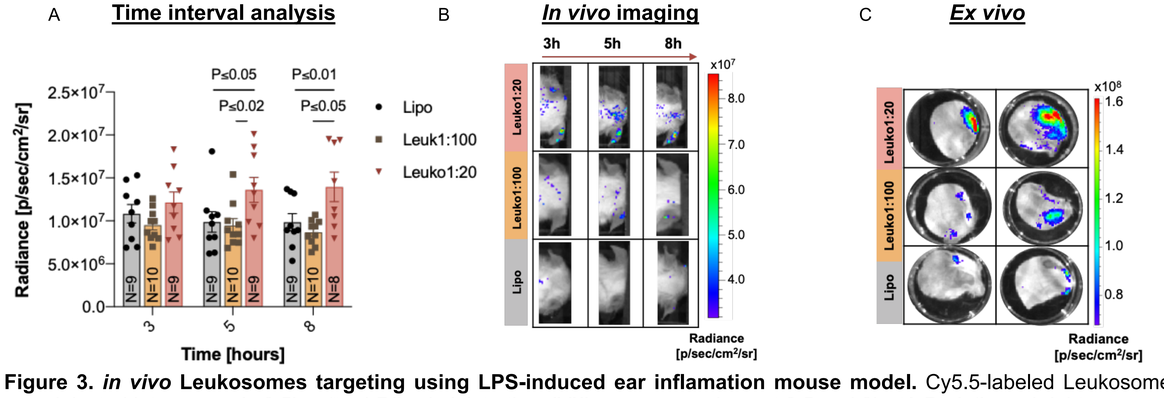


Figure 3. *in vivo* Leukosomes targeting using LPS-induced ear inflamation mouse model. Cy5.5-labeled Leukosomes were injected intravenously 0.5hr after LPS administration. IVIS scans were done at 3,5 and 8hr (A,B). Inflamed right ears were collected and the NP targeting ability was evaluated by IVIS (C), * p < 0.05, *** p < 0.001.

In Vivo Leukosomes Accumulation In Tumor Model

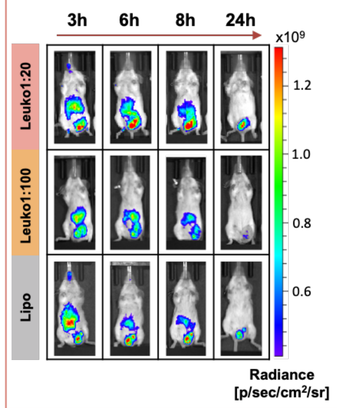


Figure 4. *in vivo* Leukosomes accumulation in 4T1 tumor bearing mice. Cy7-labeled Leukosomes were injected intravenously. IVIS scans were done at 3,6,8 and 24 hr.

Methods

- Leukosomes were **formulated** using 3 different protein:lipid amount ratio (1:20, 1:40, 1:100) by Nanoassemblr (Precision Nanosystems).
- **Size and Zeta potential** were measured by Zetasizer Nano.
- **Particles number** was measured by Nanosight.
- **Membrane proteins** on the particles were detected by SDS-PAGE and western blot.
- **Association and uptake** studies were analyzed by flow cytometry and confocal microscopy.
- ***In vivo* bio-distribution** was assessed by IVIS.

Conclusions & Future Work

1. These studies demonstrated that 100 nm Leukosomes containing **different loading amount of membrane proteins** can be fabricated in **reproducible manner.**
2. **Higher targeting effects** were assessed by increasing the membrane protein amount on the Leukosomes, tested *in vitro* by inflamed endothelia tests and two different *in vivo* models.
3. **Future work** will aim at exploring the mechanism of this targeting and the biological function by Leukosomes with higher amount of membrane protein.

Affiliation

[1] Regenerative Medicine Program, Houston Methodist Research Institute, Houston TX, USA
[2] Department of Orthopedics and Sports Medicine, Houston TX, USA

References

1. Molinaro et al., (2016). *Nature Materials, doi: 10.1038/nmat4644*
2. Zinger et al., (2020). *ACS Applied Biomaterials, https://doi.org/10.1021/acsabm.0c00685*