Engineering Polymeric Nanoparticle Systems to Modulate Leptin Synthesis and Adipocyte Metabolism

Luis Andrade, Biomedical Engineering
Sarah E. Stabenfeldt, PhD[1], Eleanna De Filippis MD, PhD[2], James D. Hernandez, M.S.[2]
Arizona State University[1], Alix Mayo Medical School Mayo Clinic Arizona[2]

Ira A. Fulton Schools of Engineering
Arizona State University
SBHSE School of Biological and Health Systems Engineering
MAYO CLINIC

Introduction

- In the U.S. alone, the prevalence of **obesity** in adults has exceeded 40% and trends have shown that the epidemic has only continued to worsen, with **diabetes** playing a major role [1].
- Obesity is marked by elevated leptin, driving **insulin resistance** (IR), diabetes, and **adipose tissue** (AT) dysfunction [2].
- IL-4 acts as a regulator for leptin production, suppressing it to reverse IR and restore AT metabolic functionality [3].
- **Nanoparticles** are small particles <1000 nm in size [4].
- **Polymeric nanoparticles** have encapsulated payloads using polymer PLGA, with the potential to encapsulate IL-4 [5].

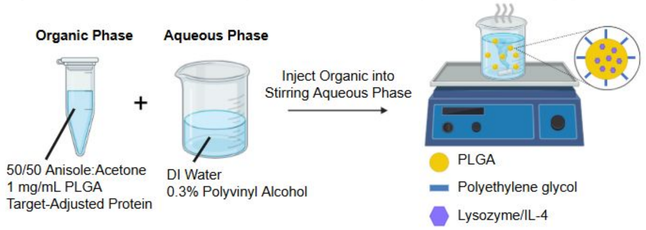

Figure 1. General Diagram of the Nanoprecipitation Method for Fabrication. [6]

Materials and Methods

Fabrication

- 1 mg/mL PLGA, 20 ng/mL Protein, 1:1 Acetone:Anisole
  - Using Nanoassemblr Ignite, 1 mL of NP formulation in center pump, 10 mL of DI water with 0.3% polyvinyl alcohol (PVA) surfactant in right pump, total flow rate of 20 mL/min
  - For washing, centrifuged suspensions at 10k RPM at 4°C for 40 minutes, and replaced supernatant with new DI for 3 cycles. Resuspended in 5 mL of new DI, vortex, and filtered through a 0.22 μm pore filter to remove large particulates.

Physicochemical Analysis

- **Dynamic light scattering (DLS)** used to determine size, polydispersity index (PDI), and zeta potential.
- Lyophilized samples resuspended in 2 mL of 1:1 ethyl acetate:DI, vortexed, centrifuged at 10k RPM for 15 minutes, and the aqueous layer collected for **UV-Vis** and **NanoOrange** assays.

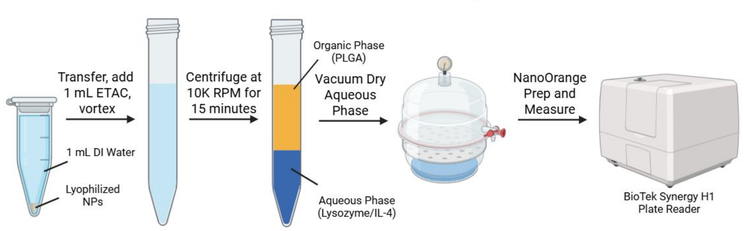

Figure 2. Extraction Protocol for Encapsulation Efficiency Determination.

Results

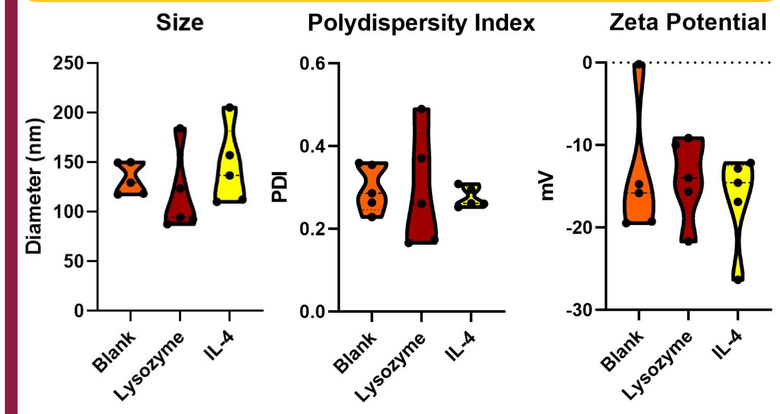

Figure 3. No Significant Differences Were Observed Between Any NP Groups. Average NP size, PDI, and zeta potential were approximately the same across groups (n=3). P-values were 0.4467, 0.9148, and 0.7660 for these respective NP properties, with Lysozyme as an IL-4 representative control.

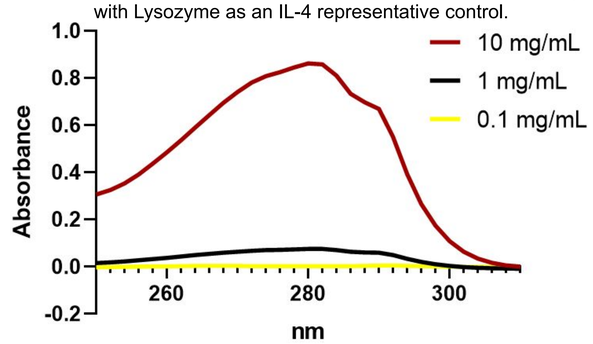

Figure 4. Linear Portion of Lysozyme Calibration Curve Using UV-Vis Spectroscopy. In the range of 0.1 to 10 mg/mL, the standard curve exhibited viable, positive absorbance readings of lysozyme with a peak around 280 nm. From this region, the linear regression analysis also demonstrated a high R^2 coefficient of 0.9925, sufficient for evaluating concentrations of unknowns.

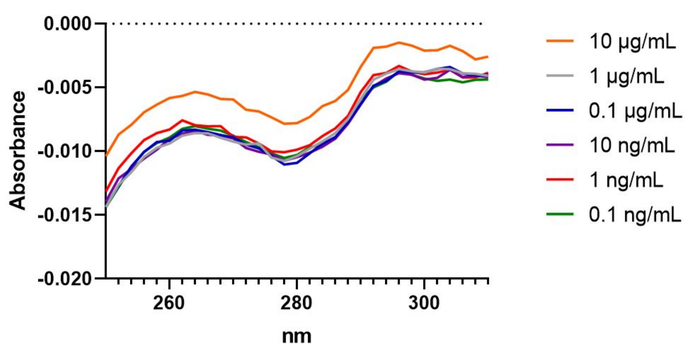

Figure 5. Nonlinear Portion of Lysozyme Calibration Curve Using UV-Vis Spectroscopy. In the range of 0.1 ng/mL to 10 μg/mL, the standard curve exhibited negative absorbance values. Additionally, clear distinguishable peaks could not be seen from 1 μg/mL and lower.

Results

RFU	10	6	3	1	0.6	0.3	0.1	0.06	0.03	0.01	0 (µg/mL)
BSA	69195	85135	83698	11747	29734	54140	10437	28940	42609	62760	83191
BSA	OVER	99373	OVER	11792	29041	68457	11234	45749	87741	OVER	OVER
LSZ	OVER	OVER	OVER	13751	38865	64088	12146	25052	49707	66722	81619
LSZ	OVER	OVER	OVER	14557	40172	63550	12027	34258	82460	OVER	OVER

Figure 6. Relative Fluorescent Intensity (RFU) Was Nonlinear Across Standard Curves of Bovine Serume Albumin (BSA) and Lysozyme (LSZ). Using a Biotek Synergy H1 plate reader to follow NanoOrange user guide, obtained RFU for standard curves of BSA and LSZ from 10 μg/mL to 0.01 μg/mL with 200 μL volume in each well. Gain was adjusted on the first well with the highest BSA concentration, excitation at 485 nm, emission at 590 nm, and were bottom-read using a 96-well plate.

Summary, Conclusion, and Future Directions

From the DLS measurements and statistical analysis, it was found that the fabricated PLGA NPs fell within reasonable ranges for all variables, with no statistically significant differences between any groups. The **mean size** for all groups was at least **under 150 nm**, **PDI** is roughly **0.3** for all groups, and **zeta potential** around **-15 mV**.

While these are promising results in terms of physicochemical analysis, the approaches used to derive encapsulation efficiency for these NPs were **incompatible**. The current and future steps are to discover a **high-sensitivity assay** that can be used to derive encapsulation efficiency, **optimize** the formulation protocol based on encapsulation data, then evaluate the **impact of NPs** in vitro/in vivo.

References

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