

SUPPORTING INFORMATION

Elucidating shape mediated drug carrier mechanics of Hematite Nanomaterials on Breast Cancer therapeutics

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1.1 Analysis of the effect of Cis-EHNP and Cis-SHNP on MCF-7 Breast cancer cells for 48 h and 72 h

We analyzed the effect of Cis-EHNP and Cis-SHNP on MCF-7. MCF-7 cells were seeded with a density of 5000 cells/well and allowed to reach the confluent of 80%. Then, the cells were treated with Cis-EHNP and Cis-SHNP at varying concentrations (0.1, 0.25, 0.5, 0.75, 1, and 1.25 μM). MTT assay was performed by washing the treated cells with 1X PBS, followed by adding 100 μL of di-methyl thiazolyl diphenyl tetrazolium reagent (MTT) and analyzed after incubating for four h.

The cell viability of Cis-EHNP treated samples decreased from $64 \pm 2\%$, $31 \pm 4\%$, $12 \pm 2.8\%$, $3 \pm 0.82\%$, $2 \pm 0.05\%$, $2 \pm 0.004\%$, with the increased concentration from 0.1 μM to 1.25 μM (Figure SI 1-A). Similarly, on treating the cells for 72h, a significant decrease in the cell viability compared to 48 h was observed till 0.25 μM . Next, in the case of Cis-SHNP treated cells, the cell viability % declined from $92\% \pm 3\%$, $81\% \pm 2\%$, $68\% \pm 3.3\%$, $48\% \pm 2.5\%$, $24\% \pm 2\%$ and $16\% \pm 1\%$ at the varying concentrations. The cell viability further decreased with an increase in the incubation time to 72h. (Figure SI-1B)

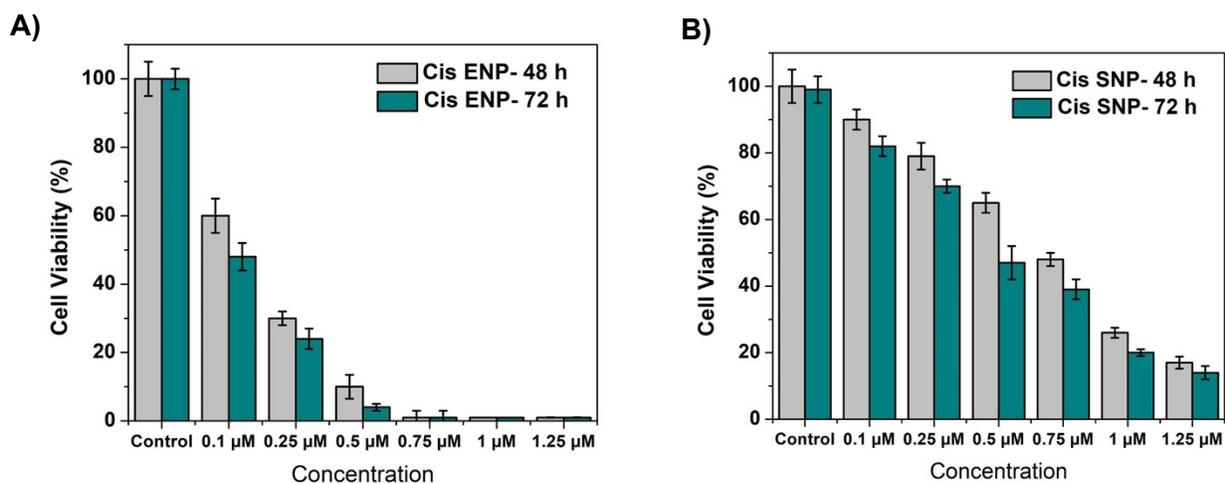


Figure SI-1: MTT assay shows the effect of (A) Cis-EHNP and (B) Cis-SHNP treated MCF-7 cells at the end of 48 h and 72 h.

1.2 Effect of EHNP and SHNP in MCF-7 Cancer cells

We also tested the effects of hematite nanoparticles alone (EHNP, SHNP) on MCF-7 cancer cells (Figure SI-2) with the same procedure mentioned in section 1.1. The cell viability percentage of control, EHNP, and SHNP-treated MCF-7 cells was observed to be $100 \% \pm 4 \%$, $99 \% \pm 3 \%$, and $99 \% \pm 3 \%$, respectively. T-test analysis showed that there is no significant difference between control and EHNP, SHNP-treated MCF-7 cells.

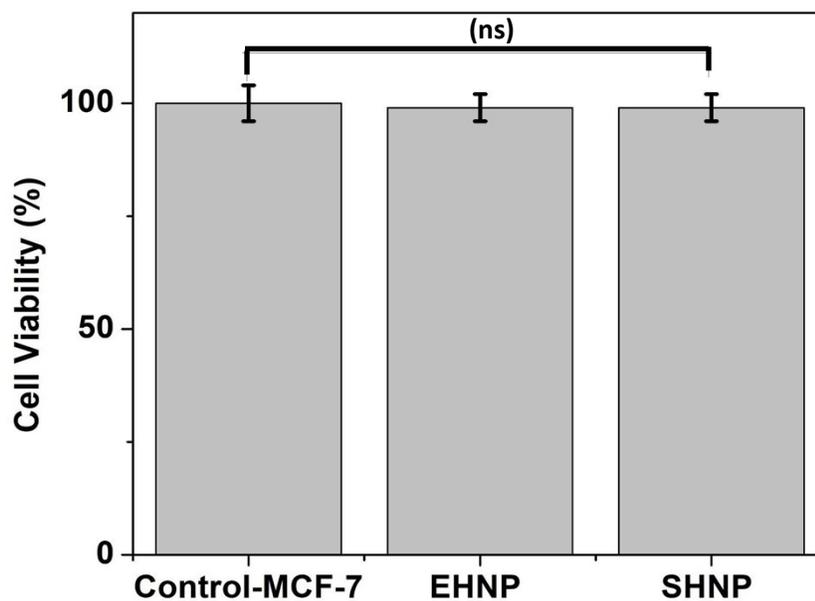
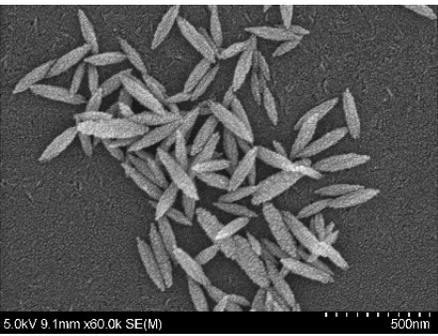
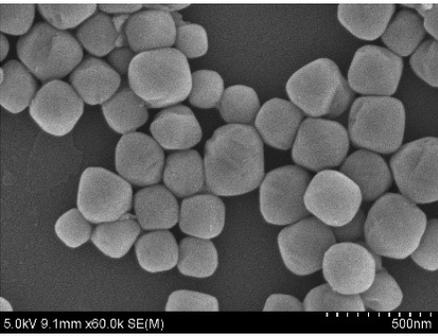
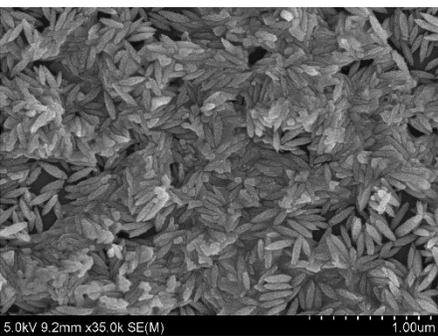


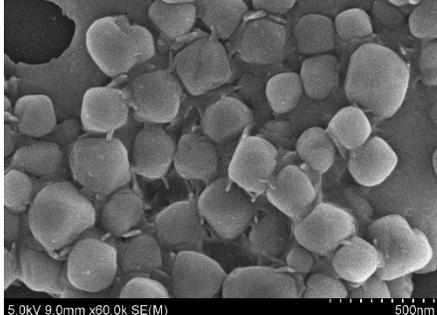
Figure SI - 2: The bar graph depicts the effect of EHNP and SHNP alone in MCF-7 breast cancer cells. Statistically analyzed using t-test $p < 0.05$, shows no significant difference between the control and SHNP, EHNP treated MCF-7 cells.

1.3 Size, zeta potential and SEM images of bare and cisplatin-loaded hematite nanoparticles

We performed the zeta potential analysis of cisplatin-loaded ellipsoidal and spherical hematite nanoparticles. The zeta potential values were observed to be 2.6 ± 0.87 mV and -23.84 ± 2.36 mV for Cis-EHNP and Cis-SHNP, respectively. The Cis-loaded EHNP and SHNP showed more positive zeta potential compared to bare nanomaterials. This could be due to the positive charge of cisplatin, as reported previously.^{1,2} Also, we characterized the cisplatin-loaded EHNP and SHNP using a scanning electron microscope (SEM) to visualize the morphology of drug-loaded nanoparticles and their sizes were measured using ImageJ software. (Table SI-1) SEM images show no morphological changes were observed for the Cis-EHNP and Cis-SHNP, indicating that the nanoparticles retain their morphological structure even after loading with cisplatin drug molecules.

Table SI-1: Size, zeta potential, and SEM images of EHNP, SHNP, Cis-EHNP, and Cis-SHNP.

S.No	Nanocarrier	Size (nm)	Zeta potential (mV)	Morphology
1.	Bare EHNP	252 ± 40	-17.72 ± 4.28	
2.	Bare SHNP	208 ± 55	-46.56 ± 2.79	
3.	Cis-EHNP	254.16 ± 29.42	2.6 ± 0.87	

4.	Cis-SHNP	244.69 ± 42.79	- 23.84 ± 2.36	
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1.4 Cytotoxicity study of free cisplatin drug molecule on MCF-7 Breast cancer cells

We have also carried out the cytotoxicity for free cisplatin in the same concentration (0.001, 0.002, 0.005, 0.01, 0.02, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 5 and 10 μM) as loaded into EHNP and SHNP on MCF-7 cell lines. We found that free cisplatin showed no cytotoxicity for concentrations less than 100 nM. The IC_{50} value of 4 μM was observed for free cisplatin, which was 20-fold higher than IC_{50} value of Cis-loaded EHNP and five-fold higher than Cis-loaded SHNP. The IC_{50} value of cisplatin alone observed in our studies is in agreement with previous studies.³

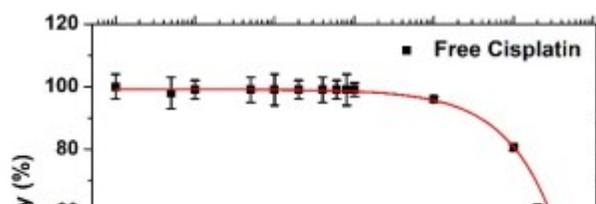


Figure SI-3 The graph represents the results of the MTT assay analyzed the cytotoxic effects of free cisplatin drug molecule using MCF-7 breast cancer cell lines.

References:

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- 2 M. Ghaferi, S. Amari, B. V. Mohrir, A. Raza, H. E. Shahmabadi and S. E. Alavi, *Pharmaceuticals*, 2020, **13**, 44.
- 3 M. Poodat, A. Divsalar, B. Ghalandari and R. Khavarinezhad, *J. Iran. Chem. Soc.*, 2023, **20**, 739–750.