**Table S1. Drug loading efficiency and exosome recovery in different studies**

|  |  |  |  |
| --- | --- | --- | --- |
| **Study** | **Drug loading method** | **Drug loading efficiency (%)** | **Exosome recovery (%)** |
| This study | Incubation | 22.8 | 53.7 |
| Bagheri et al, 2020 [1] | Incubation | 17 | - |
| Goh et al, 2017 [2] | Incubation | 16.5 | - |
| Chen et al, 2022 [3] | Incubation | 4.1 | - |
| Wei et al, 2019 [4] | Incubation and dialysis | 12 | - |
| Lennaárd et al, 2022 [5] | Electroporation | 32 | 30-50 |
| Tian et al, 2014 [6] | Electroporation | 20 | - |
| Gomari et al, 2018 [7] | Electroporation | 13 | - |
| Thakur et al, 2020 [8] | Exo-Load microfluidics | 16 | - |
| Chen et al, 2022 [9] | Sonication | 6.8 | - |
| Chen et al, 2021 [10] | Sonication and extrusion-assisted | 18.2 | 80 |

**Table S2. Cellular uptake of drug-loaded exosomes or nanoparticles in different studies**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Study | Increased uptake compared to free drug (times) | | | | Incubation time |
| **WT exos** | **Single-tagged exos** | **Dual-tagged exos** | **Nano-particles** |
| This study | 9 | 12 | 27 | - | 2h |
| Li et al, 2018 [11] | 4.3 | 7 | - | - | 4h |
| Gong et al, 2019 [12] | - | 2.8 | - | - | 4h |
| Zhang et al, 2008 [13] | - | - | - | 1.6 | 2h |

**Table S3. Potency of cytotoxic drugs loaded to exosomes and nanoparticles**

|  |  |  |  |
| --- | --- | --- | --- |
| Study | Increased potency compared to free drug (times)\* | | |
| **WT/Single-tagged exosomes** | **Dual-tagged exosomes** | **Nanoparticles** |
| This study | 1.2 – 1.6 | 3.7 – 17 | - |
| Gomari et al, 2018 [7] | no difference | - | - |
| Wei et al, 2019 [4] | 1.6 | - | - |
| Kanchanapally et al, 2019 [14] | 1.7 | - | - |
| Gong et al, 2019 [15] | 2 | - | - |
| Lou et al, 2020 [16] | 2 – 2.5 | - | - |
| Wei et al, 2022 [9] | 2.7 | - | - |
| Schindler et al, 2019 [17] | 20 | - | 0.3 – 3.3 |
| Kullenberg et al, 2021 [18] | - | - | 0.1 |

\* = fold changes in IC50/GI50 of cytotoxic drugs

**References for the tables**

[1] E. Bagheri, K. Abnous, S. A. Farzad, S. M. Taghdisi, M. Ramezani, and M. Alibolandi, “Targeted doxorubicin-loaded mesenchymal stem cells-derived exosomes as a versatile platform for fighting against colorectal cancer,” *Life Sci*, vol. 261, 2020, doi: 10.1016/j.lfs.2020.118369.

[2] W. J. Goh, C. K. Lee, S. Zou, E. C. Y. Woon, B. Czarny, and G. Pastorin, “Doxorubicin-loaded cell-derived nanovesicles: An alternative targeted approach for anti-tumor therapy,” *Int J Nanomedicine*, vol. 12, 2017, doi: 10.2147/IJN.S131786.

[3] M. Chen, Y. Li, N. Ma, and J. Zang, “Mesenchymal stem cell‑derived exosomes loaded with 5‑Fu against cholangiocarcinoma *in vitro*,” *Mol Med Rep*, vol. 25, no. 6, p. 213, 2022, doi: 10.3892/mmr.2022.12729.

[4] H. Wei *et al.*, “A nanodrug consisting of doxorubicin and exosome derived from mesenchymal stem cells for osteosarcoma treatment in vitro,” *Int J Nanomedicine*, vol. 14, 2019, doi: 10.2147/IJN.S218988.

[5] A. J. Lennaárd, D. R. Mamand, R. J. Wiklander, S. E. L. Andaloussi, and O. P. B. Wiklander, “Optimised electroporation for loading of extracellular vesicles with doxorubicin,” *Pharmaceutics*, vol. 14, no. 1, 2022, doi: 10.3390/pharmaceutics14010038.

[6] Y. Tian *et al.*, “A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy,” *Biomaterials*, vol. 35, no. 7, pp. 2383–2390, 2014, doi: 10.1016/j.biomaterials.2013.11.083.

[7] H. Gomari, M. F. Moghadam, and M. Soleimani, “Targeted cancer therapy using engineered exosome as a natural drug delivery vehicle,” *Onco Targets Ther*, vol. 11, 2018, doi: 10.2147/OTT.S173110.

[8] A. Thakur, R. K. Sidu, H. Zou, M. K. Alam, M. Yang, and Y. Lee, “Inhibition of Glioma Cells’ proliferation by doxorubicin-loaded Exosomes via microfluidics,” *Int J Nanomedicine*, vol. 15, 2020, doi: 10.2147/IJN.S263956.

[9] H. Wei *et al.*, “Mesenchymal Stem Cell Derived Exosomes as Nanodrug Carrier of Doxorubicin for Targeted Osteosarcoma Therapy via SDF1-CXCR4 Axis,” *Int J Nanomedicine*, vol. Volume 17, pp. 3483–3495, 2022, doi: 10.2147/IJN.S372851.

[10] C. Chen, M. Sun, J. Wang, L. Su, J. Lin, and X. Yan, “Active cargo loading into extracellular vesicles: Highlights the heterogeneous encapsulation behaviour,” *J Extracell Vesicles*, vol. 10, no. 13, 2021, doi: 10.1002/jev2.12163.

[11] Y. Li *et al.*, “A33 antibody-functionalized exosomes for targeted delivery of doxorubicin against colorectal cancer,” *Nanomedicine*, vol. 14, no. 7, 2018, doi: 10.1016/j.nano.2018.05.020.

[12] C. Gong *et al.*, “Functional exosome-mediated co-delivery of doxorubicin and hydrophobically modified microRNA 159 for triple-negative breast cancer therapy,” *J Nanobiotechnology*, vol. 17, no. 1, 2019, doi: 10.1186/s12951-019-0526-7.

[13] Z. Zhang, S. H. Lee, C. W. Gan, and S. S. Feng, “In vitro and in vivo investigation on PLA-TPGS nanoparticles for controlled and sustained small molecule chemotherapy,” *Pharm Res*, vol. 25, no. 8, 2008, doi: 10.1007/s11095-008-9611-6.

[14] R. Kanchanapally *et al.*, “Drug-loaded exosomal preparations from different cell types exhibit distinctive loading capability, yield, and antitumor efficacies: a comparative analysis,” *Int J Nanomedicine*, vol. 14, pp. 531–541, 2019, doi: 10.2147/IJN.S191313.

[15] C. Gong *et al.*, “Functional exosome-mediated co-delivery of doxorubicin and hydrophobically modified microRNA 159 for triple-negative breast cancer therapy,” *J Nanobiotechnology*, vol. 17, no. 1, p. 93, 2019, doi: 10.1186/s12951-019-0526-7.

[16] G. Lou *et al.*, “MiR-199a-modified exosomes from adipose tissue-derived mesenchymal stem cells improve hepatocellular carcinoma chemosensitivity through mTOR pathway,” *Journal of Experimental & Clinical Cancer Research*, vol. 39, no. 1, p. 4, 2020, doi: 10.1186/s13046-019-1512-5.

[17] C. Schindler *et al.*, “Exosomal delivery of doxorubicin enables rapid cell entry and enhanced in vitro potency,” *PLoS One*, vol. 14, no. 3, 2019, doi: 10.1371/journal.pone.0214545.

[18] F. Kullenberg *et al.*, “In Vitro Cell Toxicity and Intracellular Uptake of Doxorubicin Exposed as a Solution or Liposomes: Implications for Treatment of Hepatocellular Carcinoma,” *Cells*, vol. 10, no. 7, p. 1717, 2021, doi: 10.3390/cells10071717.