Cell-Derived Materials as Hydrogel Components

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INTRODUCTION/BACKGROUND: (250 words maximum)

Extracellular vesicles (EVs) are nanocarriers composed of a mixture of lipids, surface proteins, and membrane proteins that equip them with the necessary markers to reach their targets for cell-to-cell communication, making them attractive for targeted therapeutic delivery. [1] Additionally, protein nanocages are able to mimic the structure of viruses and are highly engineerable for antigen identities and combinations, making them very attractive for vaccine development and overall strengthening of the immune system (2). However, encapsulating cargo into these nanocarriers without compromising the cargo's activity and the nanocarrier's integrity is a challenging process. Studies have shown successful expression of cell-derived EVs and protein nanocages in Escherichia coli (E. coli). [2, 3] In this process, designed membrane proteins are encapsulated in EVs and secreted. Similarly, proteins self-assemble into icosahedral virus-like protein nanocages. Direct expression of antigen-containing nanocarriers in bacteria eliminates the need for additional engineering steps that could compromise material integrity. This study focuses on optimizing the expression, isolation, and characterization of these nanocarriers and integrating them into modified hyaluronic acid-based hydrogels. Loading into hydrogels reduces immunogenicity by limiting exposure to non-target tissues, ensures a sustained release rate, and provides an injectable medium for these nanocarriers, collectively enhancing therapeutic effectiveness. (4)

MATERIALS AND METHODS: (250 words maximum)

Miniprep and preparation. Followed standard procedures to clone genes of interest into E. coli (VNp15-NeonGreen and EPN01), propagate plasmids, and transform them into E. coli BL21 for expression and purification.

Expression of cell-derived nanocarriers. Followed standard procedures to express NeonGreen and nanocage proteins. Flasks with nanocage-expressing cells were induced at an OD of 0.8 to an IPTG final concentration of 1 mM. Cells transformed with plasmids encoding NeonGreen-encapsulated vesicles were induced ODs of 0.6 or 1 using final IPTG concentrations of 0.1, 0.5, and 1 mM to optimize expression and secretion of NeonGreen.

Protein nanocage expression, isolation, and characterization. The sample was spun down, and cells were lysed in a binding buffer (25 mM TRIS, 250 mM NaCl, 1 mM DTT, 20 mM imidazole) using sonication at 70% intensity for 10 minutes. The sample was subsequently purified using a HIS-Tag column, with binding and elution buffers (25 mM TRIS, 250 mM NaCl, 1 mM DTT, 500 mM imidazole). Samples were loaded onto SDS-PAGE to assess the purity of the samples collected from the column. Samples showing the presence of pure nanocage protein (mass of 37 kDa) were then subjected to dynamic light scattering (DLS) and transmission electron microscopy (TEM).

Extracellular vesicle expression, isolation, and characterization. Induced samples were monitored at different time points for optical density (OD) values and fluorescence to determine the secretion of green fluorescent protein (GFP) over time. On one note, vesicles were passed though titration fluid filtration (TFF) for isolation. On another note, some of the lysed vesicles were loaded on a HIS-Tag column and ran though a SDS PAGE to assess purity of the protein. Then, these samples were subjected to TEM, DLS, and an SDS PAGE.

RESULTS

Nanocage characterization. SDS characterizes method proteins based on their molecular weight. Given that the nanocages have a molecular weight of 37 kDA, we can say we purified them into the Elution 1 fraction (Figure 1). Once we determined the pure sample, we imaged it on TEM and saw nanocage-like structures, including an icosahedral hollow shape (Figure 2). Additionally, DLS shows a particle size of 27.7 nm for the nanocages, which corresponds to

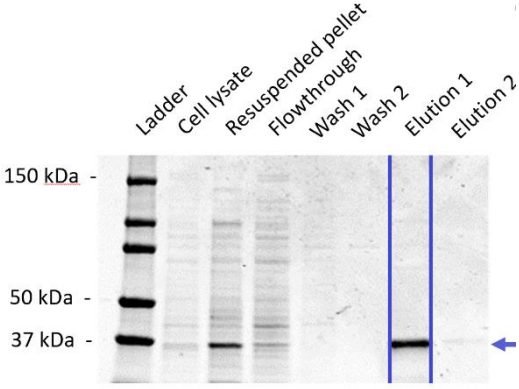

Figure 1: SDS PAGE analysis of nanocage purity.

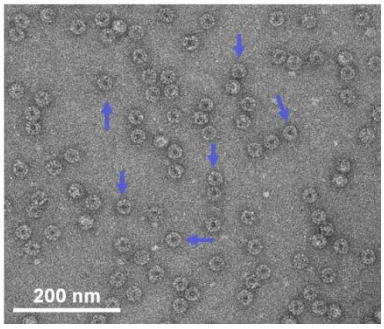

Figure 2: Transmission electron microscopy shows intact nanocages with desired morphology.

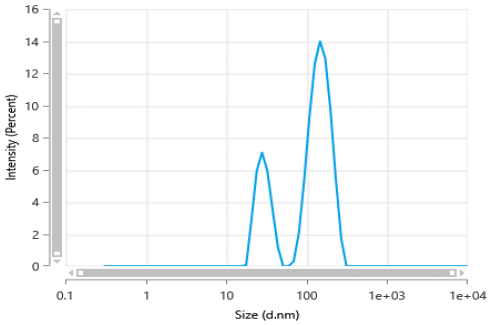

Figure 3: Dynamic light scattering shows bimodal particle size.

Vesicle characterization. Optimization data shows that inducing IPTG at an OD closer to 1 and to a final concentration of 0.1 mM secretes the most NeonGreen on a 24 hour period (Figure 4). We also saw some vesicle-like figures on the TFF, but were not fluorescent (Figure 5).

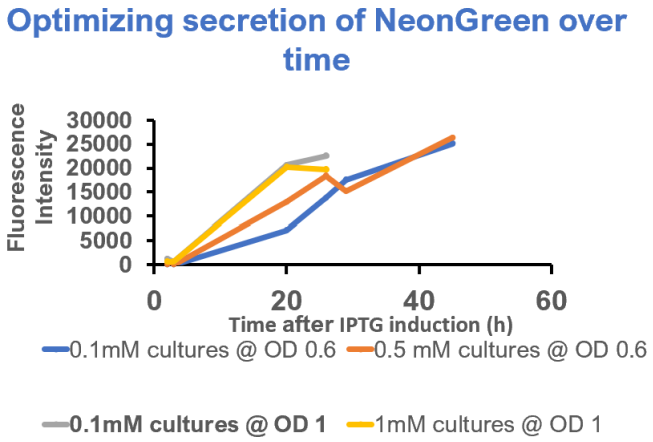

Figure 4: Optimizing secretion of NeonGreen over time.

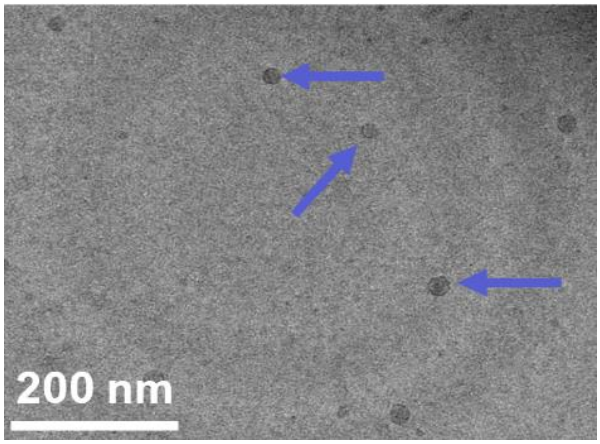

Figure 5: TEM of TFF retenta.

DISCUSSION/CONCLUSION

We used nano-characterization techniques to determine the average size of cell-derived particles following various procedures for particle isolation. Based on our SDS-PAGE (molecular weight), TEM (structure), and DLS (size) data, we can conclude that we have successfully expressed and purified protein nanocages. Additionally, we explored the use of a membrane-binding peptide to induce the secretion of cargo proteins into bacterial vesicles. However, our data are still insufficient to confirm that we are successfully secreting vesicles. Future steps include performing Nanoparticle Tracking Analysis to determine additional vesicle properties and introducing a lipophilic marker to label vesicle populations and track them by fluorescence. We also aim to improve the nanocage yield by optimizing expression conditions (e.g., density at induction, IPTG concentration, strain of cells used). We are considering incorporating antigens onto nanocages or loading biological cargo into vesicles and comparing the efficacy of these methods. Additionally, we plan to generate a library of polymer-nanoparticle hydrogels using various cell-derived polymers and monitor cargo release rates and hydrogel decomposition using fluorescent nanocages or vesicles.

REFERENCES

1. Peruzzi (2024) *Nat. Commun.*
2. Eastwood (2023) *Cell Rep.*
3. Hsia (2016) *Nature.*
4. Correa (2021) *Chem. Rev.*