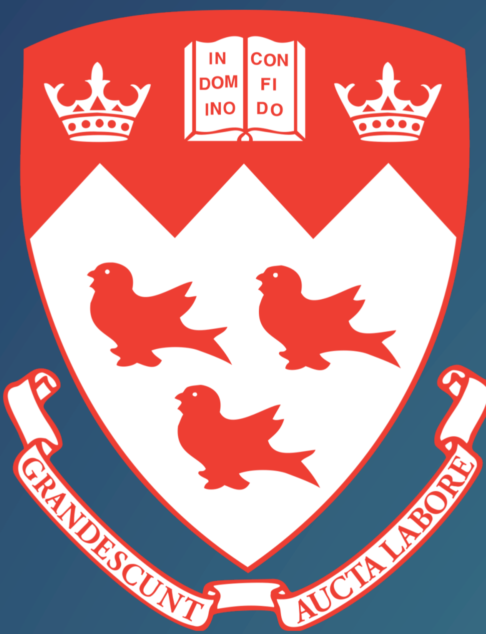

Synthesis and Stabilization of Nanoliposomal Copper Diethyldithiocarbamate using Poly (Ethylene Glycol) Carboxylate for Cancer Therapy

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Introduction

- Cancer is one of the leading causes of death worldwide.[1]
- Immune checkpoint inhibitors (ICI) significantly improve survival outcomes in some patients with advanced metastatic cancer. [2]
- However, multiple cancers are resistant to ICI therapy. [3]
- Copper diethyldithiocarbamate (CuET) has been shown to cause endoplasmic reticulum stress resulting in an accumulation of misfolded proteins, a heat shock response and cancer cell death.[4]
- Apoptotic cells release tumor antigens and could drive a localized immune response making CuET a promising neoadjuvant therapy.[5]
- Unfortunately, CuET has extremely low bioavailability, and a versatile, stable and scalable clinical formulation does not yet exist.

Materials and Methods

- Nanoliposomal CuET (LP-CuET) was synthesized using a modified ethanol injection method as shown in Figure 1. Briefly, phospholipids containing PEG-COOH, cholesterol and CuET (no CuET for control liposomes) were heated and dissolved in pure ethanol. The hot ethanol solution was then injected in rapidly stirred ultrapure water. The ethanol was then removed via rotary evaporation.
- The physicochemical parameters were measured using nanoparticle tracking analysis and dynamic light scattering - Zeta PALS. Transmission electron microscopy (TEM) was performed at 120 kV after drop casting the liposomes on carbon copper grids. SDS-PAGE electrophoresis was performed after incubating and centrifuging the liposomes with 10% heat-inactivated and normal human plasma.
- Cellular survival assays were performed using human (SK-MEL-28) and mouse (YUMM 1.7, YUMMER 1.7) melanoma cell lines using CuET dissolved in DMSO or LP-CuET.
- Cellular uptake was evaluated using live confocal imaging in YUMM 1.7 and RAW 264.7 cell lines (blue = nucleus, green = acidic vesicles, red = nanoliposomes stained with SR101)

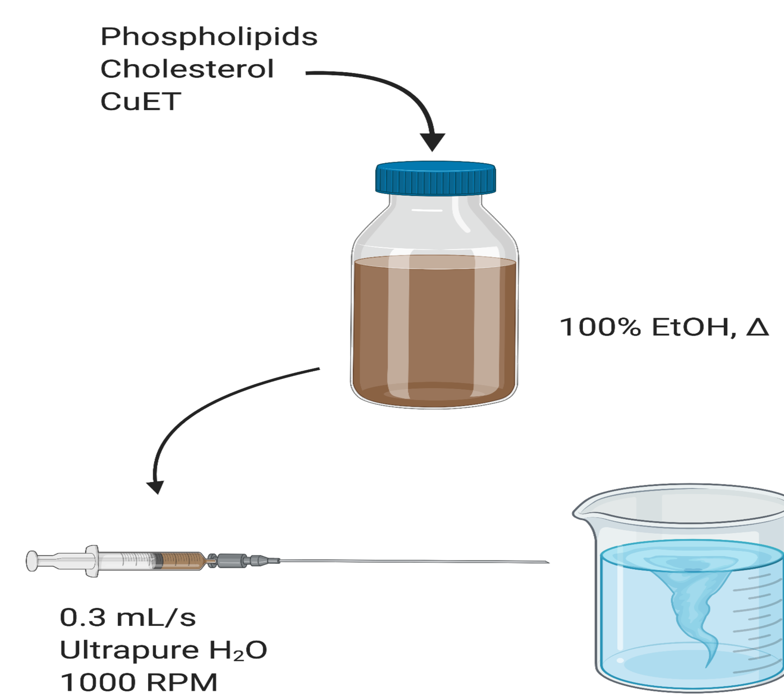

Figure 1. Illustration of the synthesis of LP-CuET

Results and Discussion

Parameters (Mean ± SD, n=3)*	LP-CuET
Diameter (nm)	111.9 ± 2.1
Hydrodynamic Diameter (nm)	191.6 ± 2.9
Concentration (np/mL)	1.75 * 10^{11} ± 2.71 * 10^{10}
Polydispersity Index	0.101 ± 0.009
Zeta Potential (mV)	- 56.60 ± 2.32
Encapsulation Efficiency (n=12)*	81.01 ± 1.16 %

Table 1. Nanoparticles' Physicochemical Characteristics

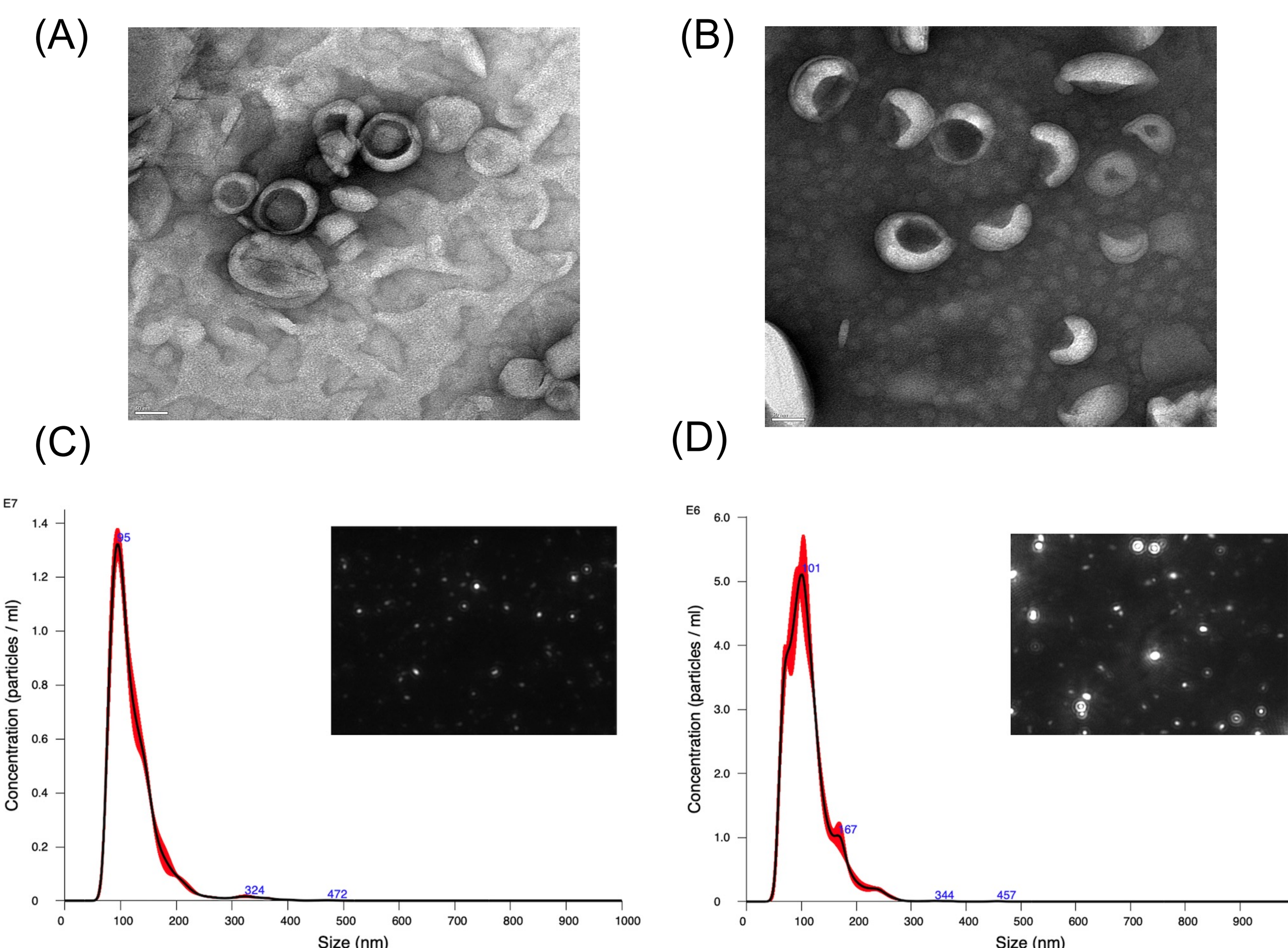

Figure 2. On average nanoliposomes are ~100 nm in size for both LP-CuET (A) and LP-Control (B) CuET is weakly stained in the center of the liposome (scale bar = 50 nm). (C-D) Representative NTA measurements of empty LP and LP-CuET.

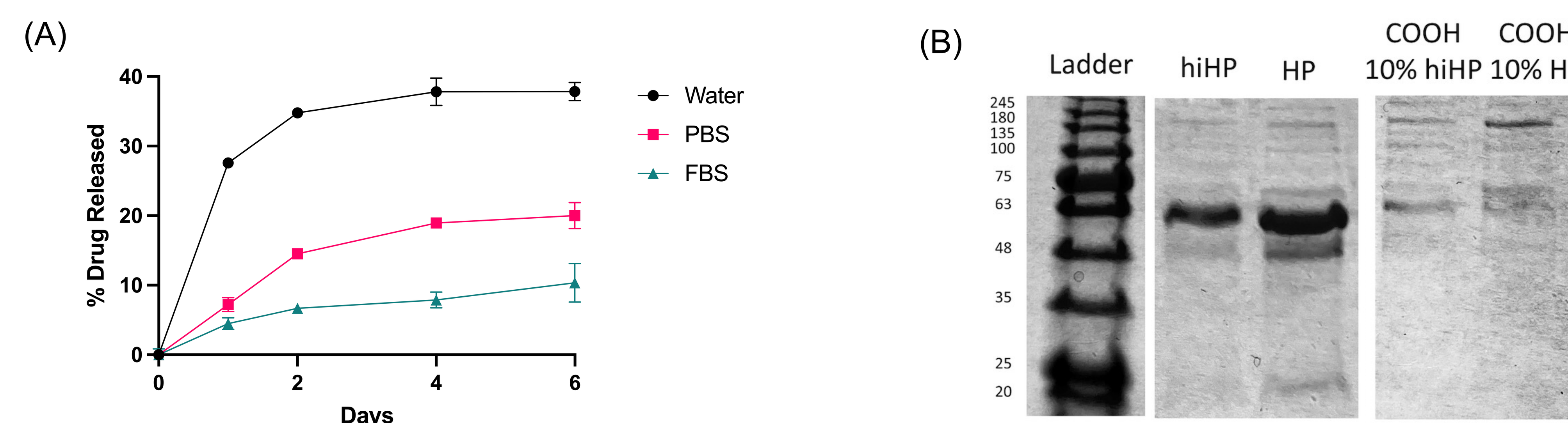

Figure 3. (A) LP-CuET demonstrates differential release kinetics *in vitro* at 37 °C depending on the solvent (mean ± SD, n=3). (B) Nanoliposomes show an accumulation of a distinct protein corona depending on their environment, which can influence their stability and cellular uptake.

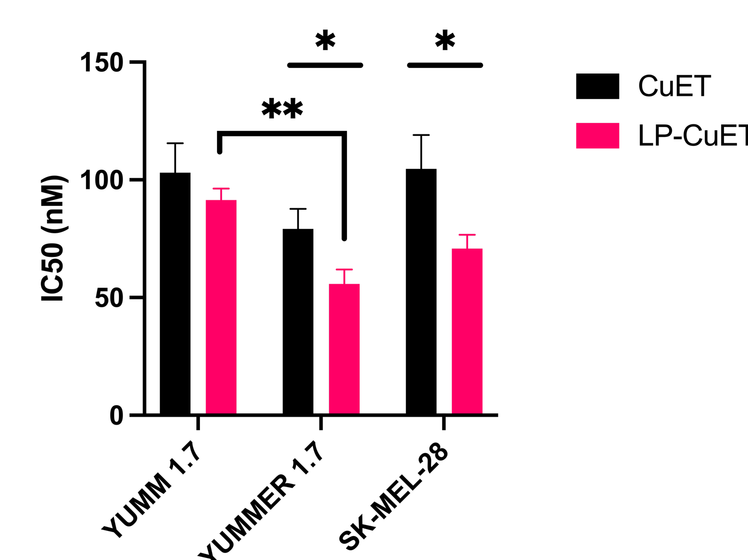

Figure 4. LP-CuET exhibits significantly increased cytotoxicity in melanocytes with a higher mutational burden (unpaired t-test with Welch's correction, where n = 3, * p<0.05, ** p<0.01).

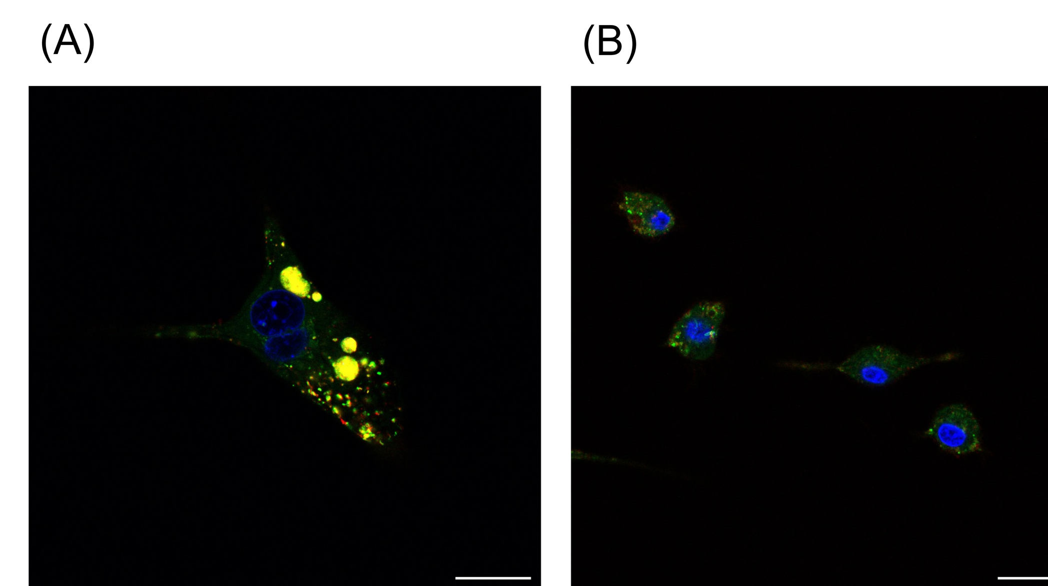

Figure 5. Live confocal imaging showing the preferential uptake of nanoliposomes (red) into intracellular acidic vesicles (green) in (A) YUMM 1.7 compared to (B) RAW 264.7 cells (nucleus is stained in blue, scale bar = 20 μm)

Results and Discussion

The synthesis protocol consistently yields nanoparticles with a mean size of 111.9 ± 2.1 nm, a polydispersity index of 0.101 ± 0.009, and zeta potential of -56.60 ± 2.32 mV, highlighting the colloidal stability of the system (Table 1, n=3). Negative staining with uranyl acetate allows the visualization of individual nanoparticle morphology, which remains relatively spherical when CuET is encapsulated (Fig. 2A) in direct contrast to the collapsed cup-like structure seen with LP-Control (Fig. 2B). NTA analysis confirms the size distribution (Fig. 2C,D). The nanoparticles exhibit multicomponent release kinetics (Fig. 3A) at 37ºC depending on the solvent. CuET encapsulation efficiency is over 80% and the nanoliposomes exhibit the lowest release of the drug over a week when dissolved in 10% FBS as a result of the adsorption of a distinct protein corona (Fig. 3B). Our results demonstrate the cytotoxic ability of LP-CuET against melanoma cancer cell lines, with IC50 values ranging from 28.64 nM to 125.10 nM (Fig. 4). Uptake experiments show that liposomes are delivered to intracellular acidic vesicles to a higher extent in YUMM 1.7 as compared to RAW 264.7 (Fig. 5).

Conclusions

- We were able to synthesize monodisperse nanoliposomes encapsulating CuET with high encapsulation efficiency and excellent stability.
- The nanoliposomal formulation exhibits multicomponent release kinetics that was stabilized by the addition of saline or serum at 37 ℃.
- LP-CuET solutions could be stored in the fridge or freezer for one month with no significant drug release or size change (data not shown).
- The formulation was able to significantly kill human and mouse cancer cells, especially those harboring significant mutagenic burdens.
- The nanoliposomes accumulate a distinct protein corona and show higher uptake in endocytic vesicles in YUMM 1.7 compared to RAW 264.7 cells.
- This data suggests that LP-CuET should be further explored as a neoadjuvant therapy in conjunction with ICI *in vivo*.

Acknowledgments

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