Supplementary Materials

Use of a Lipid-Coated Mesoporous Silica Nanoparticle Platform for Synergistic Gemcitabine and Paclitaxel Delivery to Human Pancreatic Cancer in Mice

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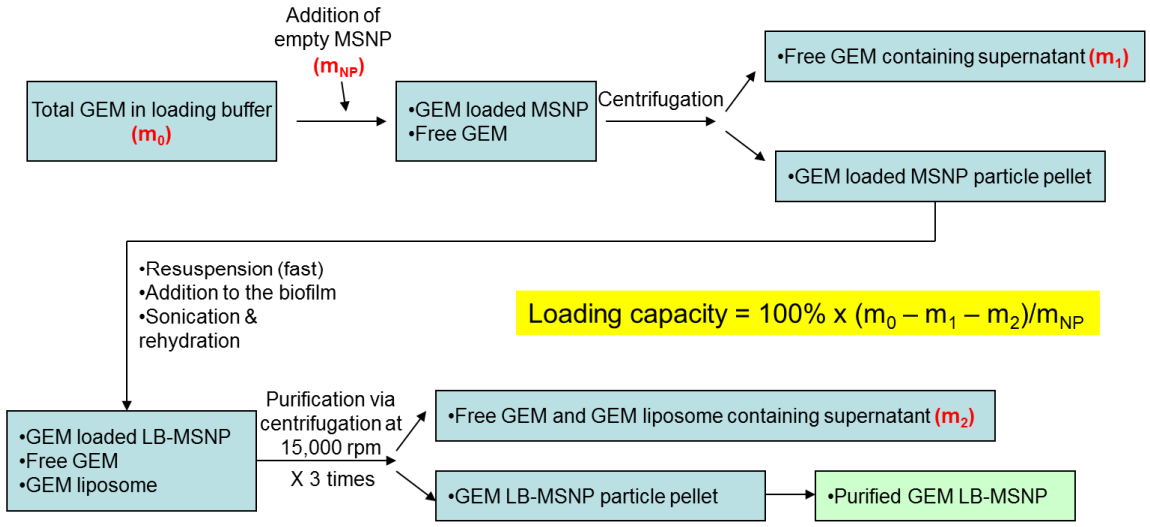
S1. Experimental determination of drug loading capacity in lipid bilayer coated mesoporous silica nanoparticle (LB-MSNP) and the theoretical calculation of maximal loading capacity based MSNP pore volume.

The following diagram explains how we achieved the calculation of 40 wt% GEM loading:



This diagram illustrates that the original amount (weight) of GEM introduced in the loading medium (before the addition of the carrier) is defined as m_0. The weight of the MSNP is referred to as m_{NP}. Following drug loading, the nanoparticles were centrifuged at 15,000 rpm for 20 minutes to collect the supernatant for determining the GEM content, using a microplate reader and OD value of 265 nm. This quantity of non-encapsulated drug before pore sealing is referred to as m_1. The GEM-laden MSNP pellet was rapidly re-suspended and added on top of the continuous coated lipid film to provide surface coating and pore sealing upon sonication. This yields a particle suspension containing a mixture of GEM loaded LB-MSNP, free GEM, and liposomes. The GEM-encapsulated LB-MSNPs were purified from free GEM and GEM liposomes by centrifugation at 15,000 rpm for 10 minutes. The supernatant was collected and the pellet washed 3 times in a saline buffer. These washings were combined and used to quantify the GEM content by microplate reader and by HPLC. The

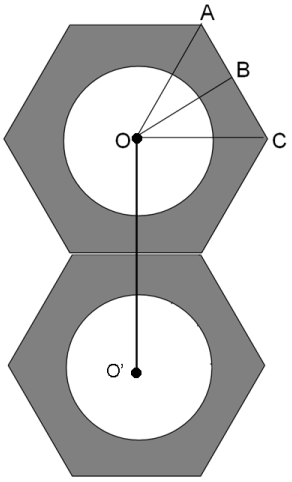
weight of the free GEM and liposome-associated GEM collectively is referred to as m_2. This allowed us to determine loading capacity, according to the formula: Loading capacity = $[m_0 – (m_1 + m_2)] / [m_{NP}]$ x 100%. For example, in an experiment using 5 mg empty MSNP (m_{NP}) incubated with 5 mg GEM (m_0) (0.25 mL of a 20 mg/mL GEM solution), we showed m_1=1.04 mg and m_2=1.94 mg, leading the calculation of loading capacity as = (5-2.98)/5 × 100% = 40% (w/w). While it is difficult to determine the exact physicochemical state of GEM trapped in the pores at this loading capacity, it is reasonable to speculate that the high interior surface area of the pores contribute to drug loading by providing a template for interaction by H-bonding, Van der Waals interactions, and electrostatic binding. While we do not have any evidence of drug precipitation, we know from the biological experiments that the GEM can be released and is bioavailable. Our results are in agreement with Dr. Brinker's studies showing a high loading capacity in 'protocells' (containing an aerosol-assisted synthesized MSNP core; Nature Materials, 2011, 10, 389). For example, the loading capacity of doxorubicin was determined as high as 57% (w/w) in the 'protocell' platform.

In order to answer the question about the porosity of the particles, we have also performed calculations to determine the total pore volume, which can be used to calculate a theoretical maximum loading capacity. The lattice spacing of our 65 nm MSNPs was measured as 3.3 nm, based on TEM images. The pore diameter was 2.75 nm. The accompanying figure shows the structure of MSNP pores. We know that OB= ½ OO', therefore the area of the hexagon

$$S_{hex} = 6 \times S_{AOC}$$

$$= 6 \times 0.5\ OO' \times 0.5\ OO'/\sqrt{3}\ nm^2$$

$$= 9.4312\ nm^2$$



We know that the area of the pore opening

$$S_{pore} = \pi r^2 = \pi(2.75/2)^2 = 5.936\ nm^2.$$

The percentage of the hexagon area covered by the pore area:

$$S_{pore} / S_{hex} = 5.936 / (9.4312) = 63\ \%$$

The percentage of the silica wall area in hexagon area:

$$S_{silica\ wall} / S_{hex} = 100\% - 63\% = 37\ \%$$

Because the MSNP can be considered as an array of hexagon tubes, the areal percentage can be directly used to calculate volume percentage. Thus,

$$V_{pore} / V_{particle} = (S_{pore} \times \text{tube height}) / (S_{hex} \times \text{tube height})$$

$$= S_{pore} / S_{hex} = 63\%$$

$$V_{silica\ wall} / V_{particle} = 100\% - 63\% = 37\%$$

Thus, the total % of silica volume in MSNP is 37%. The total % of interior pore volume in MSNP is 63%.

In order to determine the number of particles per gram of MSNP, we assessed the weight of a single particle. Given that the density of amorphous silica is 2.5 g/cm^3, we can calculate the mass of a single MSNP:

$$m_{one\ particle} = V_{silica\ wall} \times \rho_{silica} = 37\% \times V_{particle} \times \rho_{silica}$$

$$= 37\% \times [4/3 \times 3.14 \times (65/2)^3]\ nm^3 \times 10^{-21}\ cm^3/nm^3 \times 2.5\ g/cm^3$$

$$= 1.3294 \times 10^{-16}\ g$$

Particle number per 1 g MSNP

$$= 1 / (1.3294 \times 10^{-16}) = 7.522 \times 10^{15}$$

Thus, the pore volume per 1 g MSNP is:

Pore volume for one particle × particle number per gram MSNP

$$= 4/3 \times 3.14 \times (65/2)^3 \times 63\% \times 7.522 \times 10^{15}$$

$$= 6.811 \times 10^{20}\ nm^3/g = 0.6811\ cm^3/g$$

This calculated pore volume was also confirmed by BET measurement. The BET results showed that the surface area of our MSNP was 850 m^2/g, with a pore volume of 0.75 cm^3/g. This is close to the calculated value of 0.6811 cm^3 /g shown above. For further discussion purposes, we will use a pore volume of ~0.7 cm^3/g.

Since the concentration of the aqueous GEM loading solution is 20 mg/mL and the pore volume is 0.7 cm^3/g in MSNP, the GEM loading capacity would be 1.4%

w/w (20 mg/mL × 0.7 cm^3/g = 14 mg per 1g MSNP) base on the assumption that the drug loading is simply a passive entrapment process. However, if one assumes that the MSNP pores are completely filled by GEM precipitate, one should expect a maximal loading capacity of 129% w/w (GEM density of 1.84 g/cm^3 × 0.7 cm^3/g / 1 g MSNP = 129%). Thus, we believe that an experimental loading capacity of 40% (w/w) is a reasonable value.

S2. *In vitro* dosimetry calculations for free drug mixtures and achieving ratiometric design of GEM/PTX delivering LB-MSNPs.

In order to explain the *in vitro* synergy data in Fig. 1E, we provide the following methodology including the *in vitro* dosimetry calculation using free GEM/PTX mixtures and dual delivery nanoparticles. In order to demonstrate the synergy of free drug combination, a series of GEM/PTX mixtures were prepared in which we initially used a fixed amount of GEM (50 μg/mL) plus different amounts of PTX to yield GEM/PTX ratios over the range 100:1 to 0.2:1. Each mixture was considered as a starting concentration to make a series of dilutions. Thus, from the 100:1 starting solution we made seven dilutions (#1 to #7) to yield GEM/PTX concentrations of #1: 50 μg/mL GEM + 0.5 μg/mL PTX, #2: 25 μg/mL GEM + 0.25 μg/mL PTX, ……, and #7: 0.78 μg/mL GEM + 0.0078 μg/mL PTX (all at the same ratio). Using the samples to conduct cell viability (MTS) experiments, we obtained a 50% killing effect for the 100:1 mixture. The free GEM/PTX concentrations in mixtures providing 50% killing were calculated, such as a GEM and PTX doses of 4.9 μg/mL and 0.049 μg/mL, respectively, at this ratio. Subsequently, we also prepared a series of dilutions for GEM/PTX ratios of 10:1, 5:1, 1:1 and 0.2:1 to calculate the 50% killing concentrations of these mixtures as shown in the upper panel in Fig. 1E1. In order to keep the dose of PTX in the low range, we decided in further experimentation to use a ratio of 10:1. At this ratio, the free PTX concentration is 0.25 μg/mL and that of GEM is 2.5 μg/mL (Fig. 1E, upper panel). The free drug mixture data was further processed by using CompuSyn software for drug synergy calculation. We obtained a combination index (CI) of 0.5; according instruction software, which suggested a synergistic effect when CI<1 and a strong synergy if CI≤0.5 (www.combosyn.com). Thus 0.5 is indicative of strong synergy.

We continued to use the free drug data as a reference point to design the dual delivery LB-MSNP, including a 10:1 ratio, which was compared against 100:1 and 5:1. The necessity of synergy confirmation in nanoparticle mediated dual

delivery has been discussed in the result section. In order to evaluate the GEM/PTX synergy in the nano formulation, a series of GEM/PTX LB-MSNPs were prepared in which we used a fixed amount of GEM (25%, w/w) in the presence of 0.25-5% wt% PTX to yield GEM/PTX ratios of 100:1, 10:1 and 5:1. Each particle type was used to assess cytotoxicity and determine the concentration of each drug in the mixture for a 50% killing effect (Fig. 1E1, lower panel). We also performed independent experiment using GEM-only LB-MSNP and PTX-only LB-MSNP. This allowed us to show at an encapsulation ratio of 10:1, one could reduce the GEM concentration required for 50% cell killing from 5.2 μg/mL (using GEM-only particle) to 2.3 μg/mL (using dual delivery particle). Since the 50% killing occurred in the presence of a PTX dose of 0.23 μg/mL (Fig. 1E1, lower panel), this falls into the non-toxic dose of PTX based on the killing experiment using PTX-only LB-MSNP (Fig. 1E2). The nanoparticle dual delivery data was also processed by CompuSyn software. It showed a CI of 0.5 for this ratio, which indicates strong synergy. The particle using an encapsulation ratio of 5:1 also showed potent killing, but we did not want to use this mixture due to the high dose of PTX (0.48 μg/mL), which was relatively close to a PTX cytotoxic dose (Fig. 1E2). The particles with the 100:1 encapsulation ratio were not effective in killing and had a CI of 1.03, which means an additive effect. All considered, we decided to use a 10:1 ratio for the further cellular and animal experiments.

S3. Assessment on the stability of GEM encapsulation and GEM release kinetics in LB-MSNP with and without the inclusion of PTX.

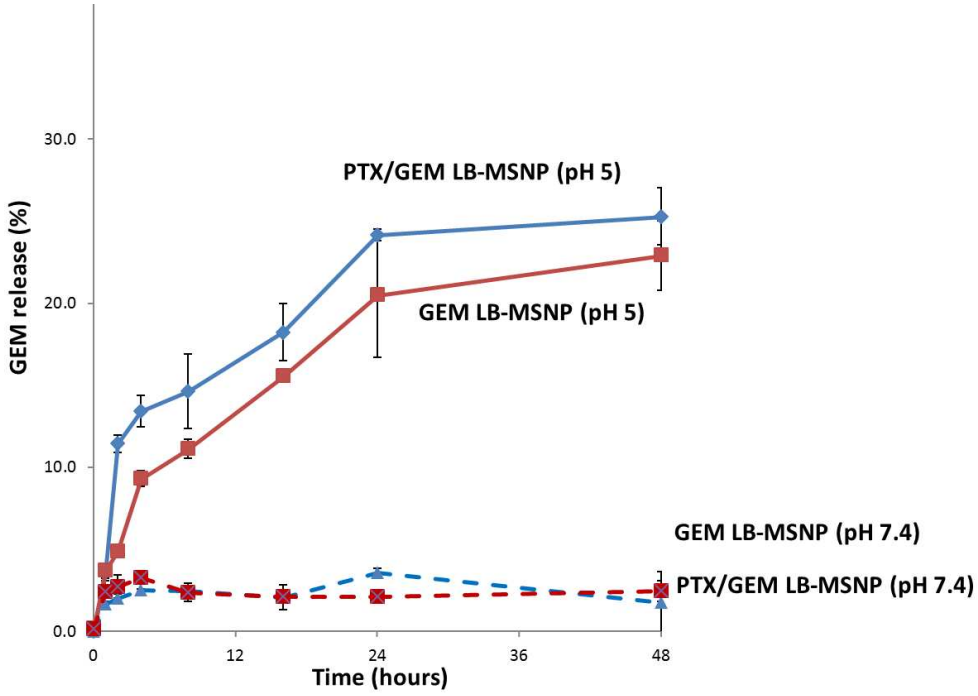


Figure S3: The stability testing of the LB by comparing the stability of GEM encapsulation in our particles with and without the inclusion of PTX. In both particle types, GEM encapsulation was stable in PBS (pH 7.4) for 48 hours, *i.e.,* <3.6% release. We also performed a comparison of GEM LB-MSNP and PTX/GEM LB-MSNP to determine GEM release kinetics in simulated lysosomal fluid, at a pH of 5. This demonstrated that the % GEM release from LB-MSNP in the presence or absence of PTX was 25.3% and 22.9%, respectively.

Figure S4.

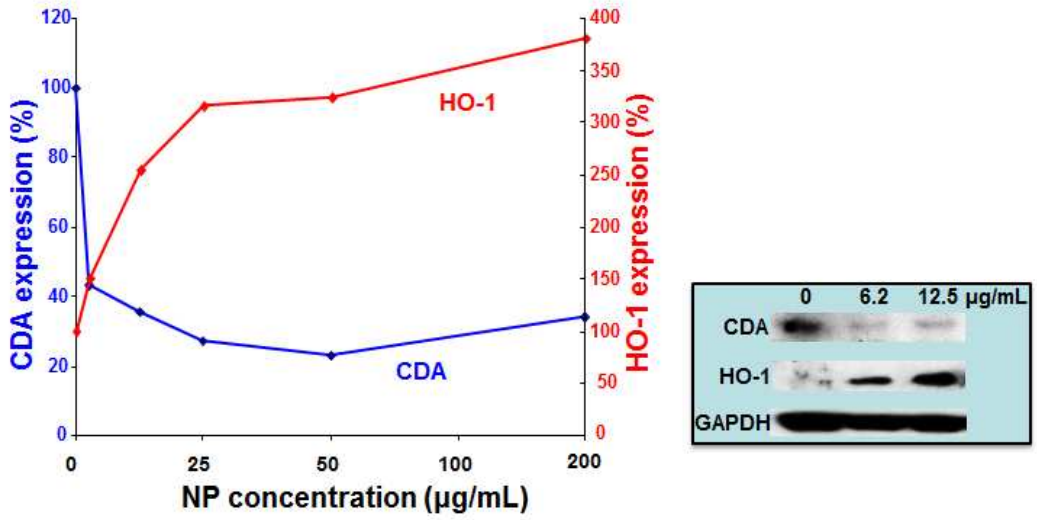


Figure S4: Dose-dependent effect of PTX/GEM co-delivery on CDA and hemeoxygenase 1 (HO-1) expression in PANC-1 cells. The levels of HO-1 and CDA expression were determined using western blotting, as described in the methods section. Representative immunoblot data are shown in the insert. Use of incremental particle doses (0-200 µg/mL) over 24 hours demonstrated a rapid decline in CDA in parallel with a more gradual increase in HO-1 expression.

Table S1.

Treatments	ALB (g/dL)	ALP (U/L)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Ca (mg/dL)	CHOL (mg/dL)	CREAT (mg/dL)	Phos (mg/dL)	TRIG (mg/dL)
Saline	3.0±0.1	47.0±5.8	38.9±11.1	171.5±48	30.0±15.1	9.7±0.2	82.7±14	0.2±0	6.5±0.3	127.0±21
Free GEM	2.8±0.1	50.4±8.6	25.2±3.9	90±31.5	18.7±0.6	9.5±0.4	78.3±3.2	0.2±0	8.2±1.6	125.3±15
Abraxane	2.8±0.1	48.2±5.1	37.4±14.9	202.2±118.5	22.3±2.1	9.5±0.3	70.7±11.8	0.2±0	7.5±1.3	118±27.2
GEM LB-MSNP	2.8±0.1	44.1±15	22.1±4.5	139.1±52.3	20±4.6	8.9±0.9	63.7±5.5	0.1±0	4±3.9	52±36.6
PTX/GEM LB-MSNP	2.9±0.1	36.9±18.6	40.3±23.4	180.2±25.6	20±4.6	9.5±0.7	77.3±2.1	0.2±0.1	5.7±1	110.3±861
GEM/Abraxane (1X)	2.9±0.2	68.4±17.8	32.6±10.6	126.5±76.8	24±9.5	9.9±0.6	84.7±4	0.2±0	7.3±1	118.7±964
GEM/Abraxane (12X)	2.9±0.2	82.1±18.4	36.8±14.2	170.9±50.6	21.7±2.1	10.2±0.6	76.3±3.2	0.2±0.1	7.7±2	131±11.5

Blood was collected from the sacrificed animals, and the serum obtained by centrifuging the whole blood at 5000 rpm for 15 min. The biochemical parameters were assayed by UCLA Division of Laboratory Animal Medicine (DLAM) diagnostic laboratory services. These parameters include albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium (Ca), cholesterol (CHOL), creatinine (CREAT), inorganic phosphorus (PHOS), and triglycerides (TRIG).