Electronic Supplementary Material (ESI) for Biomaterials Science

Supplementary Data

The Composition and End-group Functionality of Sterically Stabilized Nanoparticles Enhances the Effectiveness of Co-administered Cytotoxins.

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Supplementary Table 1. Zeta potential values of the SPIONs.

Abbrev. Name	Particle coating (molar % of individual polymer stabilizers)	Zeta potential in water (mV)
NP1	100 % MPEG	-42
NP7	100% NH_2	-29
NP8	100% COOH	-39

Supplementary Table 2. 72hr IC_{50} values for the active compounds used in this study. Paclitaxel was growth inhibitory at concentrations of less than 10nM in both DLD-1 and PA-1 cells. Doxorubicin was cytotoxic to PA-1 cells at all concentrations tested to a minimal concentration of 10 nM.

Active Compound	DLD-1	PA-1
Doxorubicin (Dox)	1 +/- 0.1 μM	N/A
Mitoxantrone (Mito)	40 +/- 2nM	20 +/- 1nM
Paclitaxel (Pac)	N/A	N/A
Cisplatin (Cis)	10 +/- 1 μM	1 +/- 0.2 μM
5-Fluorouracil (5FU)	12 +/- 1 μM	14 +/- 1 μM

Supplementary Table 3. 72hr IC_{50} values of drug (μM) and nanoparticle (ppm) combinations used in human DLD-1 colorectal carcinoma cells grown in monolayer culture. Nanoparticle concentration was kept constant at 50 ppm for the co-administration experiments

Doxorubicin	1 ± 0.1	
SPION	Without Dox	With co-administered Dox
NP1	>100 ppm	1 ± 0.1
NP3	>100 ppm	1 ± 0.1
NP4	>100 ppm	1 ± 0.1

Supplementary Scheme 1: Chemical structures of cytotoxins used in this study.

Supplementary Figure 1: Nanoparticle size distribution. Plot of representative nanoparticle size distribution before and after steric stabilization as determined by dynamic light scattering.

Supplementary Figure 2: The fluorescence spectrum of Doxorubicin is not altered by the presence of nanoparticles. Fluorescence spectra of 1 μM Doxorubicin in the presence of 10 ppm nanoparticles. No quenching of the fluorescence intensity was observed at this concentration of nanoparticles.

Supplementary Figure 3: Nanoparticles alone do not affect cellular outgrowth. Plot of cellular outgrowth of DLD-1 spheroids dosed with SPIONs as well as gold and silica nanoparticles. The values obtained were normalized to the outgrowth of untreated DLD-1 spheroids. The shading represents the percentage of NH_2 functionalized polymers on the particle.

Supplementary Figure 4: Inhibition of cellular outgrowth requires specific nanoparticle/drug combinations. Plot of cellular outgrowth of PA-1 spheroids co-administered with cytotoxins and SPIONs normalized to the outgrowth of the cytotoxin alone treated PA-1 spheroids. a) Mitoxantrone, b) 5-Fluorouracil, c) Cisplatin and d) Paclitaxel. The shading represents the percentage of NH_2 functionalized polymer on the particle. Error bars represent standard error with n=6. * indicates $p<0.01$ compared to cytotoxin alone.

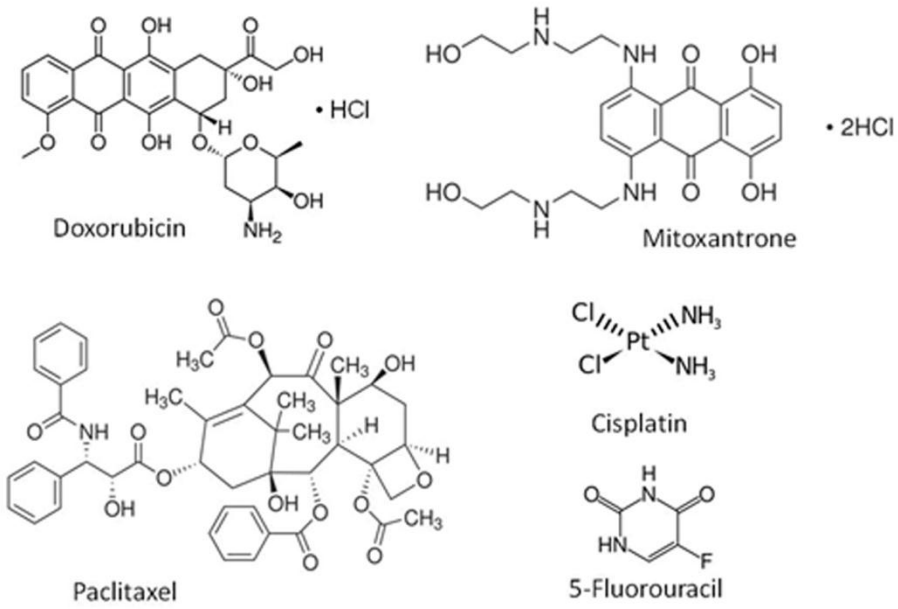
Supplementary Figure 5: Inhibition of cellular outgrowth requires specific nanoparticle/drug combinations. Plot of cellular outgrowth of DLD-1 spheroids co-administered with cytotoxins and SPIONs normalized to the outgrowth of the cytotoxin alone treated DLD-1 spheroids. a) 5-Fluorouracil, b) Cisplatin and c) Paclitaxel. The shading represents the percentage of NH_2 functionalized polymer on the particle. Error bars represent standard error with n=6. * indicates $p<0.01$ compared to cytotoxin alone.

Supplementary Figure 6: Gold and Silica nanoparticles. Transmission electron micrographs of 95% MPEG/5%NH_2 polymer stabilized, gold (a) and silica (b) nanoparticles. Scale bar 100 nm.

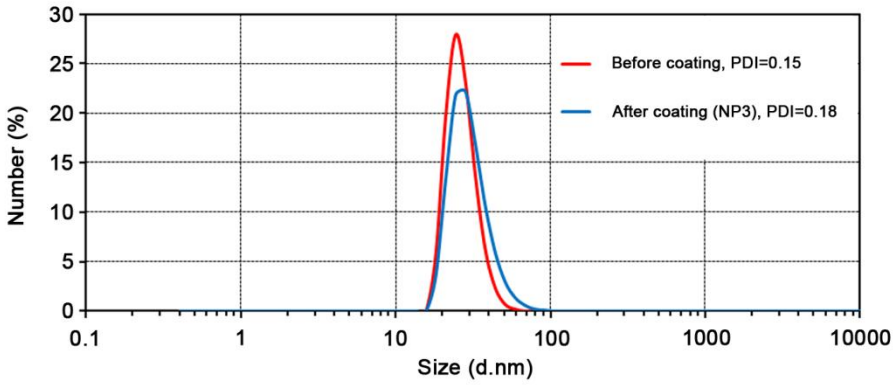
Supplementary Figure 7: The localization of Dox is unchanged in the presence of nanoparticles in 2-dimensional cell culture. Confocal images of DLD-1 cells treated with Dox alone, Dox + 100%MPEG or Dox + 95% MPEG/5% NH_2 coated particles. Scale bar 10 μm.

Supplementary Movie 1: Long term live cell imaging of the outgrowth assay. Brightfield imaging of untreated DLD-1 spheroids placed onto tissue culture plastic and allowed to adhere over a period of 1430 minutes. Images were taken every 10 minutes and the movie is displayed at 6 fps. The total width of the image is 700 μm.

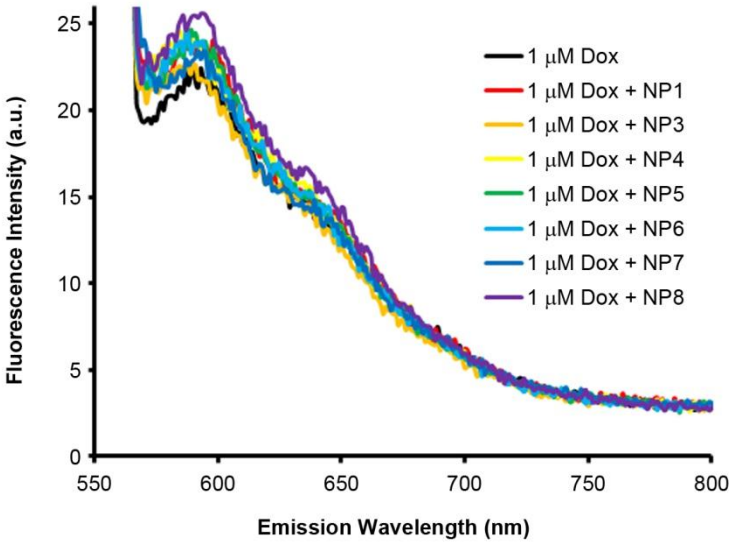
Supplementary scheme 1



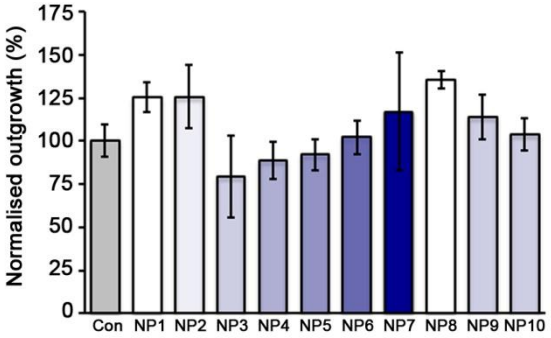
Supplementary Figure 1



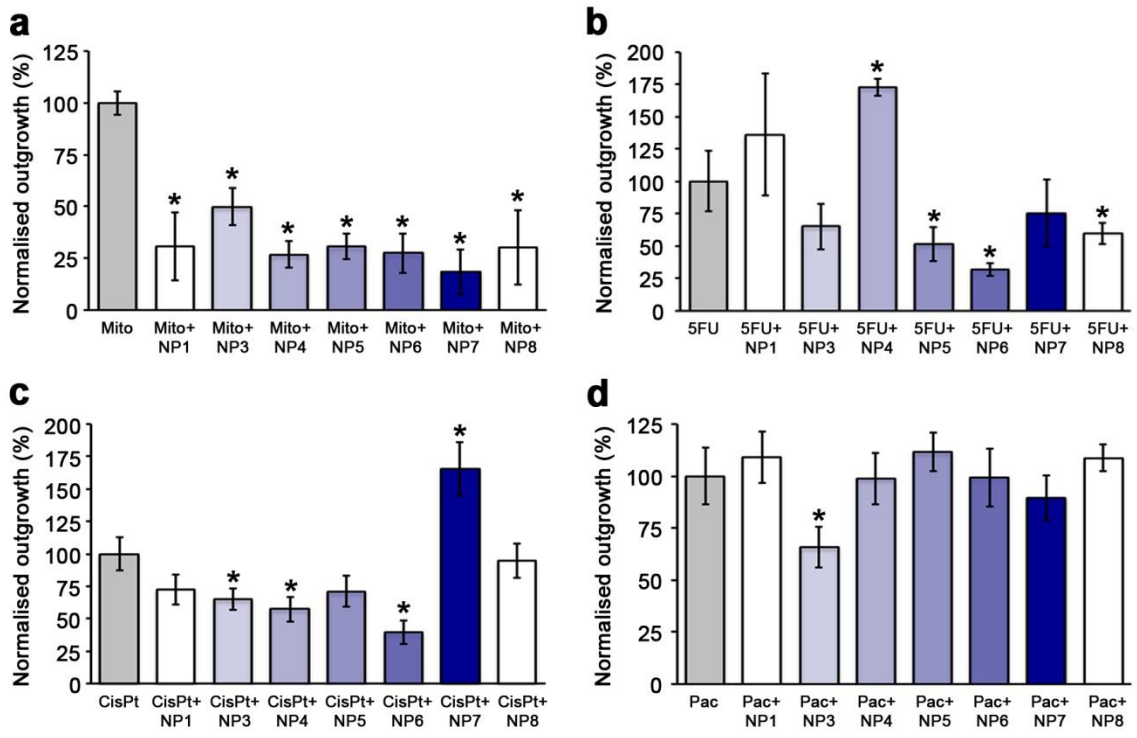
Supplementary Figure 2



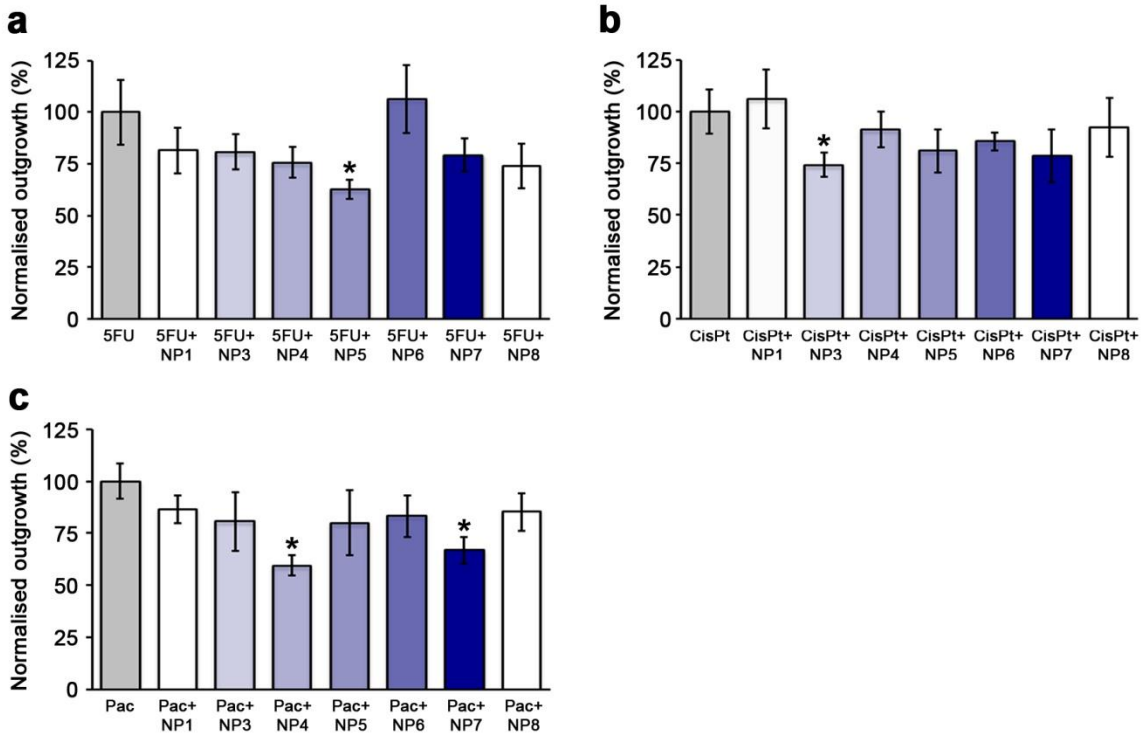
Supplementary Figure 3



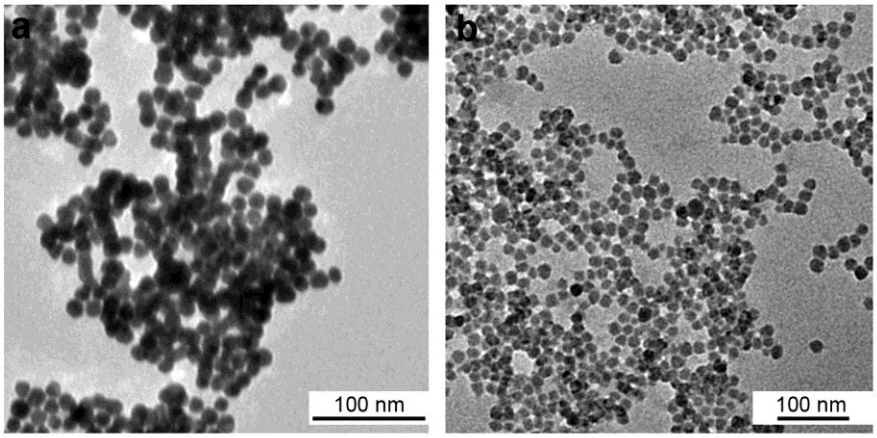
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7