Characterization of Extracellular Vesicles Produced from Glycocalyx-Engineered Cells *in vitro*

CNF Project Number: 2272-14

Principal Investigator(s): Dr. Matthew Paszek

User(s): Erik Chow

Affiliation(s): Department of Biomedical Engineering, Cornell University

Primary Source(s) of Research Funding: National Science Foundation Graduate Research Fellowship

Contact: paszek@cornell.edu, ec829@cornell.edu

Primary CNF Tools Used: Malvern NS300 NanoSight

Abstract:

Extracellular vesicles are key mediators of intercellular communication and are a rising area of biomedical research in disease diagnostics and therapeutics. However, surprisingly little is known about the glycobiology of extracellular vesicles, despite the fact that the cell glycocalyx dictates many of a cell's interactions. Specifically, very little is known about the role of the glycocalyx in EV production and function or whether EVs can be engineered through rational manipulation of the glycocalyx. Here we describe recent efforts to investigate the effects of glycocalyx engineering on the production of extracellular vesicles *in vitro*.

Summary of Research:

Extracellular vesicles (EVs) play a key role in intercellular communication. They have been shown to carry a wide range of cargoes, including DNA, coding and non-coding RNAs, and proteins. Because of the diverse nature of their cargoes, and their innate biocompatibility, EVs have quickly become a prominent focus in numerous areas of biomedical engineering research, including disease pathogenesis, diagnostics, drug delivery, and targeted therapies.

However, there are still many aspects of EV biology which are still poorly understood. One largely unexplored area is the significance of the glycocalyx — a polymer meshwork of proteins, nucleic acids, and glycans, which dictates numerous cellular interactions — on EV biogenesis and function. Specifically, the capacity for the production of rationally designed EVs through engineering of the glycocalyx remains poorly understood. It has been previously shown that engineering the glycocalyx can result in membrane morphologies which are favorable for the formation of certain types of EVs [1]. This report summarizes research from the last year which demonstrates that glycocalyx engineering, specifically be the overexpression of the mucin glycoprotein MUC1, results in an increase in production of EVs with size characteristics consistent with classical exosomes and microvesicles.

To engineer the glycocalyx, MCF10A cells were genetically engineered to overexpress a MUC1.mOxGFP construct on the cell membrane, hereafter referred to as MCF10A-1E7 cells. Expression of MUC1-mOxGFP in MCF10A-1E7s was tied to a tetracycline-inducible promoter, and cells were treated with doxycycline (Dox) for 24 hours at a concentration of either 0.1 ug/mL or 1 ug/mL to induce MUC1-mOxGFP overexpression. MCF10A cells engineered with only the promoter but no MUC1-mOxGFP construct, hereafter referred to as MCF10A-rtTA cells, were used as a negative control. After Dox treatment, the cells were switched to serum-free media and cultured at 37°C, 5% CO_2 for 15 hr to 18 hr. EV-containing media was harvested and EV concentration was measured by nanoparticle tracking analysis (NTA) using the Malvern NS300 NanoSight.

Figure 1 shows that media harvested from MCF10A-1E7 cells contained a higher concentration of EVs of sizes consistent with exosomes (50-150 nm) and microvesicles (100-1000 nm) compared to MCF10A-rtTA cells. Additionally, these data suggest that EV production in MCF10A-1E7 cells increased in a Dox concentration-dependent manner. These findings are further supported by Figure 2, which shows the EV concentrations of each of nine fractions obtained by density gradient ultracentrifugation

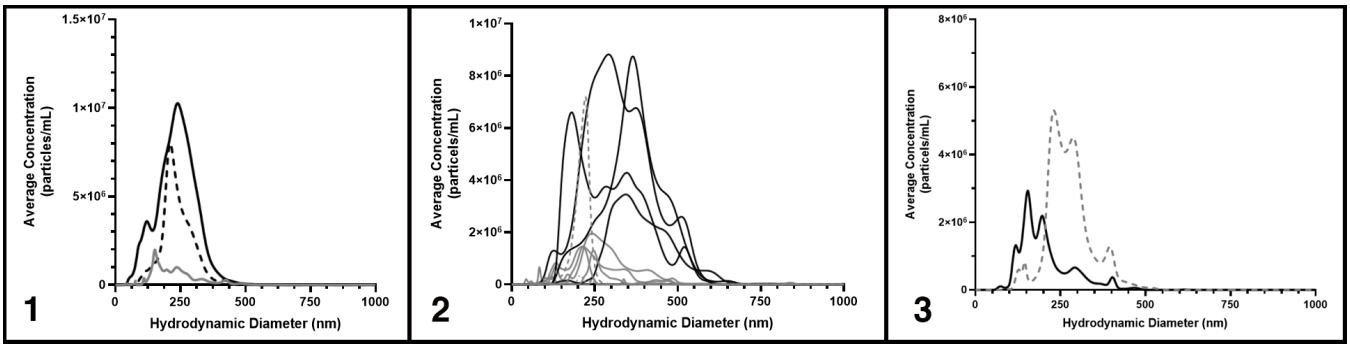

Figure 1. left: EV preparations from glycoengineered MCF10A cells. EV particle concentrations measured by NTA from MCF10A-rtTA cells (solid gray line), MCF10A-1E7 cells induced with 0.1 ug/mL Dox (dashed black line), and MCF10A-1E7 cells induced with 1 ug/mL Dox (solid black line). Curves represent the average reported concentration from five video recordings.

Figure 2, middle: Density gradient centrifugation of EVs from MCF10A-1E7 cells. EV particle concentrations measured by NTA from deionized water (neg. control, gray dotted line); 240 nm NIST nanospheres (pos. control, gray dashed line); density gradient fractions F1, F2, F7, F8, and F9 (solid gray lines); and density gradient fractions F3, F4, F5, and F6 (solid black lines). Curves represent the average reported concentration from five video recordings

Figure 3, right: Triton X-100 Treatment of EVs from MCF10A-1E7 cells. EV particle concentrations measured by NTA from mock-treated sample (neg. control, gray dotted line) or sample treated with Triton X-100 (solid black line). Curves represent the average reported concentration from five video recordings.

of EVs from MCF10A-1E7 cells using a protocol adapted from Jeppesen, et al. [2]. The vast majority of EVs were contained in fractions F3, F4, F5, and F6, which is consistent with previously published data of exosome samples [2]. Deionized water and NIST 240 nm nanospheres were used as negative and positive controls for EVs, respectively.

In order to validate that the particles analyzed by NTA were biological in origin, EVs from MCF10A-1E7 cells were treated with 0.1% Triton X-100 at room temperature for 10 min to solubilize lipid membranes. Mock-treated MCF10A-1E7 EVs were used as a negative control. Figure 3 shows the dramatic reduction in both the EV concentration and the average EV size profile following Triton X-100 treatment, demonstrating that the samples do in fact contain EVs bound by biological lipid membranes.

Conclusions and Future Steps:

Altogether, these data provide the first evidence that EV production can be controlled by engineering the glycocalyx of cells. Further experiments are needed to conclusively prove that the particles detected by NTA contain the classical exosome or microvesicle markers, such as CD63, ALIX, TSG-101, and Annexin A1.

Additionally, future experiments will strive to study the glycobiology of these engineered EVs. More NTA experiments will be conducted at the CNF as part of an effort to reliably segregate exosomes and microvesicles in order to more precisely study the exosome and microvesicle glycocalyx properties.

References:

[1] Shurer, C. R., Kuo, J. C.-H., Roberts, L. D. M., Gandhi, J. G., Colville, M. J., Enoki, T. A., Pan, H., Su, J., Noble, J. M., Hollander, M. J., O'Donnell, J. P., Yin, R., Pedram, K., Möckl, L., Kourkoutis, L. F., Moerner, W. E., Bertozzi, C. R., Feigenson, G. W., Reesink,

H. L., and Paszek, M. J. (2019). Physical Principles of Membrane Shape Regulation by the Glycocalyx. Cell, 177(7), 1757-1770. https://doi.org/10.1016/j.cell.2019.04.017.

[2] Jeppesen, D. K., Fenix, A. M., Franklin, J. L., Higginbotham, J. N., Zhang, Q., Zimmerman, L. J., Liebler, D. C., Ping, J., Liu, Q., Evans, R., Fissell, W. H., Patton, J. G., Rome, L. H., Burnette, D. T., and Coffey, R. J. (2019). Reassessment of Exosome Composition. Cell, 177(2), 428-445. https://doi.org/10.1016/j.cell.2019.02.029.