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Thermosensitive Gold-liposome Hybrid Nanostructures for Photothermal Therapy of Cancer

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Abstract — **Thermosensitive liposomes with self assembled gold nanostructures have been developed for photo-thermal therapy. These liposomes (DSPC: CH: POPG, w/w ratio 7:2:1) have been prepared by thin film hydration and incorporated with gold nanostructures in two different ways. In one of the types, the liposomes were synthesized to encapsulate near infrared (NIR) absorbing gold nanocages within them. In another type, gold nanoclusters were self assembled on to the surface of these preformed liposomes. Among the two systems, the gold cluster coated liposome was chosen for further analysis owing to its better degradability criteria. It was found that the presence of a liposome template helps in tuning the absorbance of gold nanoclusters to NIR wavelength. NIR laser mediated temperature rise experiments were performed and the photo-thermal efficiency of these gold-coated liposomes was compared with gold nanoshells. It was found that gold – cluster self assembled liposomes were equally efficient as gold nanoshells in photo-thermal conversion. In addition, they have the capacity to act as carriers for drugs for targeted drug delivery. Gold nanocluster self-assembled thermosensitive liposomes are bio-compatible and have tremendous potential in killing cancer cells by dual modes of photo-thermal effect and temperature triggered drug delivery.**

Index Terms – Nanomedicine , Photothermal therapy , Liposomes , Gold nanoparticles , Drug delivery

I. INTRODUCTION

Photothermal therapy for cancer is gaining importance in the recent past owing to emergence of drug resistance and tumor recurrence observed in the conventional strategies of chemotherapy and surgery [1]-[3]. Though many groups have synthesized various gold nanostructures for photothermal applications [4], [5], the cytotoxicity and biodegradability of these particles in the physiological system is yet to be understood. In the recent past, cancer cell resistance to chemotherapy is on the rise [6], [7]. A new multimodal strategy is warranted to address the issue of these chemo resistant cancer cells. We have synthesized a unique biocompatible gold cluster-liposomal nanosystem that is observed to be equally efficient to that of conventional gold nanoshells. This system has tremendous potential to kill cancer cells in a bimodal way i.e. both by delivering drug and inducing heat to kill cells that are otherwise resistant to chemotherapy.

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II. EXPERIMENTAL

A. Materials

Distearoyl phosphatidylcholine (DSPC) with > 99% purity and palmitoyl-oleoyl-phosphatidyl-glycerol (POPG) were obtained from Lipoid and used without further purification. Cholesterol (CH) was purchased from Sigma Chemical Company (St.Louis, USA). Tetrachloroauric acids (HAucl4) and Silver Nitrate ($AgNO_3$) were also purchased from Sigma. Polyvinyl pyrrolidone(PVP) and Sodium sulphide were purchased from Merk. All other reagents were purchased from Spectrochem India Pvt. Ltd. All chemicals were reagent grade and used as received. All glasswares were cleaned with freshly prepared aqua-regia and rinsed with water before use. The water used in the experiment was of Millipore Milli-Q quality.

B. Synthesis of gold liposome hybrid nanostructures

The liposomes (DSPC: CH: POPG) were prepared by thin film hydration method and were subjected to characterization. The gold nanocages and nanoshells were prepared by galvanic replacement of silver templates [8]. The liposomes encapsulating gold nanocages were synthesized by incubating preformed liposomes with gold nanocages in water bath under continuous stirring and heat. The gold cluster- liposomes were prepared by reducing $HAuCl_4$ on the surface of preformed liposomes with the aid of ascorbic acid.

C. Characterization of gold liposome nanostructures

The liposome – gold hybrid nanostructures were characterized using using Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM) and UV-Visible spectrophotometry. Elemental analysis was done using Energy dispersive X –ray spectroscopy (EDAX). Temperature rise experiment was done with the aid of 750nm NIR laser (650mW-PMC, India). The concentration of gold in nanocages, nanoshells and gold cluster – liposomes were determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) analysis.

Biocompatibility study was done in L929, mouse fibroblast cell line (alamar blue assay). Photothermal cytotoxicity was done in MDA-MB 231, breast cancer cell line. Dead cells were analysed using propidium iodide stain under fluorescent microscope (Olympus, IX51,USA).

III. RESULTS AND DISCUSSION

A. *Characterization*

The size of liposome was observed to be less than 100nm in DLS analysis. After encapusulating nanocage or coating with gold nanoclusters, the size was observed to be 100 - 150nm in TEM analysis (Fig. 1). The presence of gold was confirmed by EDAX analysis (Fig. 2).

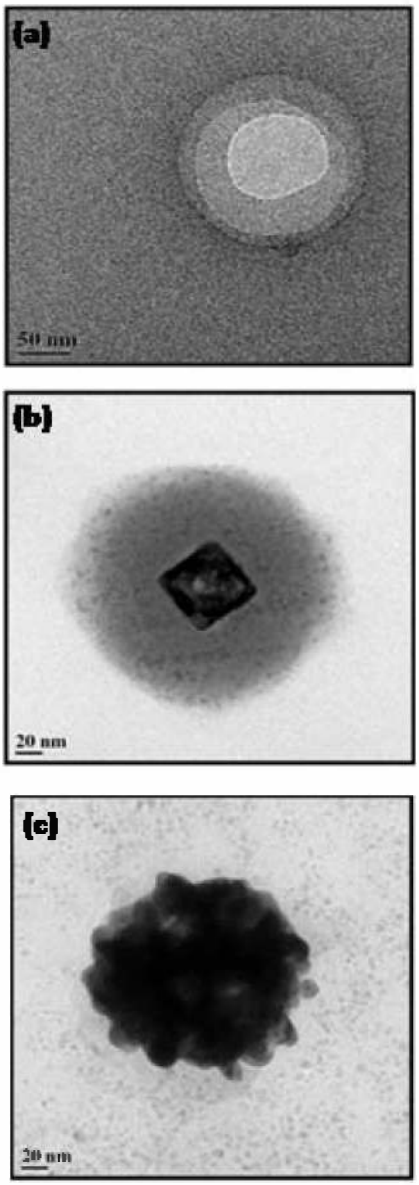

Fig. 1 TEM images of (a) liposome, (b) liposome encapsulating gold nanocage, (c) gold cluster coated liposomes.

The degradability of nanocages was uncertain. But the gold clusters have good chance of getting cleared through the renal system [9], [10]. Hence, the gold cluster – liposomes were taken up for further analysis.

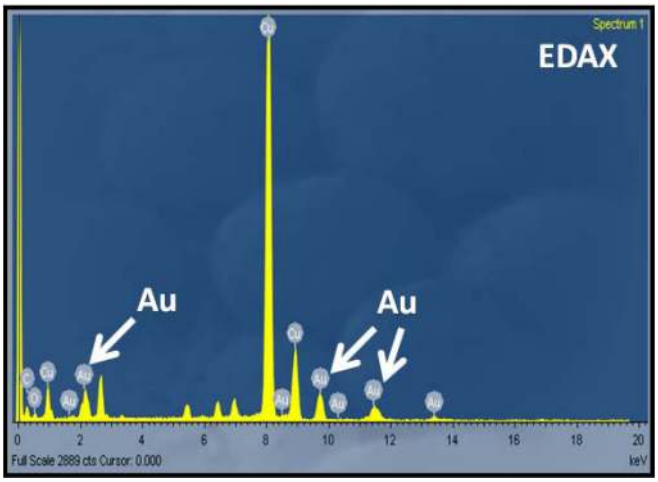

Fig. 2 EDAX analysis of gold cluster - liposome.

B. *NIR absorbance*

. The concentration of $HAuCl_4$ and ascorbic acid was varied to optimize the NIR absorbance of these gold clusters. It was observed that the blank liposomes (control) did not have any absorbance in the NIR region, whereas the gold cluster- liposomes gave a good absorbance peak in the NIR region (650-900nm) (Fig. 3).

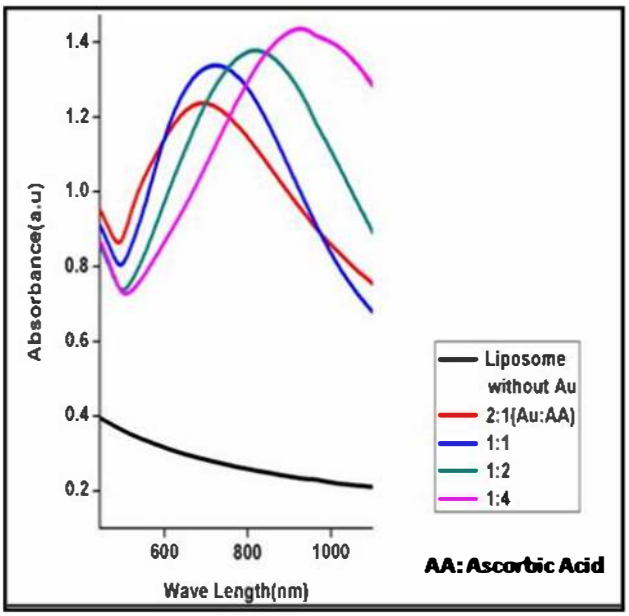

Fig. 3 UV- Vis spectroscopic analysis of gold cluster liposomes showing absorbance in the NIR region (ratio of Au:AA in mM conc.).

C. *Photothermal conversion experiment*

The photothermal conversion efficiency was determined by temperature rise experiment by irradiating a 750nm diode laser (650mW power) through the nanoparticle solution. It was observed that the gold cluster- lipsome solution was able to reach 43°C at slightly lesser time interval when compared with that of gold nanoshells containing same concentration of gold (50µg/ml – based on ICP AES analysis) (Fig. 4).

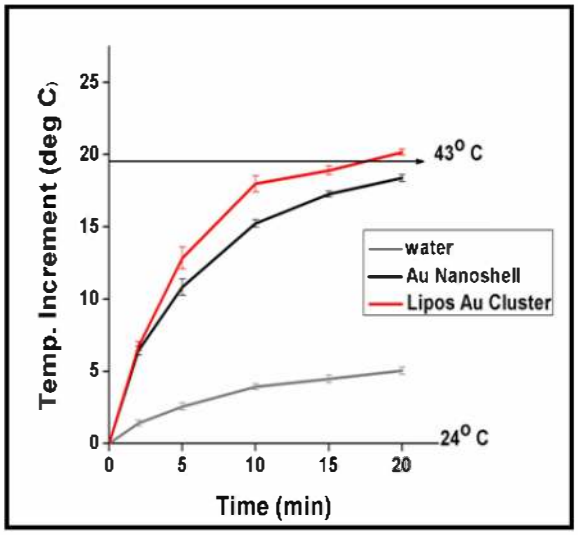

Fig. 4 Temperature rise experiment comparing the photothermal conversion efficiency of gold cluster liposomes with that of gold nanoshells.

D. *Biocompatibility study*

The gold cluster - liposomes were observed to be extremely biocompatible with normal cell line (Fig. 5). Even at a higher concentration of 1mg/ml of lipid (containing gold cluster) the cell viability was more than 90 %.

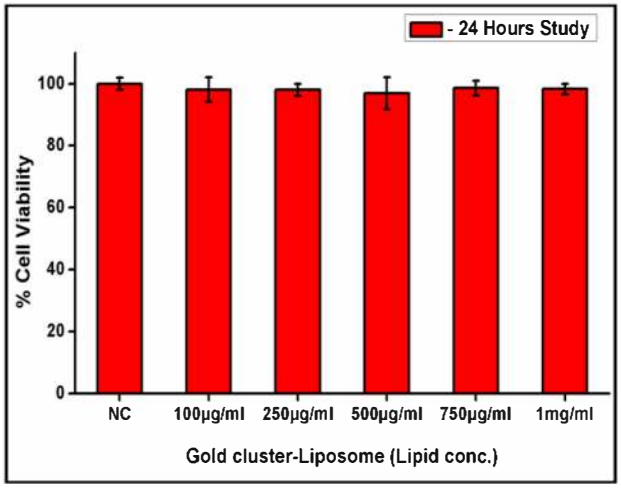

Fig. 5 Biocompatibility of gold cluster- liposomes in L929 cell line (mouse fibroblast) evaluated at the end of 24 hours (alamar blue assay).

E. *Degradation studies*

The gold cluster- liposome nanoparticles were subjected to sequential laser irradiation. It was observed that at the end of 20-30 mins of laser exposure, the particles got degraded and their spherical morphology was lost (Fig. 6a). The degraded particles contained tiny gold clusters of size approximately around 4 – 6nm as shown in Fig. 6b. This study proved that the liposome – gold cluster nanoparticles were thermosensitive and responded well to photothermal heat. This also gives an insight into the ability of the system to get cleared through the renal route, as it is well established that nanoparticles of size around 5nm can easily pass through the glomerular apparatus and undergo urinary excretion [10].

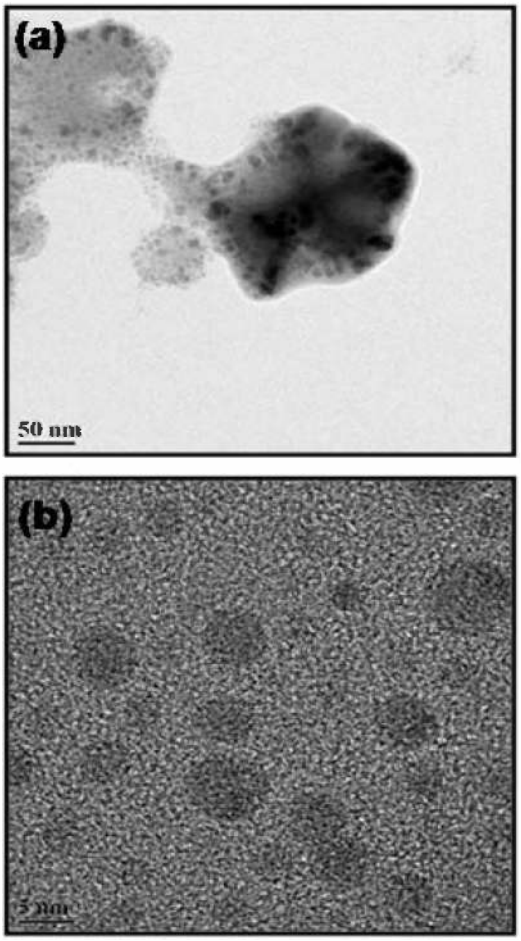

Fig. 6 TEM images of degraded gold cluster- liposomes (a,b) subjected to sequential NIR laser irradiation.

F. *Photothermal cytotoxicity*

Qualitative analysis of laser mediated photothermal cytotoxicity in cancer cell line was performed (Fig. 7). The controls showed no cell death. When the laser was irradiated on cancer cells incubated with gold cluster –liposomes, cell death started at the end of 5 mins of irradiation. When irradiated for 10 mins almost all the cells became dead

proving the photothermal cytotoxicity of gold cluster – liposomes.

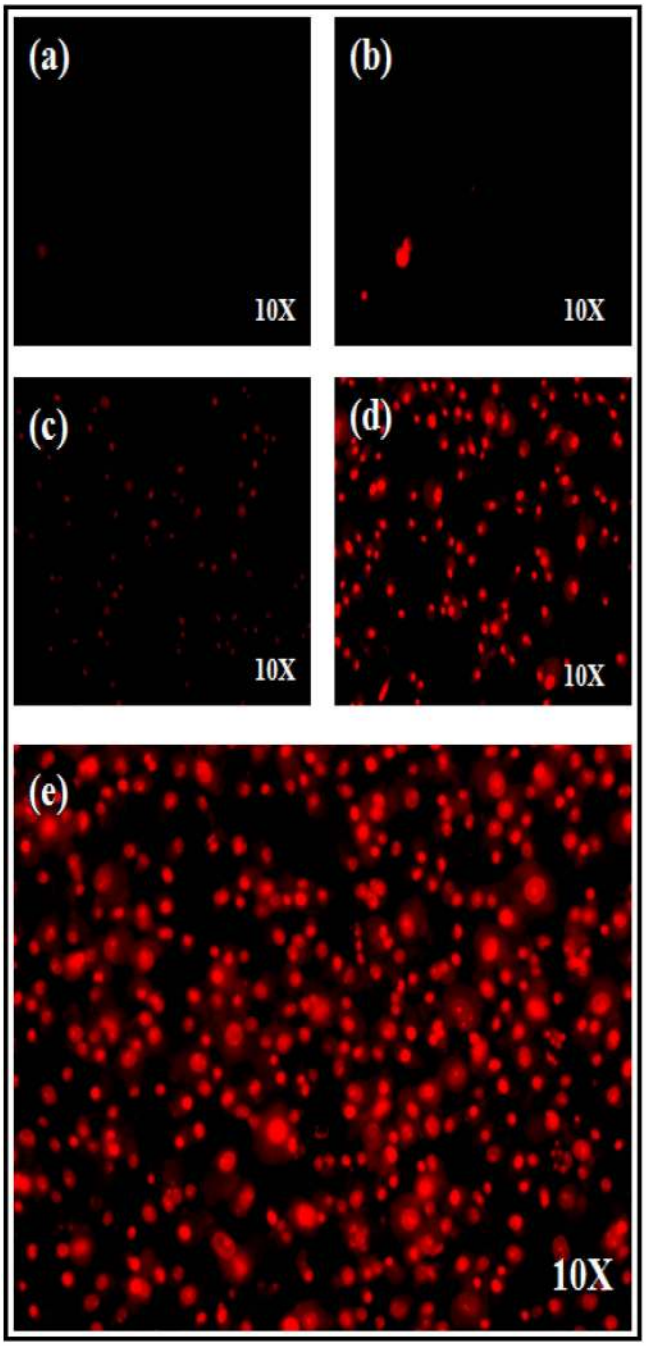

Fig. 7 Fluorescent microscopic images of MDA-MB 231 cell line (breast cancer) stained with propidium iodide (dead cell stain). (a) Cells treated with only gold cluster –liposomes, (b) cells irradiated with laser alone for 10 mins, (c-e) cells treated with gold cluster liposomes and laser irradiation for 5 mins(c), 7.5 mins(d) and 10 mins (e).

IV. CONCLUSIONS

In this study, gold cluster – liposome hybrid nanostructures were synthesized for photothermal application. They were characterized using using DLS, TEM, ICP-AES and UV-VIS spectrophotometry. They were found to be extremely biocompatible with normal cell line and were also biodegradable, when subjected to sequential NIR laser irradiation. It was observed that beyond certain cut off point (5 mins approx), cancer cell death was linear to NIR laser exposure time. This gold cluster – liposome nanoparticles have multifunctional capabilities that can be put to use in the field of cancer drug delivery and imaging as well.

Our future work would involve drug encapsulation to achieve bimodal therapy i.e. photothermal therapy combined with drug delivery. The efficacy of this system needs to be evaluated in small animal model as well.

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