

## **Supplementary Information**

### **Biodegradable Folate-Mediated Chitosan-CQD Nanocarriers for Targeted and Stimuli-Responsive Cisplatin Delivery in Melanoma**

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## S1. Drug release kinetic models:

To evaluate the release mechanisms of the drug composite from the chitosan biopolymer, the obtained release data at different pH values, ionic strengths, and different drug concentrations were fitted with six kinetic models: zero-order, first-order, Korsmeyer Peppas, Higuchi model. The description of the zero-order kinetic model is provided by Eq. (1). Zero-order kinetics were used when the drug release kinetics were dependent on concentration.

$$m_t = m_b k_0 t \quad (1)$$

Where  $m_t$  is the amount of drug released in time  $t$ ,  $m_b$  is the initial concentration of the drug in the solution before release, and  $k_0$  is the zero-order release rate constant.

When there is a concentration dependence in the drug release, first-order kinetics (Eq. (2)) is applied to describe the release.

$$\text{Log}m_t = \text{log}m_b - kt/ 2.203 \quad (2)$$

Where  $m_t$  is the amount of drug released in time  $t$ ,  $m_b$  is the initial concentration of the drug, and  $K_t$  is the first-order rate constant.

The Korsmeyer - Peppas kinetic model is as follows (Eq. (3))

$$\log(Mt/M_\infty) = \log k_{K-P} + n \log t \quad (3)$$

Where  $k_{K-P}$  is the Korsmeyer- Peppas rate constant,  $n$  is the diffusional exponent used to characterize the drug release mechanism, and  $m_\infty$  is the amount of drug released after an infinitive time . Eq. (4) describes the Higuchi kinetic model, which states that the amount of medication released is proportional to the square root of time.

$$M_t = K_H t^{1/2} \quad (4)$$

Where  $M_t$  is the amount of drug released at time  $t$ , and  $K_H$  is the Higuchi constant.

## S2 Biochemical Assays

To estimate anti-oxidant enzymes post experiment, several biochemical assays were performed on the prepared tissue homogenate and serum for estimating the levels of LDH, NO, GSH, GST and SOD.

### ***S2.1 Lactate dehydrogenase release Assay***

Lactate dehydrogenase (LDH) is a stable cytoplasmic enzyme that is found in all cells. LDH is rapidly released when there is damage in plasma membrane, a main feature of cells undergoing apoptosis, necrosis, and other forms of cellular damage (Kumar et al 2018). Each tissue sample homogenate and serum (100  $\mu$ L) was added to the substrate solution (30 mM sodium pyruvate) and then 6.6 mM NADH was added. The absorbance was measured at 340 nm using a spectrophotometer. The LDH release of the tissue and serum were measured by calculating the decreasing rate of NADH absorbance over time (Bergmeyer et al 1974). The values were expressed in  $\mu$ M/min/mg protein.

### ***S2.2 NO Assay***

The main principle of this assay was diazotization reaction basically illustrated by Griess (Griess, 1879). The chemical reaction between N-1-naphthylethylenediamine dihydrochloride and sulfanilamide in acidic conditions because of orthophosphoric acid (88% purity) was the base for working of Griess reagent system. Nitric oxide (NO) was detected by the system in different experimental and biological liquid matrices such as serum, urine, tissue culture media and plasma. Shortly, Reagent A is composed of sulphanilamide (1%) in 2.5% ortho-phosphoric acid and Reagent B is 0.1 % NEDD in 2.5 % ortho-phosphoric acid, were taken in 1:1 ratio. 100  $\mu$ l of freshly prepared Griess reagent was added into 100  $\mu$ l of supernatant fraction making final assay mixture volume of 200  $\mu$ l. The absorbance was measured at 540nm against blank of PBS in a UV-Vis spectrophotometer. The values are conveyed as  $\mu$ M/mg protein.

### ***S2.3 Superoxide dismutase (SOD) assay***

The activity of SOD was measured by following the protocol of Kakkar et al (Kakkar et al 1984). The reaction mixture consisted of 0.025 M sodium pyrophosphate (pH 8), 180  $\mu$ M phenazine methosulfate, 300  $\mu$ M nitroblue tetrazolium (NBT). In the final assay mixture of 100  $\mu$ L, 20  $\mu$ L of CFE along with 60  $\mu$ L of reaction mixture was added and the reaction was initiated by the addition of 20  $\mu$ L of 780 $\mu$ M NADH. The absorbance was measured at 560 nm immediately for 5

min against a blank containing phosphate buffer instead of the sample. SOD activity was expressed as unit/mg of protein.

#### ***S2.4 GSH activity***

Tissue homogenate and serum sample were used for GSH activity analysis and method of Moron, et al was followed for this analysis (Moron et al., 1979). Shortly, 100µl of sample was precipitated using 5% TCA (20µl). To remove the proteins, the tubes were centrifuged at 1200 rpm at RT for 5 min. 45 µl of PBS (0.2 M, pH-8), 45µl of sample (treated with TCA) and 20µl of 10mM of DTNB was added making the final assay mixture of 110 µl. After 10 mins, read the absorbance at 412nm immediately for 5mins. The GSH activity was articulated in µM/min/mg of protein.

#### ***S2.5 GST Activity***

For GST activity analysis, the tissue homogenate and serum was used and the experiment was done according to the procedure of Habig *et al* (Habig et al., 1974). The reaction mixture contained 100mM GSH (100µl), 100mM CDNB (100µl), and 9.8ml of PBS (pH 6.5). 180µl of cocktail was added in 20µl of supernatant and the change in absorbance was observed at 350nm immediately for 5 mins. The values were expressed as µM/min/mg protein.

### **S3. Histology and immunohistochemistry studies:**

The tissues were collected and fixed in paraformaldehyde for hematoxylin and eosin (H&E) staining. Meanwhile, immunohistochemistry staining including Beta-Catenin, BCL2, P53 were conducted according to standard protocols.

NO	NO ( $\mu\text{M}/\text{mg Protein}$ )						
	Liver	Kidney	Spleen	Heart	Lung	Serum	Tumor
Control	13.35195	12.78106	10.46791	10.34283	7.399615	6.918255	7.63274
FA-CCNPs	14.45186	13.24743	10.94015	10.24184	7.592163	7.242248	7.79005
Cis	16.96596	15.18214	13.73574	13.24725	9.751387	10.3492	10.13097
FA-CisCC	13.42546	13.08844	10.79761	9.928622	7.526022	7.121457	14.42089
stdev	Liver	Kidney	Spleen	Heart	Lung	Serum	Tumor
Control	0.469232	0.084077	0.349157	0.300599	0.867192	1.311042	0.13006
FA-CCNPs	0.954379	0.213357	0.815322	0.496739	1.40704	0.472967	1.19544
Cis	0.931874	0.465388	0.486335	0.812269	0.818809	1.265882	0.80685
FA-CisCC	0.126715	1.553347	1.827001	0.654087	0.873288	0.113577	1.3462

LDH	LDH (nM/mg Protein)						
	Liver	Kidney	Spleen	Heart	Lung	Serum	Tumor
Control	11.45313	6.833874	2.063548	7.249574	2.145794	2.467124	2.40869
FA-CCNPs	13.0546	7.462423	2.904007	7.996422	2.660807	3.401946	3.20257
Cis	16.85315	11.71025	4.862802	11.03326	4.209034	5.005466	4.69679
FA-CisCC	11.85122	7.598199	2.575213	8.070005	2.626827	3.085409	7.25837
stdev	Liver	Kidney	Spleen	Heart	Lung	Serum	Tumor
Control	1.512185	1.041701	0.790532	1.51471	0.71186	0.330917	1.11911
FA-CCNPs	0.035683	0.929048	0.694098	0.600948	0.614019	0.648286	1.07623
Cis	2.299817	1.445248	1.244729	1.88573	1.101725	0.892777	0.84773
FA-CisCC	1.584833	0.841793	0.361537	1.777913	0.310482	0.485321	0.65607

GSH	GSH (nM/mg Protein)						
	Liver	Kidney	Spleen	Heart	Lung	Serum	Tumor
Control	41.13995	33.87416	57.52065	37.05293	46.25664	40.50134	49.31285
FA-CCNPs	36.63413	29.59644	52.59072	32.59362	41.4123	37.65279	46.28742
Cis	26.51167	25.67377	43.07505	26.76232	34.15548	32.0841	44.2179
FA-CisCC	31.19078	29.57609	49.23968	33.55457	40.07151	37.81605	36.81617
stdev	Liver	Kidney	Spleen	Heart	Lung	Serum	Tumor
Control	2.152187	2.487294	2.464832	0.840786	2.072157	2.77734	3.2017
FA-CCNPs	1.672852	1.10977	2.247943	1.938558	0.642828	1.659947	0.31828
Cis	0.655665	0.647355	0.550562	1.239399	2.244414	1.489842	1.77348
FA-CisCC	1.407433	0.952182	0.92774	2.154024	1.51123	1.641716	1.58791

GST							
	Liver	Kidney	Spleen	Heart	Lung	Serum	Tumor
Control	43.43334	40.46823	59.64859	40.58741	51.47525	45.94362	50.95898
FA-CCNPs	45.77889	42.29402	62.32888	42.26679	53.96753	48.60303	51.01183
Cis	48.3813	45.05551	64.45351	44.41181	56.6156	51.1117	51.06396
FA-CisCCNPs	51.57292	50.15928	69.78312	49.46399	60.26945	55.19984	44.25215
stdev	Liver	Kidney	Spleen	Heart	Lung	Serum	Tumor
Control	0.405043	0.346245	0.828214	1.088801	2.149325	0.704166	2.45896
FA-CCNPs	0.692035	0.675906	1.312447	0.487826	0.752962	1.654469	1.83174
Cis	0.467577	0.479313	1.446736	2.032643	1.941221	0.444456	1.15787
FA-CisCCNPs	1.759193	2.253302	2.243699	0.861286	1.469849	0.74495	1.95279

SOD(ng/mg Protein)							
SOD	Liver	Kidney	Spleen	Heart	Lung	Serum	Tumor
Control	4.000505	3.121502	3.083495	2.544226	2.257291	2.095957	2.05133
FA-CCNPs	4.165267	3.195323	3.19414	2.677985	2.253669	2.102712	2.05133
Cis	4.201194	3.326837	3.233154	3.071942	2.280568	2.282495	1.87114
FA-CisCCNPs	6.614682	4.751329	4.035384	4.860145	2.609653	3.055797	0.98535
stdev	Liver	Kidney	Spleen	Heart	Lung	Serum	Tumor
Control	0.138857	0.244394	0.198304	0.274311	0.113645	0.093081	0.06679
FA-CCNPs	0.183363	0.209725	0.106398	0.143419	0.164697	0.18972	0.06679
Cis	0.155948	0.321063	0.359686	0.315734	0.284564	0.070851	0.21415
FA-CisCCNPs	0.58044	0.464022	0.3143	0.262734	0.143914	0.328192	0.0705

**Table ST1: data of oxidative stress for vital organs liver,kidney,spleen,heart,lung,serum and tumor**

Element	FA-CCNPs	FA-CisCCNPs
	Weight%	Weight%
C	40.31	36.02
N	12.02	10.00
O	40.90	41.75
S	6.77	4.03
Cl	--	0.59
Pt	--	7.61
Total	100.00	100.00

**ST2: EDS element analysis of FA-CCNPs & FA-CisCCNPs.**

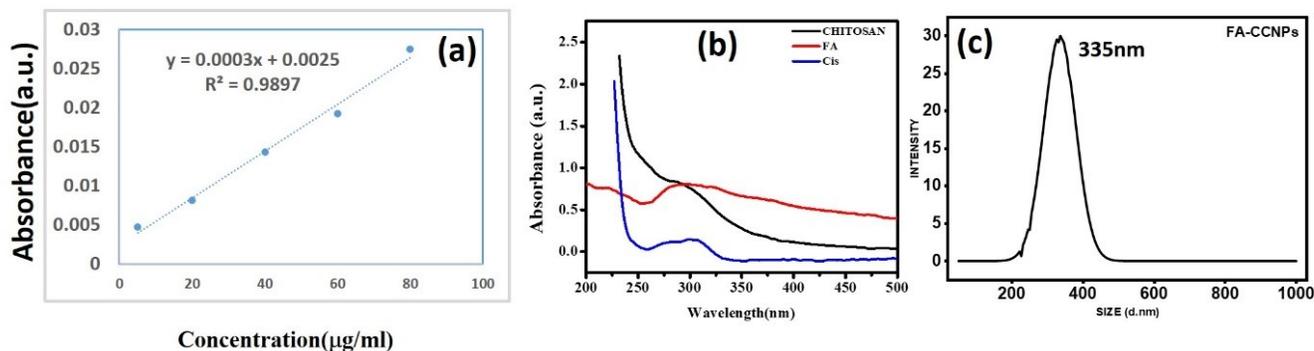
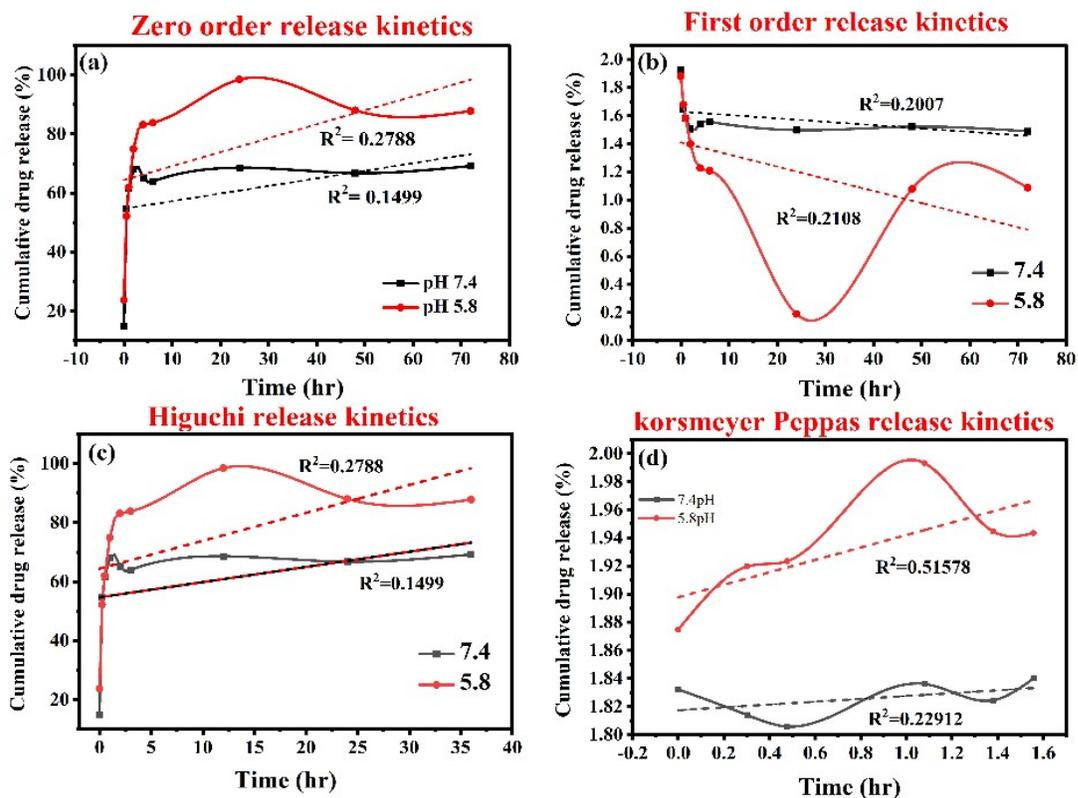
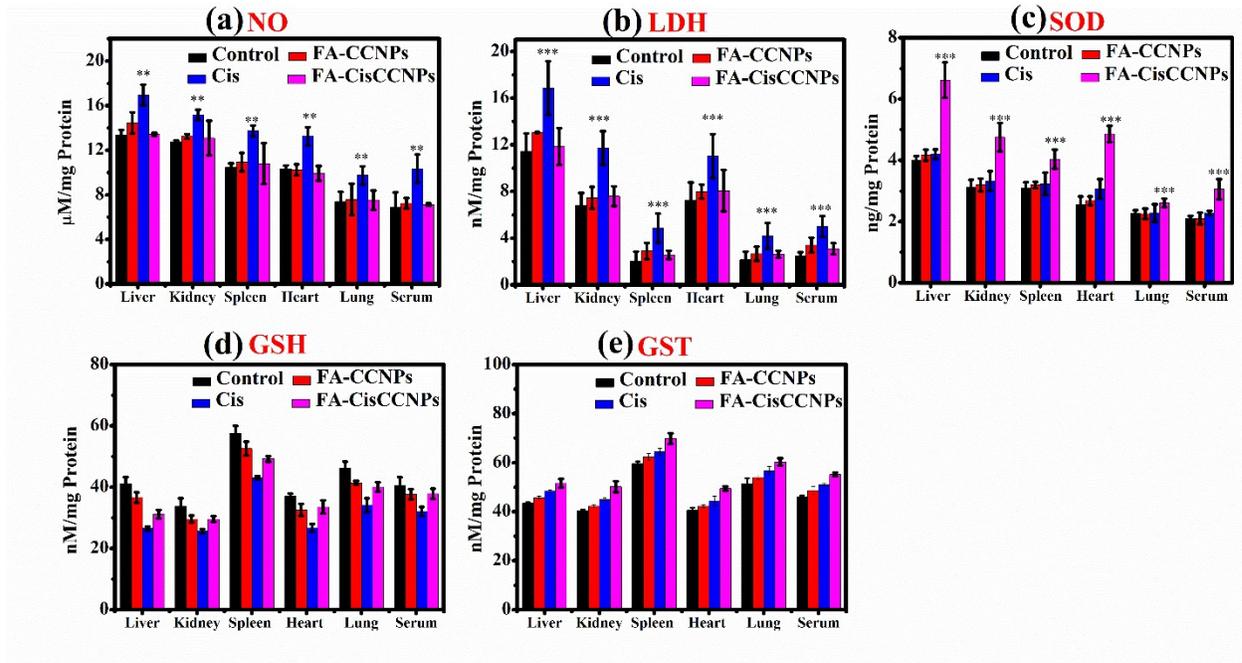


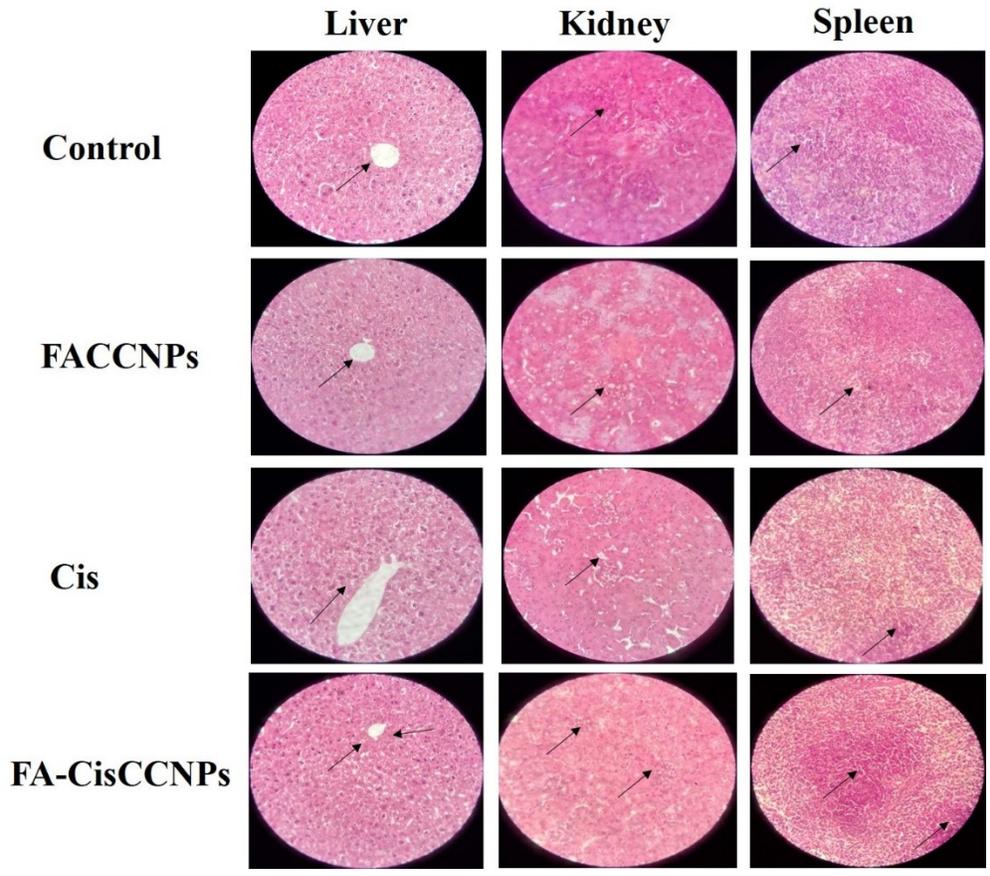
Fig S1(a) Cisplatin drug standard curve (b) Uv-Vis spectra of Chitosan, FA and Cis (c) DLS of FA-CCNPs



**FigS2 Release kinetic curve for cisplatin drug from FA-CisCCNPs at Ph 5.8 & 7.4 (a) zero order kinetics release (b) first order kinetic release (c) Higuchi kinetics release (d) Korsmeyer Peppas kinetic release.**



**FigS3 : Estimation of SOD, GSH, GST, NO and LDH in tissue homogenate and serum of control, void FA-CCNPs, Cis and FA-CisCCNPs after the end of experiment on day 21.**



**FigS4 : H/E image of liver, kidney & spleen for void FA-CCNPs, Cis and FA-CisCCNPs.**