

Supplementary Information

Water stable, red emitting, carbon nanoparticles stimulate 3D cell invasion via clathrin-mediated endocytic uptake

Udisha Singh^{1#}, Aditya Teja^{2#}, Shanka Walia^{1#}, Payal Vaswani¹, Sameer Dalvi^{2,3} and Dhiraj Bhatia^{1,3}

¹Biological Engineering Discipline, Indian Institute of Technology Gandhinagar, Palaj, Gujarat 382355, India

² Chemical Engineering Discipline, Indian Institute of Technology Gandhinagar, Palaj, Gujarat 382355, India

³ Center for Biomedical Engineering, Indian Institute of Technology Gandhinagar, Palaj, Gujarat 382355, India

US, AT, SW contributed equally

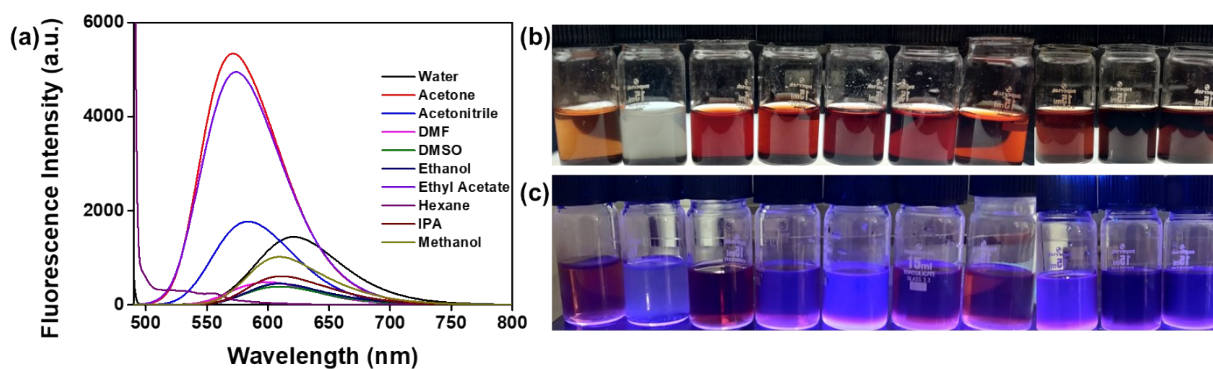
*Correspondence to – dhiraj.bhatia@iitgn.ac.in

SI table 1: Different red emissive carbon nanoparticles synthesised in recent years

No.	Reactants	Method of synthesis	Time	Temp (in °C)	Emission wavelength	Size	Ref
1	PEG400, with EuCl ₃ · 6H ₂ O	Microwave synthesis	1-6 hrs	150 - 200	615 nm	3-5 nm	(28)
2	pulp-free lemon juice in	Solvothermal	10 hrs	190	631 nm	~4.6 nm	(29)

	ethanol						
3	Citric acid, urea in formamide	solvothermal	12 hrs	180	630 nm	2-3 nm	(30)
4	Ru-Aphen and Citric acid	Hydrothermal	3hrs	185	606-616nm	3-6 nm	(31)
5	Dicyandiamide, o-paraphenylene diamine and sulfuric acid	Hydrothermal	10 hrs	200	630nm, 680 nm	~5.7 nm	(32)

SI 2: Fluorescence intensity of CNPs in different solvents



SI 2(a) Emission spectra of CNPs (0.5 mg/mL) dispersed in different solvents (Water, Acetone, Acetonitrile, DMF, DMSO, Ethanol, Ethyl Acetate, Hexane, Isopropinoic Acid, Methanol) **(b) & (c)** Images of CNPs dissolved in different solvents under white light (above) and UV light (below). (From left to right – Water, Hexane, Acetone, Acetonitrile, DMSO, DMF, Ethyl acetate, IPA, ethanol, methanol)

SI 3: Stability check of CNPs in water for seven days

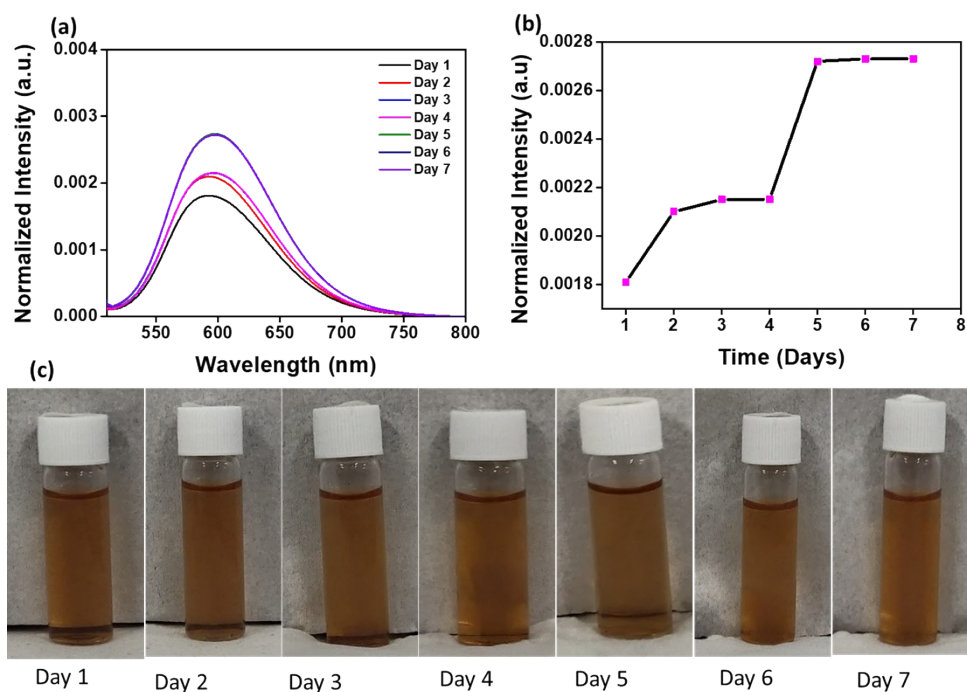
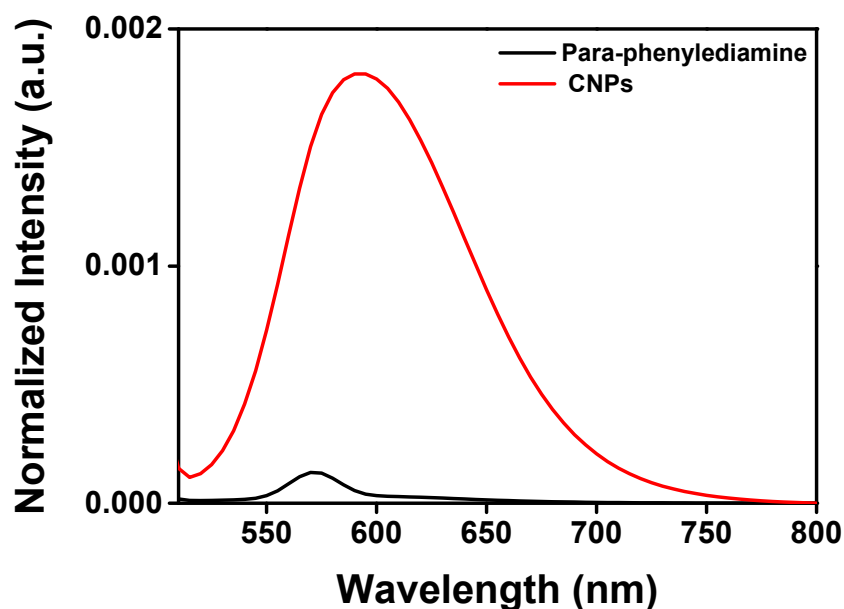


Figure SI 3: Stability studies of CNPs. **(a) and (b)** normalized fluorescence intensity reading taken for seven days, **(c)** Images of 0.5mg/ml CNPs dispersed in water for seven days to check their stability.

SI 4: Fluorescence intensity of CNPs and PPDA



SI 4: Fluorescence intensity comparison of PPDA and CNPs at 480 nm excitation wavelength.

SI 5: MTT assay

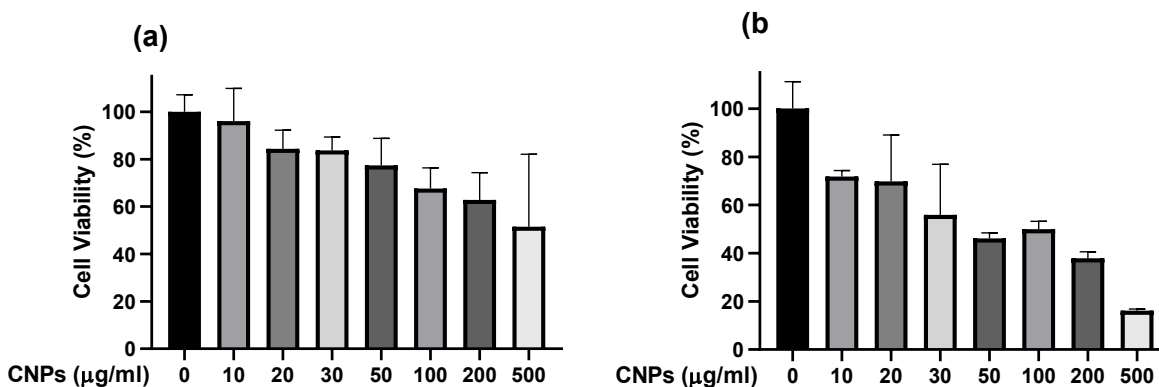


Figure SI 5: Cytocompatibility studies. Cell viability was determined by MTT assay using (a) SUM 159 and (b) MeFs. The cells were treated with CNPs at 10, 20, 30, 50, 100, 200 and 500 µg/mL for 24 h.

SI 6: 2D Cellular studies of CNPs via confocal microscopy

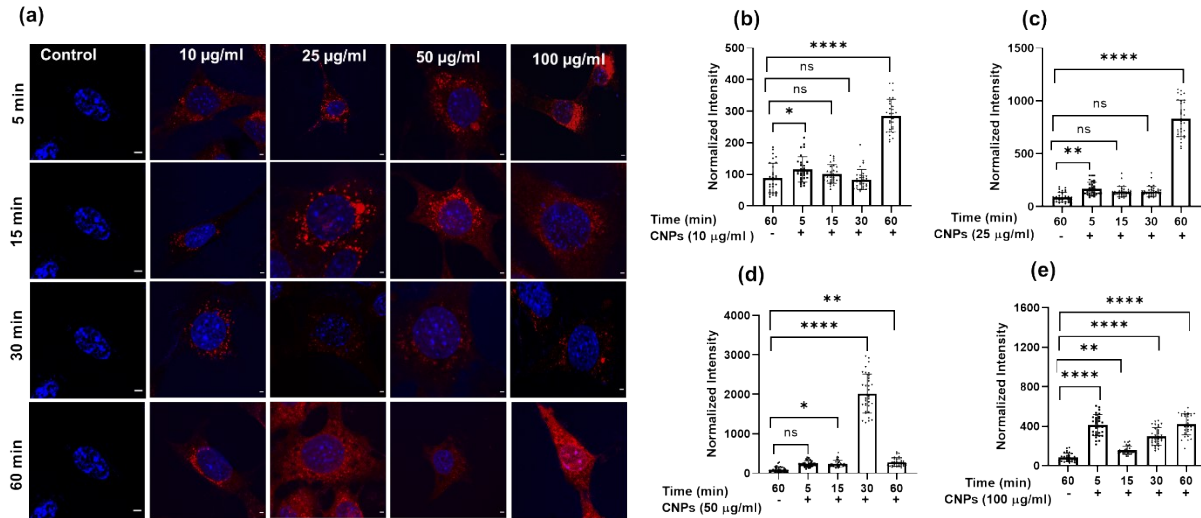


Figure SI 6(i): Concentration dependent cellular uptake studies of CNPs at different time intervals. (a) Confocal images of MeFs incubated with 10, 25, 50 and 100 µg/ml of CNPs respectively. First column in the figure represents untreated MEF cells imaged after 60 min of incubation. Scale bar is 5 µm. (b-e) Quantification of cellular uptake of at 10, 25, 50 and 100 µg/ml of CNPs at 5, 15, 30 and 60 min respectively. **** Indicates statistically significant value of $p < 0.0001$. ** Indicates statistically significant value of $p = 0.002$, * Indicates statistically significant value of $p = 0.03$ and ns indicates non-significant value of p (one-way ordinary ANNOVA).

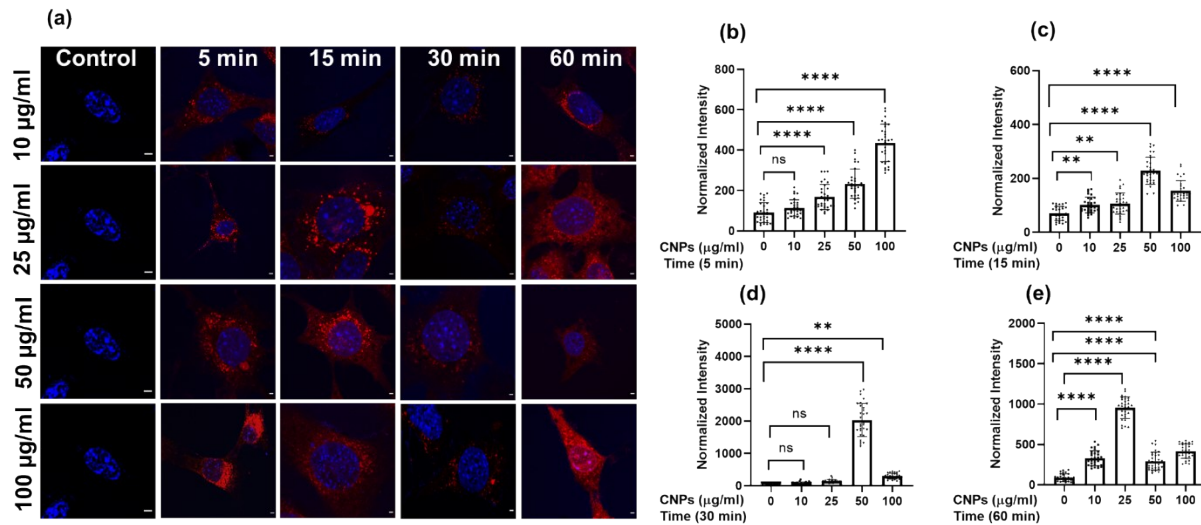


Figure SI 6 (ii): Time dependent cellular uptake studies of CNPs at different time intervals. (a) Confocal images of MEFs incubated with 10, 25, 50 and 100 µg/ml of CNPs respectively. First column in the figure represents untreated MEF cells imaged after 60 min of incubation. Scale bar is 5 µm. (b-e) Quantification of cellular uptake of CNPs at 10, 25, 50 and 100 µg/ml respectively. **** Indicates statistically significant value of $p < 0.0001$, ** Indicates statistically significant value of $p = 0.005$ and ns indicates non-significant value of p (one-way ordinary ANNOVA).

