Nanovesicles From Red Blood Cells: A Potential Dual-Mode (Chemo Photothermal/Photodynamic) Therapeutic Approach

***Rishi Kumar Mishra, **Dr. Riyazul Hasan Khan**

**Research Scholar, **Research Supervisor*

Department of Microbiology

Himalayan University,

Itanagar, Arunachal Pradesh

ABSTRACT

*Micelles, polymeric nanoparticles, and liposomes are all examples of nanoparticles with cancer treatment approval. The main aim of the study is Nanovesicles from red blood cells: a potential dual-mode (chemophotothermal/photodynamic) therapeutic approach**.** All of the cell culture reagents, including foetal bovine serum (FBS), trypsin, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide), and dichlorofluorescein diacetate (H2DCFDA), were purchased from Invitrogen. Both the hydrophobic drug CPT and the amphiphilic photosensitizer ICG have been integrated into the bilayer membranes of the target cells, RBCs are able to re-assemble into stable nanovesicles.*

Keywords: *Micelles; Theraputic; Hydrophobic; Amphiphilic; Photosensitizer*

INTRODUCTION

Glimpse of nanomedicines approved for cancer treatment

Liposomes, polymeric nanoparticles, and micelles are all examples of nanoparticles with cancer treatment approval. For the treatment of breast cancer, DOXIL was the first nanomedicine to get FDA approval, which contains the active drug component doxorubicin hydrochloride (DOX) encapsulated in liposomes prepared from hydrogenated soy phosphatidylcholine (HSPC) and methoxy polyethylene (mPEG) conjugated distearyl phosphoethanolamine (mPEG-DSPE). The DNA-binding activity of the cytotoxic drug doxorubicin is considered to halt cell division. Nevertheless, it has potentially fatal adverse effects such as cardiotoxicity, the likelihood of which rises with the cumulative dosage. Liposomal doxorubicin was proven to be as effective as free doxorubicin in a phase III clinical study, with less cardiotoxicity. Since the nano formulation circulates for a longer period of time than free drug molecules, a greater concentration of the medicine is delivered to the tumour. The simultaneous encapsulation of the medication molecules within the liposomes decreases their direct accessibility to the heart tissue.

DaunXome, which encapsulates daunorubicin for the treatment of Kaposi sarcoma, Onco-TCS, whose active drug ingredient is vincristine for the treatment of non-Hodgkin lymphoma, Mepact, which encapsulates mifamurtide for the treatment of osteosarcoma, Onivyde, which encapsulates irinotecan for the treatment of pancreatic cancer, etc. One nano-based formulation that has been approved for use in treating breast and lung cancer is Genexol-PM, a paclitaxel-loaded polymeric micelle. Abraxane, an albumin-bound form of paclitaxel created to avoid the toxicity caused by its solvent, is another significant case in point. Although its effectiveness against cancer, paclitaxel is notoriously difficult to dissolve in water. Although non-ionic surfactant is linked to dose-limiting

hypersensitivity, it was formerly used to dissolve the medication before administration. Hence, albumin, a natural carrier of non-polar molecules, was employed to improve the solubility of taxanes, significantly decreasing solvent-associated hypersensitivity in the process. After being lyophilized, albumin and paclitaxel generated stable nanoparticles that could be administered without any pretreatment.

LITERATURE REVIEW

Choudhary, Dimple & Garg (2022) The abundance of heterocycles in natural products and their extensive therapeutic use make them an important type of substance in organic chemistry. One of the most important N-heterocycles, pyrazine is an important organic chemical with many practical uses. As it is so widely used as a template in the synthesis of bioactive components, catalysts, and reactions in a wide range of media, it has recently been the subject of a number of different techniques. The pharmacological effects of pyrazines and their derivatives include anti-inflammatory, analgesic, anti-cancer, anti-bacterial, and antioxidant properties. Several coupling processes, including as Suzuki, Buchwald-Hartwig, and oxidative coupling reactions, may be performed with this apparatus. This review was written with the intention of summarising the most recent six years' worth of research and progress in the field of pyrazine synthesis.

Bai, Lu & Xu, Dong & Zhou (2022) Overproduction of reactive oxygen species (in vivo oxidative stress) is linked to several degenerative disorders, including Alzheimer's, diabetes, and cardiovascular disease (ROS). Because of their potent antioxidant capabilities, natural polysaccharides have found widespread usage in biomedical and pharmaceutical applications as a kind of biomacromolecule with high biocompatibility. In this overview, By analysing the most-cited papers in the World of Science (WOS) database, we've determined that the antioxidant activity of natural polysaccharides is the most studied and sought after of their pharmacological effects. Antioxidant mechanisms using natural polysaccharides include largely enzyme activation, regulation of signal transduction pathways, and free radical scavenging. Natural polysaccharides and their derivatives have been the subject of much debate due to their potential antioxidant effects. In the meanwhile, we provide a quick rundown of how they are used in pharmaceutics/drug delivery, tissue engineering, and antimicrobial food additives/packaging materials. In sum, the data presented in this study could be useful for researchers interested in learning more about how to optimise the creation and use of natural polysaccharides that exhibit antioxidant properties.

Seddiqi, Hadi & Oliaei, Erfan & Honarkar (2021) On our planet, cellulose is found in greater quantities than any other carbohydrate. Wood and plant cell walls, bacterial and algal cell walls, and the only cellulose-containing mammals are all examples, tunicates, are also potential sources. Because of its inherent adequacy, new uses for this adaptable material are certain to be discovered. Tissue engineering, wound dressing, and medication administration are just few of the many applications of cellulose and its derivatives discussed in this article. Cellulose may occur in many different morphological forms, including fibre, microfibril/nanofibril, and micro/nanocrystalline cellulose, all of which have distinct physical qualities, dimensions, and shapes that may be selected. Particles of cellulosic materials might vary in shape and size depending on the circumstances under which they were synthesised or processed. Cellulosic particles of varying sizes and shapes may be used as building blocks to create materials with the wide range of microstructures and qualities required for a wide variety of biomedical applications. While cellulose has tremendous promise in many areas, it has so far been used mostly in the industrial sector, with less initial attention in the biological arena. This study therefore focuses on the most up-to-date techniques for preparing cellulose and its derivatives, which have led to unique features useful in certain biological applications.

S M, Basavarajaiah & Basha N, Jeelan (2021) Bioactive chemicals found in nature have many different biological functions. Some examples include antibacterial, anticancer, anti-inflammatory, and analgesic effects. Some of the natural ingredients may even be used as substitutes for pharmaceuticals. Furthermore, embelin is a cell-permeable bioactive molecule that has been found to have several biological functions, including antimalarial, anticancer, and anti-inflammatory effects. To continue our study on biologically active compounds, Based on

structural activity connection and docking studies, we have shown the target-specific anticancer and antimalarial properties of embelin and its analogues. Some recent studies have also indicated that embelin and its derivatives have therapeutic benefits for a variety of medical conditions. Recent research on the many biological actions of embelin and its analogues are the primary focus of this study.

Srivastava, Praveen & Pandey, A.K. (2015) For millennia, people have relied on the healing properties of plants and other natural items as a primary source of healthcare. According to the WHO, as many as 80% of people are still heavily reliant on traditional medications. An alternative to the pharmaceuticals, synthetic chemicals, and antibiotics now utilised in illness management, these plants are employed as immunostimulants. Use of medicinal plants and the products derived from them has the potential to treat illness by altering the body's immunological response, as well as its biological and pharmacological actions. Herbal plants that are readily accessible have been explored as potential remedies.

METHODOLOGY

Foetal bovine serum (FBS), trypsin, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide), and dichlorofluorescein diacetate (H2DCFDA) were all acquired from Invitrogen for use in cell culture. Uranium acetate was kindly provided by Dr. Manidipa's KSBS lab at IIT Delhi. Spectrum Laboratories provided us with the 8000 MWCO mini float-a-lyzer. In order to get A549 cell lines, we reached out to the National Center for Cell Sciences (NCCS) in Pune, India. Camptothecin, acetonitrile, and diphenyl isobenzofuran (DPBF) were all purchased from Merck. The sodium version of ICG was incorporated in a commercially accessible product called Aurogreen.

RESULTS

Preparation and characterization of ICG-CPT loaded vesicles (ICGNVECPT)

Red blood cells (RBCs) were extruded to create vesicles. The size and shape of these were measured and analysed using transmission electron microscopy. The particles had a core and a shell, making them virtually spherical (Figure 4.1a). The average vesicle size was between 80 and 120 nm, which is ideal for pharmaceutical use. We found that the thickness of the bilayer is around 8 nm, which is quite similar to the previously reported figures. DLS was used to assess the nanoparticle suspension's size as part of normal analysis (Figure 4.1b). CPT-ICG co-loaded vesicles (ICGNVECPT) were larger than those produced using TEM, and their sizes were comparable to those of empty vesicles (NVE). The potential values (Figure 4.1c) for NVE and ICGNVECPT were discovered to be -13.5 0.64 and -14.03 0.7 mV, respectively, which is an interesting contrast. Vesicle surface negative charge may originate from surface glycoproteins' sialic acid residues. The vesicles' capacity to remain stable in 37°C serum was next tested. Vesicle (NVE and ICGNVECPT) size was measured before and after serum incubation (t = 0 h) and normalised to the value obtained after incubation (t = 24 h). The vesicles' mean diameters were rather stable across time, indicating that they were stable in the biological media (Figure 4.1d). Significantly, neither CPT nor ICG impacted the lipid bilayer integrity, ensuring that the vesicles remained intact. Hence, it's safe to say that the stable nanoparticles are formed when ICG-CPT-loaded erythrocytes re-assemble after passing through extrusion membranes.

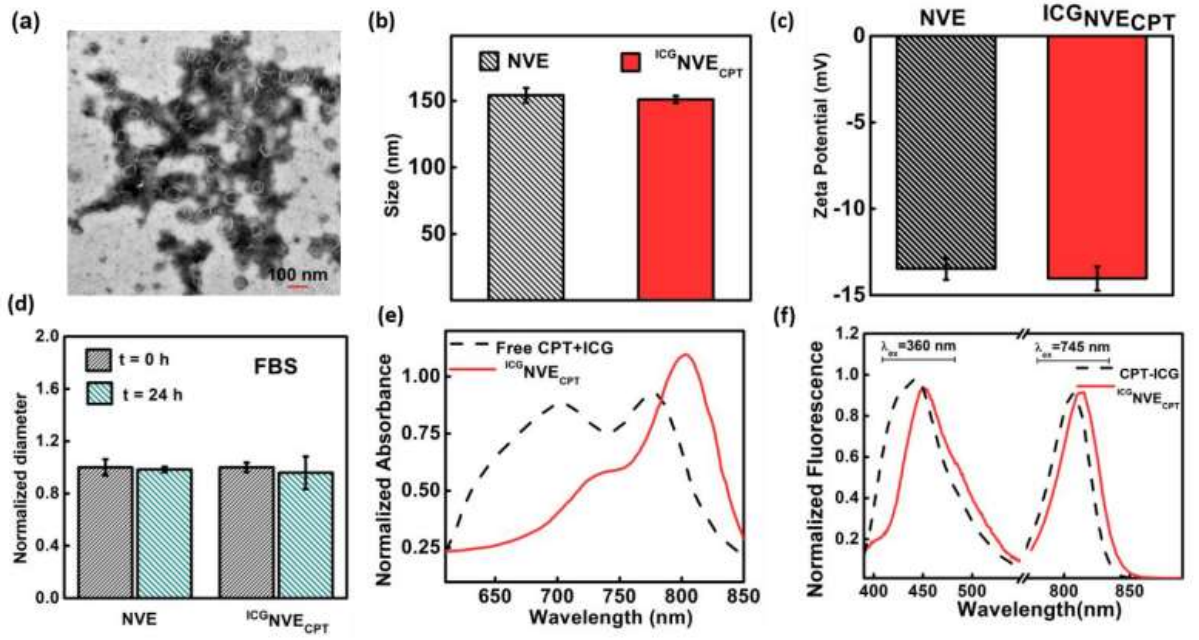

Figure 4.1: Characterization of ICG-CPT loaded vesicles (ICGNVECPT)

Physico-chemical characterization of vesicles

For vesicles, a higher concentration of ICG resulted in better loading efficiency (Figure 4.2a). Around 80% of the ICG at a concentration of 200 g/ml was partitioned inside the nano RBCs membrane. Incubation of RBCs with ICG causes the amphiphilic compound to partition rapidly inside the lipid bilayer. The red shift in the fluorescence spectra of ICG in nanovesicles (Figure 1f) is reflective of this. RBC membrane components have a high affinity for ICG, which greatly affects its stability in water (data not shown). Surprisingly, loading efficiency of CPT rose with CPT concentration immediately after ICG was loaded, but afterwards tended to decrease (Figure 4.2a). As camptothecin molecules are non-polar, it stands to reason that the RBC membrane would have a lower attraction for them than it would for ICG. ICG, which is structurally similar to membrane lipids, may have successfully adapted to its milieu thanks to the presence of both polar moieties and non-polar hydrocarbon chains. We anticipate that the integration of CPT might alter the short-range order of membrane lipids, leading to considerably decreased partitioning of CPT, due to CPT's structural rigidity. Finally, when comparing ICG and CPT for their efficacy in loading into vesicles, ICG was shown to be superior.

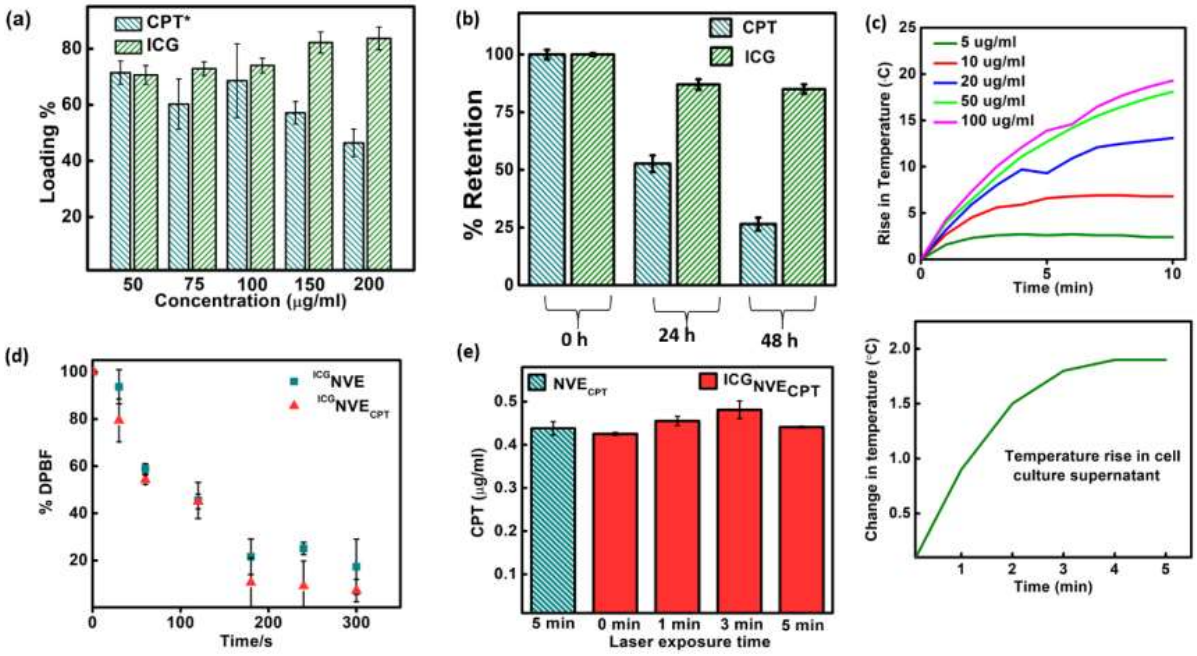

Figure 4.2 Physico-chemical characterization of vesicles

In-vitro studies

a. Cell uptake studies

The uptake of ICGNVE and free ICG was measured after incubation of the cells for various amounts of time. Figure 4.3a shows that the fluorescence intensity of the living cell population was much higher than that of the untreated cells. Cell uptake of ICGNVE was observed to rise with time and reach a maximum at 24 hours, according to a semi-quantitative analysis of cell uptake at various times. This may be because of how slowly cells absorb nanoparticles and how long ICG stays in the cytoplasm. As an alternative method, the absorbance of cells digested with ice-cold methanol was also evaluated using a multiplate reader. Spectra indicative of ICG were discovered, having a maximum wavelength (max) at 785 nm (Figure 4.3c). Additionally, by linking the gross absorbance to the ICG calibration curve and assuming a seeded cell density of 105 cells/wells, an estimated estimate of ICG concentration per cell was achieved. Each cell absorbed about 7.75 pg, 9.65 pg, 11.65 pg, and 10.2 pg of ICG after 2 hours, 4 hours, 6 hours, and 24 hours of incubation with ICGNVE. In contrast, at each time point, absorption of free ICG was three to four times greater than uptake of an equal concentration of ICG in ICGNVE (data not shown). This was likely the case due to the fact that unbound ICG molecules may diffuse into cells far more quickly than vesicles can be taken up by endocytosis.

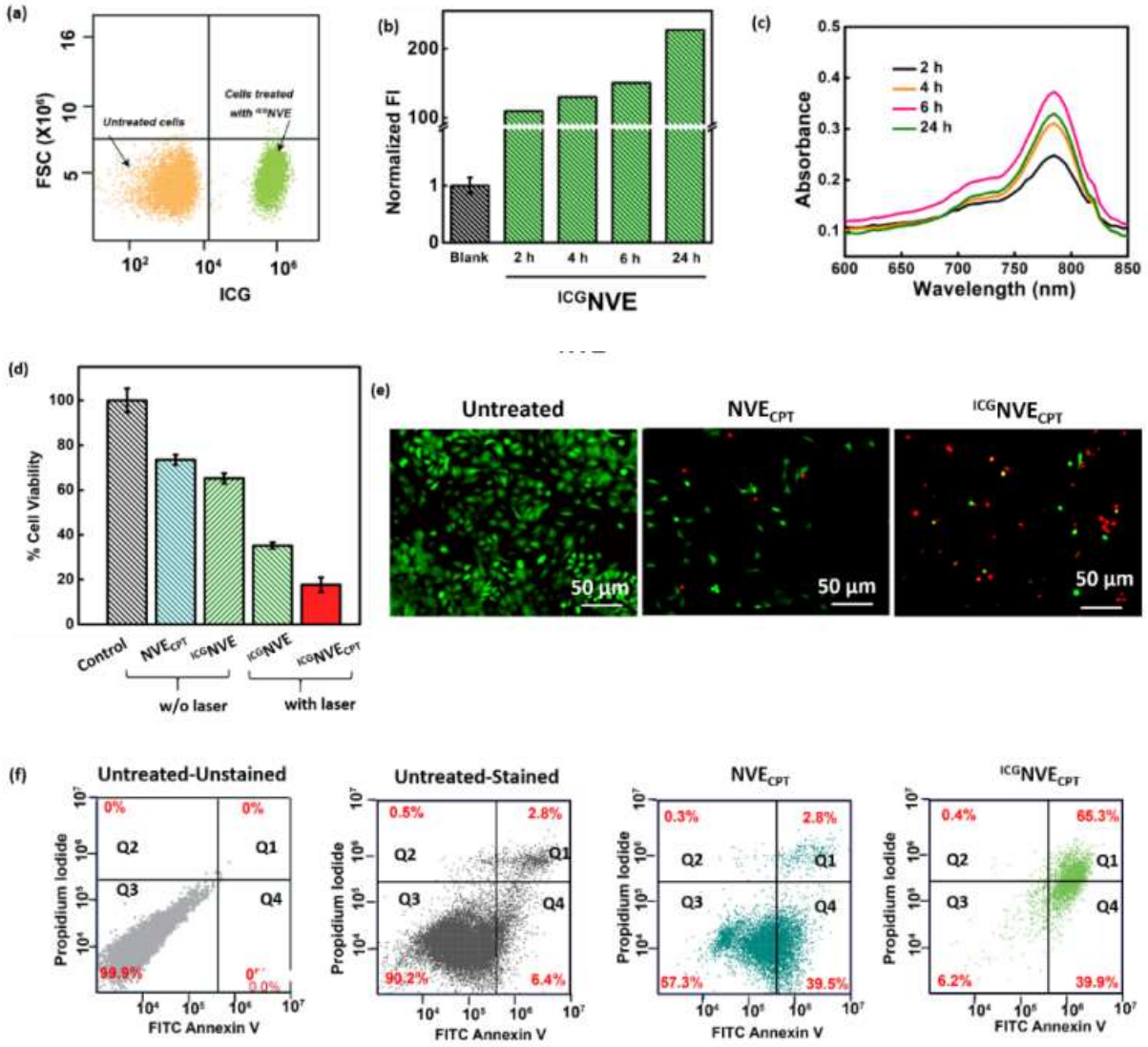

Figure 4.3. In-vitro cellular studies

b. In-vivo studies

PurposeThis research looked at how long ICG-loaded vesicles stayed inside subcutaneous EAC tumours. Subcutaneous injection of EAC cells was performed in the lower right leg, and then 50 l of ICGNVE was injected directly into the tumour. Animals were scanned for ICG fluorescence after being injected with the same amount of material into their left lower leg and excited at 745 nm. At each and every time point, tumours in mice showed strong fluorescent signals (Figure 4.5a). As a result, modest fluorescence was seen at the intramuscular injection site (left) even 15 minutes after the injection. When injected intramuscularly, nanovesicles are likely to spread quickly throughout the body. Unlike other injected substances, nanovesicles remained within the tumour for up to 72 hours after administration. Because of the absence of lymph veins and the leaky nature of tumour blood arteries, this was probably a symptom of the heightened permeability and retention effect. These results provide evidence for the safety and efficacy of intra-tumoral injection of ICGNVECPT in cases with superficial or easily accessible tumours amenable to localised laser irradiation.

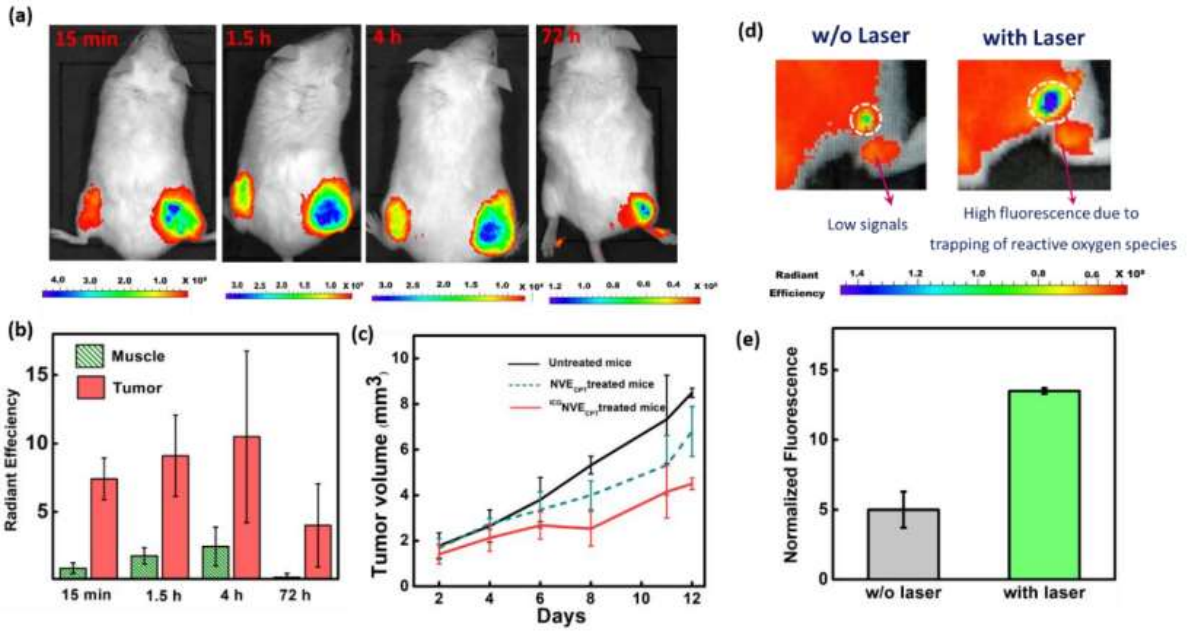

Figure 4.5: In-vivo tumor retention and efficacy studies

CONCLUSION

Re-assembly of RBCs into stable nanovesicles is facilitated by the partitioning of the hydrophobic drug CPT and the amphiphilic photosensitizer ICG into the RBC bilayer. Higher cytotoxicity is achieved because to the system's ability to create ROS in response to infrared laser irradiation, a result of the additive actions of CPT and ICG. Better effectiveness of the dual treatment was seen, and this may be attributable to the nanovesicles' prolonged retention in the mouse tumours, as a result of the EPR effect. Further research on its potential as a dual-therapy tool is needed.

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