Biomolecular Corona and Therapeutic Lipid Nanoparticles: A Workflow for High-throughput Corona Isolation and Analysis

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Background

Lipid nanoparticles (LNPs) as an excellent delivery platform for gene therapy, are increasingly utilized into routine clinical practice. To optimize the delivery efficacy in various diseases, it is necessary to acquire a mechanistic understanding of how LNPs adapt to the biological systems in both normal physiological and pathological states. A key aspect of how the biological systems affect LNPs performance, and vice versa, is the formation of a corona around the nanoparticles when they contact biological fluids. [1] Most nanoparticles acquire a corona of biomolecules derived from the biological context they are exposed to. The formation of the biomolecular corona is believed to create a new biological identity for nanoparticles, and this is what biological systems actually perceive, rather than the pristine, uncomplexed nanoparticles. However, a solid method for rapid and high-throughput corona isolation for clinically relevant LNPs from plasma proteins, extracellular vesicles, and lipoprotein particles is still absent. [2]

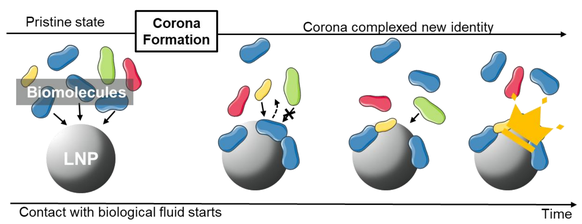
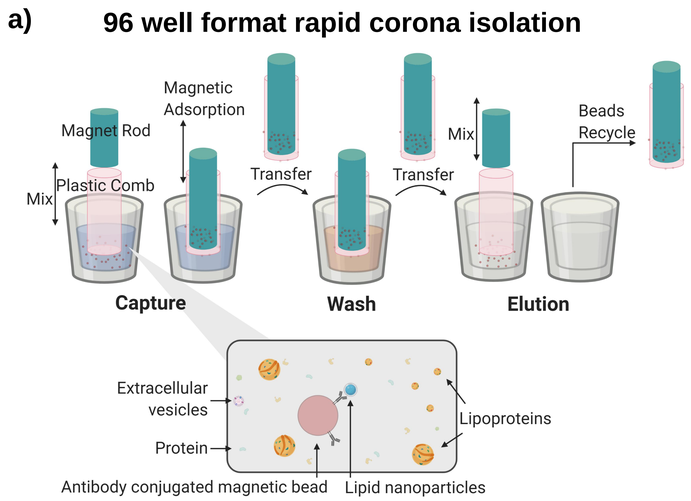
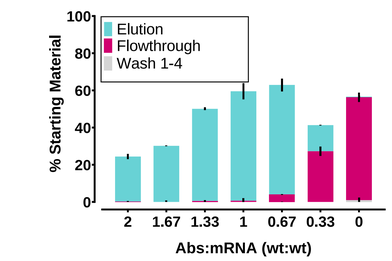


Figure 1. The evolution nanoparticle biomolecular corona. From left to right: After the administration of nanomedicine, they are in contact with biological fluids. Typically, biomolecules with high abundancy (in blue) arrive first to the surface to generate an initial-stage corona. Those adsorbed biomolecules of low-affinity (in blue) are subsequently replaced by other biomolecules with higher affinity (in yellow). Other molecules (in green), that may initially have a lower affinity for the pristine nanomedicine surface, adsorb due to interactions with the already adsorbed (yellow and blue) biomolecules. Some biomolecules (in red) may also interact with the surface, but more rarely. Figure adapted from Monopoli et al.[3]

Methods

A clinically relevant lipid nanoparticle formulation was employed to understand their corona composition within the lean and obese animal plasma. To address the separation of LNPs from lipoproteins, extracellular vesicles and plasma proteins, a novel affinity-based magnetic isolation protocol was developed, without modifying the LNPs formulation. Following corona isolation, physiochemical characterization and proteomics procedures were used to evaluate the efficacy of the corona isolation.





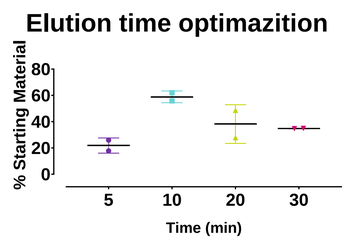


Figure 2. The isolation of LNPs/corona complex. **a)** An illustration LNPs/corona complex isolation. Antibody against ploylethylene glycol (PEG) was conjugated to magnetic Dynabeads®. After a designated period of biomolecular formation, Dynabeads® were employed to capture LNPs/corona complex in biological media. A series of PBS washes were used to remove unspecific bound proteins, extracellular vesicles and lipoproteins. Dynabeads® were removed and recycled once the LNPs/corona complex was eluted in elution buffer. The captured LNPs/corona complex associated with Dynabeads® Illustration created with BioRender.com. The antibody to LNPs ratio **b)** and the elution time **c)** were optimized to achieve optimal recovery rate.

Results

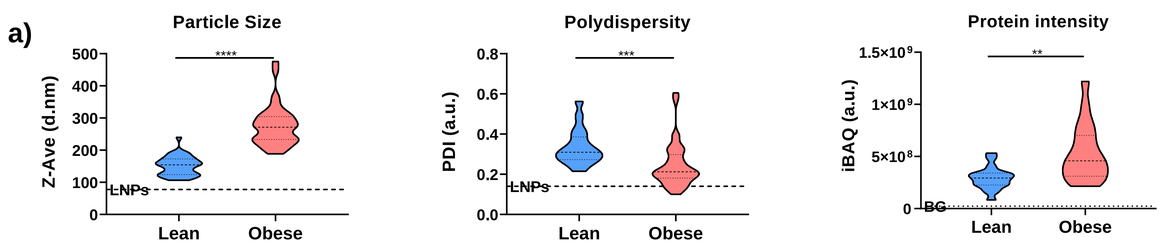
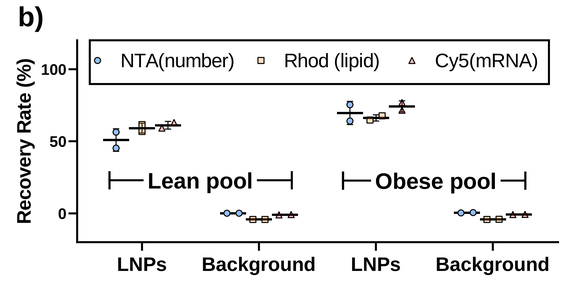


Figure 3. Corona formation in lean and obese plasma. a) A clinical relevant LNPs formulation was employed to exam the variation of corona formed in lean and obese individuals (n=9, respectively). This workflow help to identify significant differences in LNPs/corona complex size, dispersion and protein abundance between lean and obese conditions. **b)** The LNPs/corona recovery were calculated by analysing the florescent label of lipids (rhodamine) and mRNA (cy5). The number of particular objects were analyzed by nanoparticle tracking analysis (NTA). A clear separation between the LNPs/corona complex from plasma components was successfully achieved, with high LNPs/corona recovery.



Conclusion

- With this work flow, the harvest up to 96 corona samples were achieved within 50 minutes, requiring a minimal amount of LNP formulation.
- The workflow became our handle to tailor LNPs for specific therapeutic contexts. It allows us to quantify the interactions between nanoparticle features (e.g. formulation methods, novel lipids selection, helper lipids selection, components ratio matrix), and hundreds of coronal components and, how this affects the delivery system function. It holds great promise to improve LNPs' efficacy, reduces side effects, and lower costs.

References

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