A Systematic Framework for the Scalable Loading of Therapeutic siRNA and PMO into Exosomes

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Abstract

Exogenous loading methods enable the incorporation of higher concentrations of therapeutic agents, including small interfering RNA (siRNA) cargos and Phosphorodiamidate Morpholino Oligomer (PMO) cargos, into exosomes. To achieve clinically relevant dosing, scalable production of the loaded exosomes is required. Utilizing electroporation as the exogenous loading approach, we describe the evaluation of scale-out versus scale-up manufacturing strategies and identify the optimal method for further development.

Methods and Results

Methods: Engineered exosomes derived from 293F cells were loaded with PMO or siRNA by electroporation at pre-defined exosome-to-cargo ratios. Each standard reaction volume was 800 µL and after electroporation the unloaded cargo was removed by post-loading clean-up procedures. To address dosing requirements, we compared scale-out and scale-up loading strategies. In the scale-out approach, multiple 800 µL reactions were electroporated (BioRad; Gene Pulser Xcell) in parallel and processed for characterization. In the scale-up approach, a single reaction chamber was used to electroporate (Harvard Bioscience; BTX ECM 630) a 10-fold higher volume, followed by identical post-loading processing and characterization. These experiments were conducted once due to the limited availability of the BTX demo unit.

Results: Because different electroporators were required for the various reaction volumes, our initial step was to verify that both instruments produced comparable exosome loading efficiencies for the siRNA and PMO cargos. After confirmation, we observed that relative to a single loaded reaction, both the scale-out and scale-up approaches resulted in similar loaded exosome yields and corresponding cargo recoveries. These approaches can therefore be integrated, whereby scaled-up reactions are further scaled-out to enable production of substantially larger batches of therapeutic exosomes. This outcome was consistent for both the siRNA and PMO cargos.

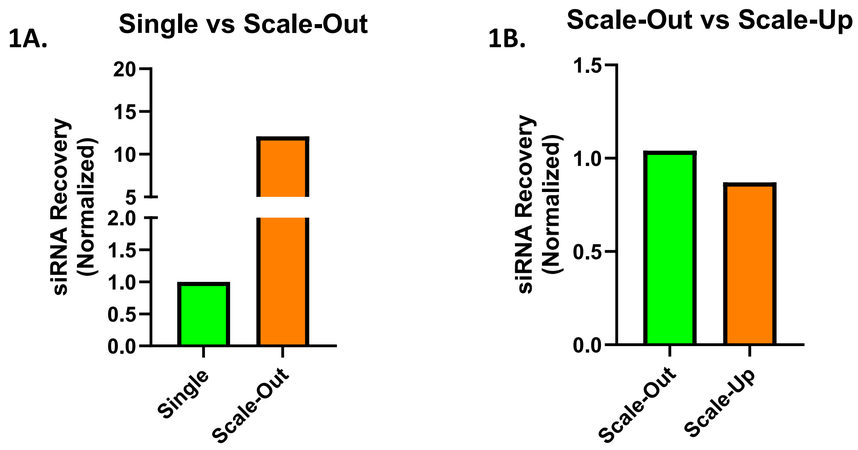

Figure 1**. RT-qPCR analysis; siRNA recovery:** Exosomes were loaded with siRNA either as individual low-volume reactions or as a single large-volume reaction and were processed for free (unloaded) siRNA removal and loaded exosome characterizations. The amount of siRNA loaded within the exosomes was determined post siRNA isolation and RT-qPCR analysis. A standard curve was generated to determine the amount of siRNA obtained within each loading method.

Figure 1A.
The siRNA yield from ten single loaded reactions processed together as one pooled sample was roughly 10 times higher than that from the single-reaction control. This demonstrates that scaling out multiple loaded reactions preserves the total amount of cargo incorporated into the exosomes, with the recovered cargo matching the input amount.
(Scale-out siRNA yield normalized to the single reaction yield)

Figure 1B. A large electroporation mixture was prepared and split in half: one portion was electroporated as a single large-volume reaction (scale-up), and the other was distributed across multiple cuvettes for individual reactions (scale-out). After electroporation, the scale-out reactions were pooled, and both methods underwent identical post loading steps to clean the loaded reactions and remove free siRNA. The results show similar amounts of siRNA obtained amongst the methods tested
(The recovered siRNA amount for both scaled reactions were normalized to their respective input amount)

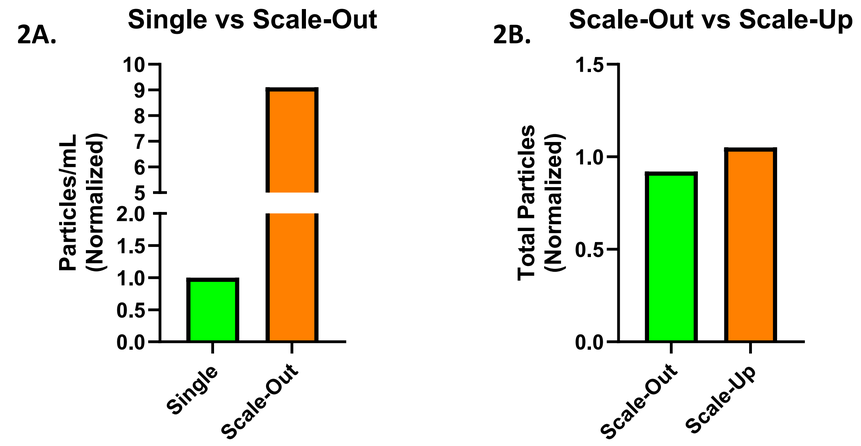

Figure 2. NTA analysis; exosome recovery: Exosomes were loaded with siRNA either through multiple small-volume reactions or a single large-volume reaction, After the post loading procedures, the samples were analyzed on the NTA.

Figure 2A. Ten individual loaded reactions were pooled, processed for siRNA removal, and concentrated to match the volume of the single-loaded reaction control. NTA showed that the scaled-out preparation reached a particle concentration 9.1 times higher than the single loaded reaction.
(Scale-out exosome concentration normalized to the single reaction concertation)

Figure 2B. A single large electroporation mixture was prepared and divided into two equal portions. One half was processed as a single large-volume reaction (scale-up), while the other was split across multiple cuvettes for individual reactions (scale-out). Following electroporation, the scale-out reactions were pooled, and both preparations underwent the same post-loading cleanup to remove unincorporated siRNA. The results show the recovered number of exosomes closely matched the initial input for both methods.
(The recovered numbers of total particles obtained were normalized to their respective input amount)

Figure 3. Nanodrop and NTA analyses, PMO and exosome recoveries: Exosomes were loaded with PMO either as individual low-volume reactions or as a single large-volume reaction and were processed for free (unloaded) PMO removal and loaded exosome characterizations. The amount of PMO loaded within the exosomes was determined using both NTA & the Nanodrop spectrophotometer.

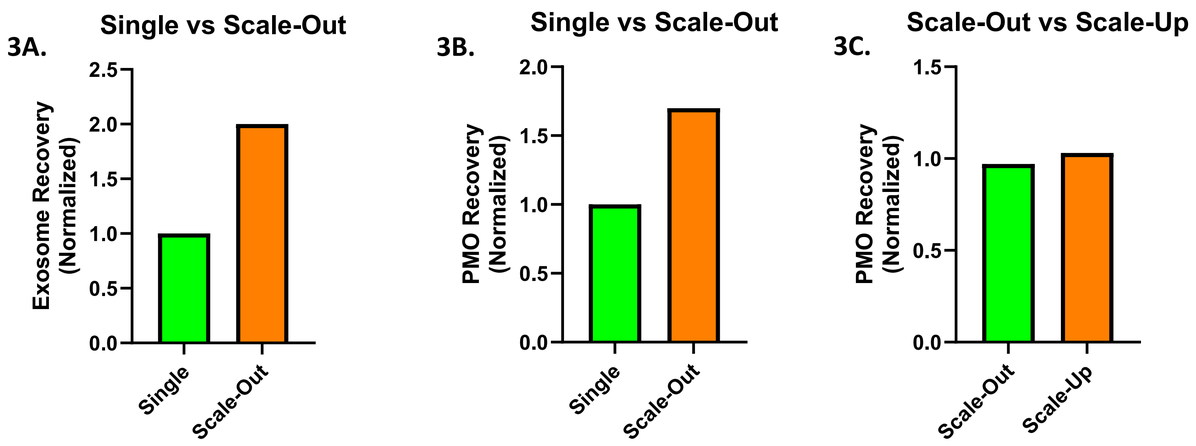

Figure 3A. PMO was loaded either in a single or in two combined electroporation reactions (scale-out). The same post-loading cleanup procedures were performed. The scale-out sample showed approximately double the exosome recovery and **3B.** nearly double the PMO recovery, compared with the single-reaction preparation. The scale-out method of loading proves effective for loading PMO cargo.
(Scale-out Exosome and PMO yields normalized to the respective single reaction yield)

Figure 3C. A large electroporation mixture was prepared and split in half: one portion was electroporated as single large-volume reaction (scale-up), and the other was distributed across multiple cuvettes for individual reactions (scale-out). After electroporation, the scale-out reactions were pooled, and both methods underwent identical post loading steps to clean the loaded reactions and remove free PMO. Similar amounts of PMO were obtained amongst the methods tested.
(The recovered PMO amount for both scaled reactions were normalized to their respective input amount)

Disclosure

This work is proprietary of Capricor Therapeutics, Inc. (NASDAQ: CAPR)

Conclusion

Both scale-out and scale-up strategies demonstrated comparable loading efficiencies, exosome and cargo recoveries relative to the individual loaded reaction control. By integrating these approaches, where we scale up individual reactions and subsequently scale them out, we can achieve substantially larger yields of loaded therapeutic exosomes to meet the clinical dosing needs. This strategy proves effective for both the siRNA and PMO cargos, establishing a practical framework for manufacturing clinically relevant quantities of loaded exosomes, sufficient to meet clinical patient dosing requirements.