CHITOSAN-COATED LIPOSOMES REDUCE TUMOR GROWTH IN PANCREATIC ADENOCARCINOMA MODEL

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INTRODUCTION

Pancreatic adenocarcinoma is one of the most lethal and treatment-resistant neoplasms. While combining gemcitabine (GEM) and olaparib (OLA) is promising—particularly for patients with BRCA mutations—dense stroma and severe hypoxia trigger resistance to conventional therapies. To optimize efficacy and reduce side effects, this study proposes developing and evaluating *in vitro* and *ex ovo* chitosan-coated liposomes for the targeted delivery of co-incorporated GEM and OLA to tumor cells.

METHODS

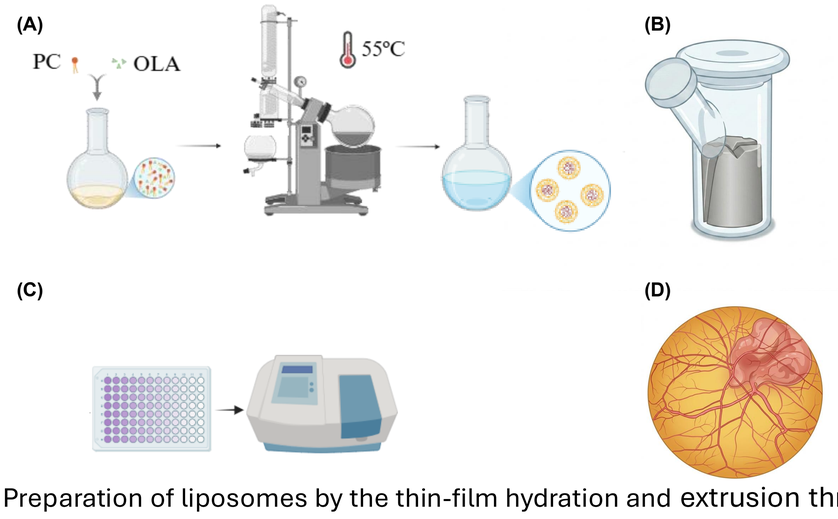

Figure 1. (A) Preparation of liposomes by the thin-film hydration and extrusion through 0.6–0.1 µm membranes and characterization by hydrodynamic size, PdI, zeta potential, pH, entrapment efficiency and transmission electron microscopy. **(B)** The drug release assay was performed over 6 h in PBS maintained under sink conditions. **(C)** MTT assay under 72 h in pancreatic carcinona cells (PANC - 1, MIA PaCa-2 anda BxPC - 3). **(D)** HET-CAM and *ex ovo* CAM assay.

RESULTS

Table 1. Characterization of empty and GEM/OLA-loaded liposomes.

Sample	Size (nm)	PdI	Zeta potential (mV)	pH	EE (%) GEM	EE (%) OLA
Uncoated empty liposome	133.3 ± 1.2	0.08 ± 0.01	-13.3 ± 0.5	7.1 ± 0.1	–	–
Coated empty liposome	134.5 ± 1.8	0.04 ± 0.02	-7.0 ± 0.4	7.3 ± 0.1	–	–
Uncoated GEM/OLA liposome	113.0 ± 0.5	0.08 ± 0.01	-12.3 ± 1.1	7.5 ± 0.1	49.7 ± 2.2	79.2 ± 0.2
Coated GEM/OLA liposome	114.9 ± 1.8	0.08 ± 0.02	-5.6 ± 0.3	7.6 ± 0.1	48.6 ± 0.3	81.5 ± 0.1

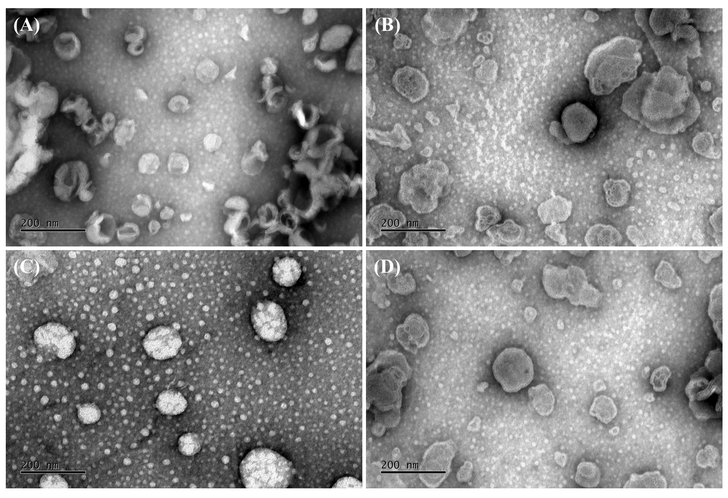

Figure 2. Transmission electron microscopy of the developed liposomal formulations. **(A)** Uncoated empty liposome; **(B)** Empty coated liposome; **(C)** Uncoated GEM/OLA liposome; and **(D)** chitosan coated GEM/OLA liposome. 10,000-fold magnification and 200 nm corresponding scale bar.

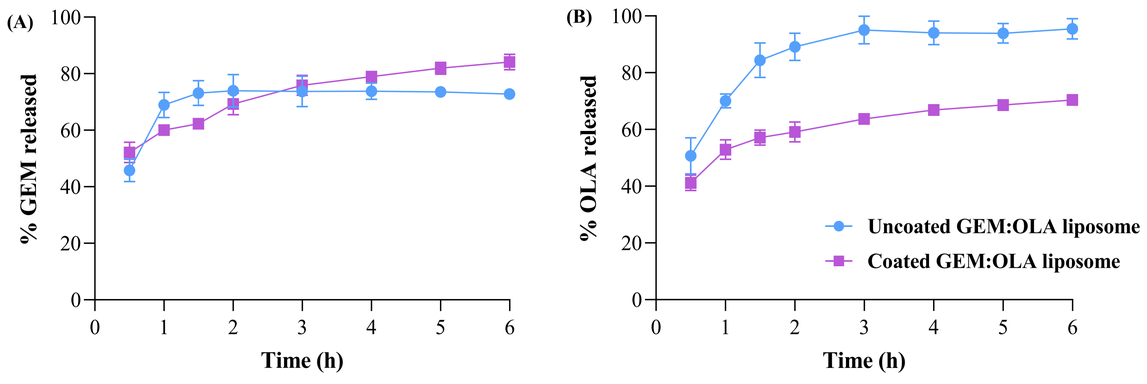

Figure 3. *In vitro* release profiles of **(A)** GEM and **(B)** OLA from liposomal formulations with and without the chitsan coating. $n = 6$ and $p < 0.05$.

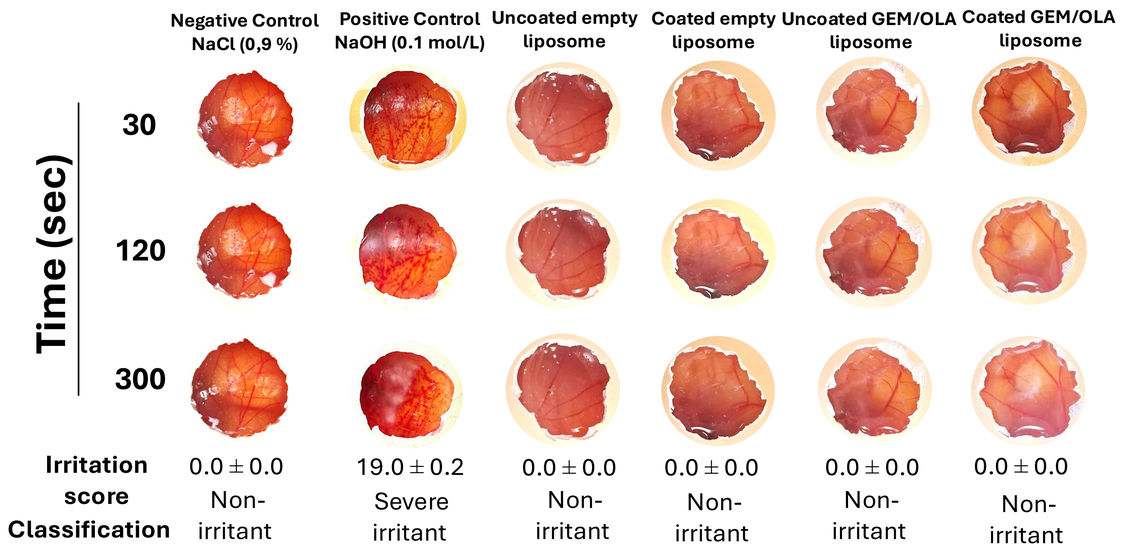

Figure 4. Representative sequential photographic record of the chicken embryo CAM toxicity and irritability assay (HET-CAM). $n = 3$ and the cumulative irritation score is presented as mean ± standard deviation.

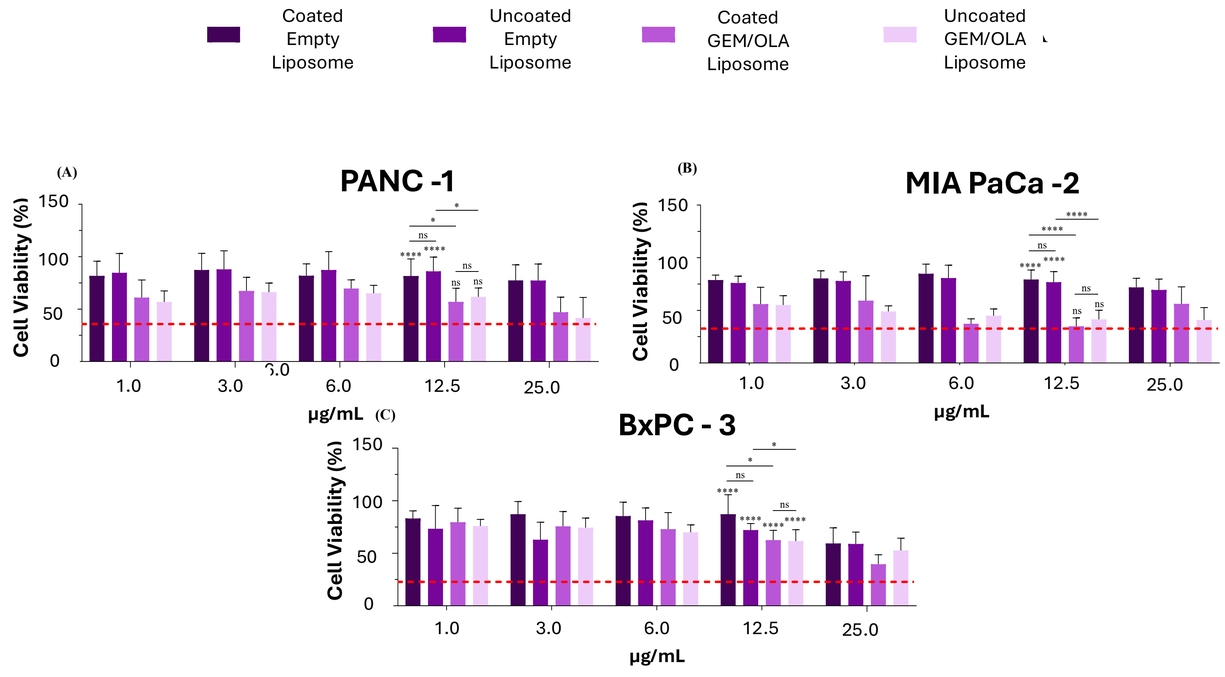

Figure 5. MTT assay after 72 hours of incubation for the **(A)** PANC-1, **(B)** MIA PaCa-2 and **(C)** BxPC-3 lines exposed to increasing concentrations (1.0 to 25.0 µg/mL). $n = 3$, ANOVA, where *(*)* $p < 0.05$, *(****)* $p < 0.0001$ and ns $(p > 0.05)$.

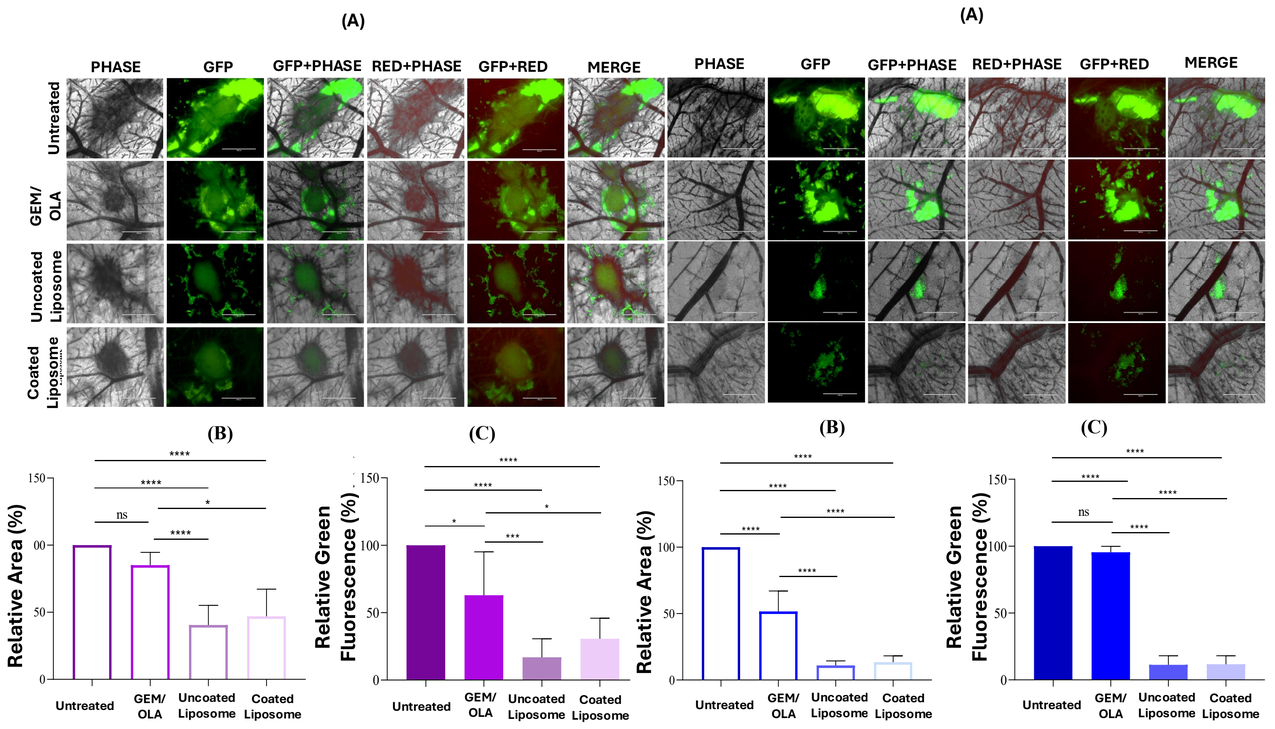

Figure 6. Representative image of the *ex ovo* CAM assay. PANC -1 and BxPC - 3 **(A)** Fluorescence microscopy, **(B)** Relative area and **(C)** Relative Green Fluorescence Intensity. ANOVA, *(*)* $p < 0.05$, *(****)* $p < 0.0001$.

CONCLUSIONS

The newly developed chitosan chitosan-coated liposomes successfully achieved the co-encapsulation of GEM and OLA. The platform demonstrated excellent biocompatibility and high antitumor efficacy within the *ex ovo* CAM model, positioning this nano-system as a highly promising translational strategy to overcome stroma-associated resistance in pancreatic adenocarcinoma therapy.

ACKNOWLEDGEMENTS