

Dr. Wing-Fu Lai 16th July 2018

Corresponding Guest Editor  
Methods and Techniques for the Development of Drug Delivery Systems

*Journal of Visualised Experiments (JoVE)*

*Institute of Pharmaceutical Science*

School of Cancer and Pharmaceutical Sciences

at Guy’s, King’s College

and St Thomas’ Hospitals

Dear Dr. Lai,

We would very much appreciate it if you could consider the following manuscript for the Methods and Techniques for the Development of Drug Delivery Systems Collection in ***Journal of Visualised Experiments (JoVE).***

**Isolation and characterisation of exosomes for siRNA delivery to cancer cells**

Farid N. Faruqu, Lizhou Xu, Khuloud T. Al-Jamal

There is an increasing interest in developing exosomes as drug delivery vectors, particularly for the delivery of siRNA in RNAi-based gene silencing applications. This is mainly due to their intrinsic capability of intercellular delivery of RNA. The protocol described in this manuscript is proposed for the production of exosomes from immortalised cell lines with high yield and purity for in vitro siRNA delivery to cancer cells.

Culture of the cells of which the exosomes were derived from were done in bioreactor flasks to increase the exosome yield. The exosomes were then isolated by ultracentrifugation onto a single sucrose cushion to achieve high purity of the exosomes. Fluorescently-tagged non-specific siRNA was loaded into the exosomes via electroporation, and were then delivered to PANC-1 (human, pancreatic adenocarcinoma) cells in vitro. Efficiency of siRNA uptake by the PANC-1 cells were analysed by flow cytometry.

Bioreactor flask culture coupled with isolation using a sucrose cushion yielded exosomes of high yield and minimal non-exosomal vesicle and protein contamination, assessed by the particle:protein (P:P) ratio. Although the encapsulation efficiency of the siRNA into exosomes were quite low (10-20%), ~40% PANC-1 cells treated with the siRNA-encapsulated exosomes were positive for the siRNA in just 4 h post-treatment. It is hoped that the proposed protocol would be applied in obtaining high quality exosomes as carriers for various siRNA against different oncogenic targets for in vitro and in vivo delivery and therapy studies.

Thank you for your consideration of our work and please do not hesitate to contact me for any further query.

I look forward to hearing your response.

Sincerely yours,

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Prof. Khuloud T. Al-Jamal, BSc (Hon), PhD, MRPharmS, FHEA

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