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ORIGINAL PAPER

Biodegradable/disintegrable nanohybrids for photothermal theranostics

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Abstract

Nanohybrids using biocompatible and biodegradable materials are explored for their photothermal mediated theranostic applications. These hybrids are so chosen that they are biocompatible and biodegradable. Gold-based different nanosystems have NIR absorption which is highly favored for PTT, but they are limited by their toxicity, stability, and clearance. To overcome these challenges, we have demonstrated thermosensitive liposome-gold hybrid nanosystem for photothermal therapy and its clearance in vivo for the first time. We have also explored the possibility of using the same hybrid as drug (curcumin) carrier for triggered delivery and were successful in using curcumin as an adjuvant to PTT. Thermoresponsive polymers coated with gold for photothermal therapy and X-ray imaging of deep seated tumors has been evaluated. Fluorescent Poly (ethylene glycol) encapsulated metal nanoparticles were also synthesized and their application for cancer theranostics has been explored. Biodegradable and biocompatible polymer (PLGA) and liposomes loaded with anticancer plant fraction and a NIR dye showed synergistic photo-chemo therapy against cancer in-vitro & in-vivo. The intracellular changes with PTT and the mechanistic action of anti-cancer agent were explored in detail.

Keywords Biodegradable nanohybrids · Photothermal therapy · Enhanced permeability and retention effect · Combinative approach · Reactive oxygen species · Autophagy

Abbreviations

EPR	Enhanced permeability and retention effect
Au NPs	Gold nanoparticles
PTT	Photothermal therapy
NIR	Near infrared region
SPR	Surface plasmon resonance
FA	Folic acid
PLGA	Poly D,L lactic-co-glycolic acid
CfAc	Chlorophyll-rich anti-cancer fraction from the plant *Anthocephalus cadamba*
Lipos-Au	Liposomes coated with gold
Au PNVCL NS	Poly (*N*-vinyl caprolactam) coated with gold nano shells
Au-Lipos Cur	Curcumin encapsulated liposome-gold nanohybrid
CT	Computerized tomography
nm	Nanometer
FDA	Food and Drug Administration
DSPC	1,2-Distearoyl-sn-glycero-3-phosphotidylcholine
CHOL	Cholesterol
PEG	Poly ethylene glycol
FL PEG	Fluorescent poly ethylene glycol
PoeM	Polymer encapsulated metal
UV	Ultraviolet
TEM	Transmission electron microscopy
CMCs	Curcumin microcrystals
HSP 70	Heat shock proteins 70
ROS	Reactive oxygen species
LC3	Light chain 3
CTAB	Cetrimonium bromide
DOX	Doxorubicin

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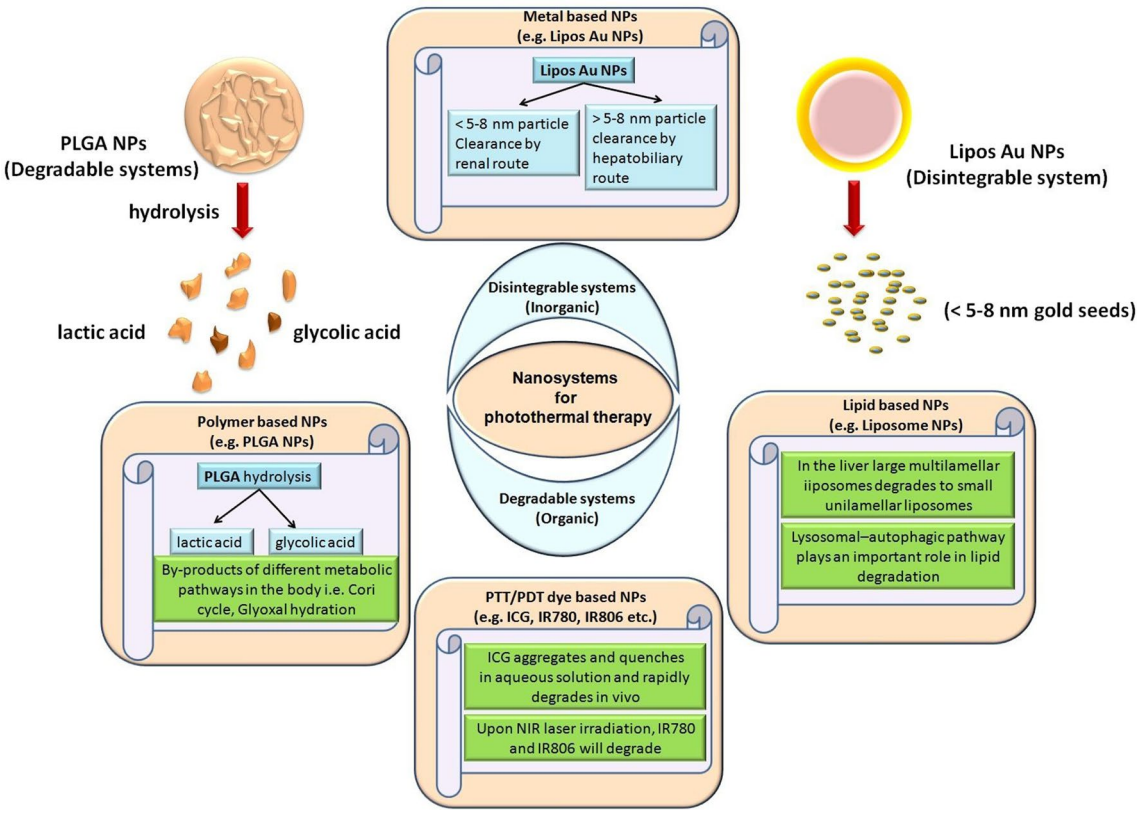
Introduction

Cancer remains one of the leading causes of mortality across the globe. According to the World Health Organization and International Agency for Research on Cancer, the number of cancer incidences is expected to rise to as many as 22 million by 2021 (Siegel et al. 2011). Therefore, cancer prevention, as well as treatment, remains one of the most important priorities in public health. Conventional strategies used for the treatment of cancer have lot of side effects and the possibility of development of resistance towards the treatment remains unaddressed. In this context, nanomaterials known for their high surface area to volume ratio that shows incredible physicochemical properties has greater scope as cancer nanomedicine. Nanomaterials show enhanced permeability and retention effect (EPR) due to leaky tumor vasculature that makes them suitable candidates for theranostic applications (Gregoriadis et al. 1977; Grislain et al. 1983; Richardson et al. 1979).

Among the metallic nanoparticles, Gold nanoparticles (Au NPs), have been widely explored in many applications like photochemistry, biomedicine, and electronics (Di Guglielmo et al. 2010; Riley and Day 2017). Au NPs are the chief mediators of photothermal therapy (PTT), with an absorption in Near Infrared region (NIR) and the ability to efficiently convert light energy to heat energy (Kennedy et al. 2011). Photothermally active nanostructures are being extensively investigated for various theranostic applications. These could be metallic nanostructures, or biodegradable nanoparticles coated by gold or loaded with NIR dyes which make them photothermally active. Au NPs are known for their Surface Plasmon Resonance (SPR) and thus their ability to be tuned to absorb in NIR range (Jain et al. 2006). The extinction coefficients of Au NPs are usually 4 to 5 times higher as compared to commonly used dyes and can be useful for imaging purpose (Huang and El-Sayed 2010). Au NPs appear in blue, red, brown or green color due to different shapes and sizes of nanoparticles formed with different precursors and reducing agents (Jain et al. 2006). Also, since tissue components do not absorb at wavelengths ranging from 700 to 1200 nm, this window is considered to be effective for PTT (Hauck et al. 2008) (Scheme 1).

PTT has been widely researched for tumor ablation in animal models and have now entered phase I of human clinical trials. The first photo thermally active nanoparticles: PEGylated silica-core Au nanoshells were prepared in the mid-1990s by Naomi J Halas and Jennifer West group



Scheme 1 Biodegradable/disintegrable nanosystems for photothermal therapy

from Rice University. The preclinical studies confirmed the higher accumulation of the nanoparticles at tumor site via the EPR effect that further advanced into clinical trials (AuroShell®) in 2008 (Hirsch et al. 2006; Loo et al. 2004). Clinical trials using a combination of Au nanoshells and laser radiation for treatment of primary or metastatic lung tumors and head & neck cancer with refractory or recurrent tumors are ongoing in United States (ClinicalTrials.gov Identifier: NCT01679470, NCT00848042) (Chen and Cai 2015). Table 1 included the lists of clinical trials based on the Au NPs.

Inorganic nanomaterials such as gold-based nanostructures of different shapes and sizes were researched extensively, but their biocompatibility and disintegration has always been a concern. For instance, Gold nanorods have an appreciable photothermal efficacy, but are limited by their toxicity. In our studies, we have compared gold nanocages and shells for their biocompatibility and efficacy and observed that cages showed significant therapeutic effect without affecting normal cells (Rengan et al. 2014b). We have also tried to reduce the toxicity of gold nanorods by encapsulating them within biodegradable liposomes, conjugated with FA for a targeted photothermal therapy. Gold is inert and biocompatible, but not biodegradable. A photothermally active biocompatible and biodegradable nanosystem that could effectively be cleared from the body is considered an ideal PTT agent. We developed one such system: a biodegradable liposome-gold hybrid that can absorb in NIR region. We have not only demonstrated the therapeutic efficacy and multifunctional capabilities of this biodegradable nanosystem but also have successfully demonstrated the clearance of the nanosystem for the first time.

Photothermal therapy has always been a stand-alone therapy but its combination with anti-cancer agents is expected to result in synergistic & improved therapeutic effect. We have successfully proved this with our studies: 1. PLGA loaded with chlorophyll-rich anti-cancer fraction from the plant *Anthocephalus cadamba* (CfAc) and NIR dye (IR780) resulted in a synergistic therapeutic system, 2. Liposomes encapsulated with NIR light-sensitive dye (IR780) and bioactive chlorophyll-rich fraction, CfAc resulted in enhanced therapeutic benefit and 3. Curcumin encapsulated liposome-gold nanohybrid (Au-Lipos Cur) where curcumin acted as adjuvant and resulted in sustained therapeutic effect.

This review discusses our research addressing the issues of biocompatibility, toxicity, stability, and clearance of photothermally active materials. It also covers the development and application of multifunctional nanomaterials for cancer theranostics. This review includes our approach to cancer theranostics by combination of photothermal therapy and anti-cancer agents for synergistic and sustained therapeutic effects.

Biocompatible and biodegradable nanohybrids for drug delivery

Au NPs have versatile applications both in therapeutics and imaging. The nanostructures with different shapes and sizes have promising optical and physical properties. They are widely used for applications in photothermal therapy (PTT), in vivo X-ray/CT contrast, biosensing, etc.

Two different nanosystems, namely gold nanoshells and gold nanocages tuned to absorb at 750 nm wavelength were chosen for our study. These systems were explored for their biocompatibility and photothermal efficacy in vitro. Both the systems showed equivalent photothermal therapeutic efficiency, but nanocages were more biocompatible compared to the nanoshells, when all the other important parameters were constant (concentration, laser power, and irradiation time). This could be attributed to the % of silver present in shells compared to cages. Gold nanocages were more effective than gold nanoshells against breast cancer cells without any significant cytotoxicity to normal cells (Rengan et al. 2014b).

Biodegradability is a very important parameter to be considered for translation of any nanosystem for imaging or therapeutic applications. Gold is inert and biocompatible which satisfies the prime criteria, but its biodegradability and clearance are not understood very well. According to the guidelines of the Food and Drug Administration (FDA), nanoparticles administered through the body should be cleared within a reasonable time from the body. Towards this aim, a distinctive lipid combination of 1,2-distearoyl-sn-glycero-3-phosphotidylcholine: cholesterol (DSPC: CHOL) along with gold (Lipos Au NPs) for photothermal treatment has been explored (Rengan et al. 2014a). In vitro biocompatibility studies were performed by using the Alamar Blue assay in L929 cell line. Lipos Au NPs were found to be biocompatible upto 1 mg ml^{-1} of Lipos Au (lipid concentration). The pharmacokinetics study of Lipos Au NPs indicated that it is biocompatible under in vivo conditions without any toxicity to the normal tissues. The biodistribution and pharmacokinetic studies performed in mouse model exhibited in situ biodegradation in liver cells and showed clearance via hepatobiliary and renal route. The Lipos Au NPs showed photothermal transduction efficiency, owing to the presence of Au coating over the liposome bilayer. The lipid bilayer disintegrates resulting in the release of drugs, when subjected to laser irradiation (Rengan et al. 2014a). These novel nanosystems passively accumulated at tumor site due to poor lymphatics drainage and leaky tumor vasculature that

Table 1 Lists of clinical trials of Au NPs

Name	Materials	Condition/disease	Application	Intervention/treatment	First posted/last update posted	Phase	Recruitment status	Clinical trials.gov Identifier
NU-0129	Spherical Nucleic Acid (SNA) AuNPs	Gliosarcoma, Recurrent Glioblastoma	Targeting BCL2L12 in recurrent glioblastoma	Laboratory biomarker analysis, pharmacological study, targeted therapy	January 13, 2017/August 22, 2018	Early Phase 1	Active, not recruiting	NCT03020017
Silica-Gold Nanoparticles	Si-AuNPs	Stable angina, multivessel coronary artery disease	Nanophotothermal therapy of atherosclerosis	Transplantation of nanoparticles	January 5, 2011/June 18, 2019	NA	Completed	NCT01270139
AuroLase®	Silica-Au nanoshells coated with PEG	Neoplasms of the prostate	Nanoparticle directed focal therapy for ablation of prostate tissue	AuroShell particle infusion to ablate neoplasms of the prostate	February 11, 2016/August 28, 2019	NA	Active, not recruiting	NCT02680535
CNM-Au8	Au nanocrystal	Healthy volunteers—male and female	Clinical trial of CNM-Au8 in healthy male and female volunteers	CNM-Au8, placebo	April 29, 2016/June 3, 2019	Phase 1	Completed	NCT02755870
AuroLase®	Silica-Au nanoshells coated with PEG	Head and neck cancer	Therapy in refractory and/or recurrent tumors	AuroLase therapy	February 20, 2009/February 9, 2017	NA	Completed	NCT00848042
AuroLase®	Silica-Au nanoshells coated with PEG	Metastatic lung tumors	Primary and/or metastatic lung tumors	AuroLase therapy	September 6, 2012/November 3, 2016	NA	Terminated	NCT01679470

leads to EPR effect. The therapeutic potential of these NPs tested in mouse tumor xenograft model using NIR laser (750 nm) irradiation showed complete ablation of the tumor mass, thus prolonging disease-free survival. Further, the pharmacokinetic study of NPs performed in a small animal model indicated in situ degradation in hepatocytes and further getting cleared through the hepatobiliary and renal route. Lipos Au NPs made up of liposome-gold nanoparticle hybrid system endures multifunctional capabilities, as contrast agents in X-Ray imaging (Rengan et al. 2015).

Similar to thermosensitive liposomes, Chauhan et al. developed a NIR responsive nanoshells using thermoresponsive and biocompatible polymer poly (N-vinyl caprolactam) to harness the maximum potential of NIR light-mediated PTT. NIR light-triggered thermoresponsive nanoshells associated with chitosan-grafted poly (*N*-vinyl caprolactam) as core and biocompatible gold as shell (Au PNVCL NS) were prepared. Chitosan coating helped elevate the lower critical temperature up to ~43 °C. The polymer nanoshells coated with gold resulted in 90% of cell death within 4 min of laser irradiation and the contrast observed in X-ray imaging is 1.6 times greater than that observed with iodine. This multifunctional polymeric nanosystem was used for both imaging and therapy when tested against breast cancer cell line, MCF-7 (Chauhan et al. 2017a). Hainfeld et al. reported a technique that unites mild hyperthermia (42–43 °C) with radiotherapy to significantly decrease the X-ray radiation dose. Au NPs with 15 nm sizes were used as hyperthermia agent and experiments performed over the radioresistant subcutaneous squamous cell carcinoma (SCCVII) in mice showed synergistic effects. The dose escalation studies confirmed that the dose was reduced to < 15 Gy in synergy whereas X-rays alone required ~ 55 Gy to control 50% of the tumors (Hainfeld et al. 2014).

Poly (ethylene glycol) (PEG) coating could enhance the circulation time of the nanoparticles by preventing its opsonization. PEG is biocompatible as well as biodegradable. The conversion of non-fluorescent PEG to fluorescent PEG (FL PEG) was employed for tracking nanoparticles. Our group synthesized FL PEG by a one-pot synthesis method. The enhanced systemic circulation was confirmed by the delayed uptake of FL PEG by zebrafish embryos. Fluorescent polymer encapsulated metal (Au/Ag) nanoparticles (PoeM NPs) showed the photothermal as well as antibacterial effects. PoeM (Ag) NPs were converted to PoeM (Au) nanoshells (NS) by galvanic replacement reaction (Fig. 1a). The galvanic replacement reaction incorporates redox reactions between a metal that is working as a votive template, and metal ions present in solution. Here, PoeM (Ag) NPs acted as a template for the synthesis of PoeM (Au) NS. PoeM (Au) NS were formed via replacement of Ag in PoeM (Ag) NPs with Au. This was further confirmed by the TEM contrast images. Presence of Ag and Au was confirmed by elemental investigation of PoeM NPs by EDAX study. PoeM (Au) NS, showed blue color fluorescence under UV light (Fig. 1b). The live/dead cell-based assay confirms ~ 67% of cancer cell death after NIR laser irradiation (Fig. 1c). FL PEG-based PoeM NPs could be useful for both imaging and therapeutic applications (Thomas et al. 2020). The stability

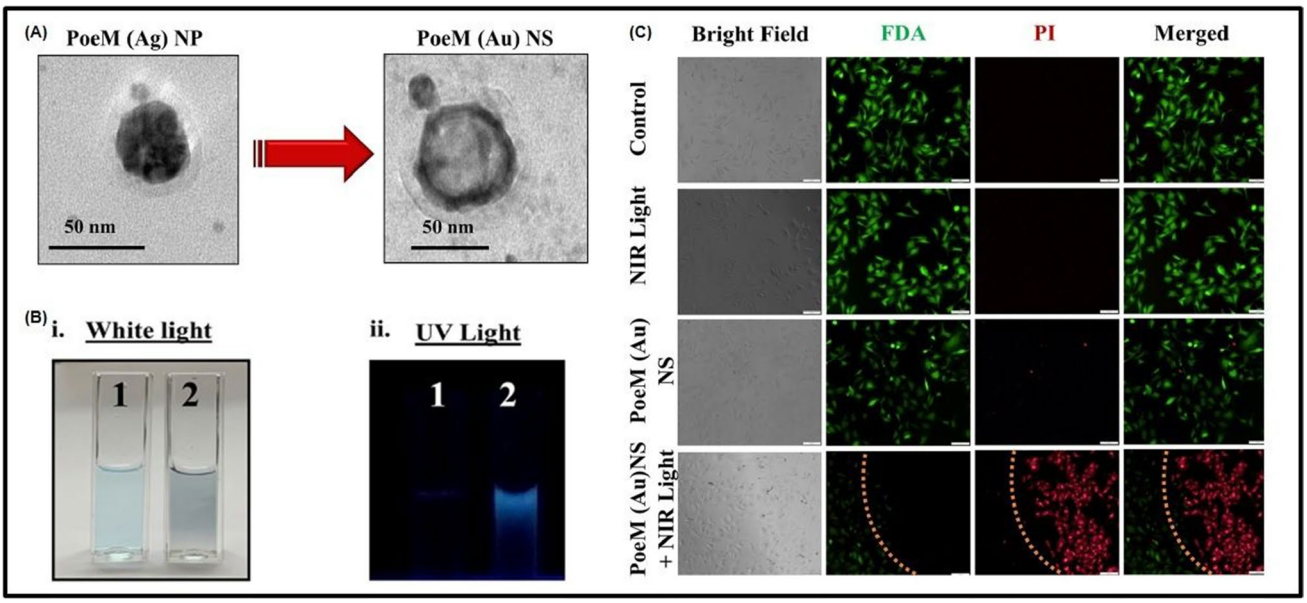


Fig. 1 **a** TEM images of PoeM (Ag) and PoeM (Au) NS (**b**) (i) White light and (ii) UV light images (1) control (Au) and (2) PoeM (Au) NS (**c**) live/dead cell assay (scale bar represents 50 µm). Reprinted with permission from Thomas et al. (2020, Copyright (2020) American Chemical Society)

and storage conditions were also evaluated for both PoeM (Ag) NPs and PoeM (Au) NS. These nanoformulations were stable upto 6 months without any observable aggregations. The hydrodynamic diameters and absorbance intensity of these nanoparticles, monitored at different time intervals depicted their improved stability (Thomas et al. 2020).

Mechanism of drug release and cell death by biodegradable nanosystems

The efficacy of nanosystems also depends upon the drug release pattern under physiological condition. On-demand drug delivery systems were considered better alternatives for cancer therapeutics (Kneidl et al. 2014). Triggered drug delivery systems based on the thermosensitive liposomes were extensively researched (Ali et al. 2008). Lipid-based nanosystems are sensitive to phase transition temperatures, where the phospholipids destabilize and release the encapsulated drug (Rengan et al. 2015, 2014b). We have evaluated the PTT mediated drug release and its therapeutic effect against different cancer cell lines using biodegradable nanosystems.

The gold-coated liposome acted as a better nanocarrier for the encapsulation of hydrophobic drugs. The photothermal transduction via NIR light irradiation has a dual role; it not only leads to hyperthermia-induced cell death but also facilitates the release of the encapsulated drug. We reported adjuvant therapeutic effect by the combination of curcumin and NIR sensitive gold-liposome nanoparticles (Fig. 2a). Curcumin is a well-studied anti-carcinogenic, and anti-inflammatory agent (Singh et al. 2014). However, the therapeutic effectiveness of the curcumin molecule is very poor because of its poor solubility and stability at physiological pH (Singh et al. 2015). Curcumin when encapsulated with liposome resulted in enhanced bioavailability. The combination of curcumin with NIR sensitive liposome gold nanoparticles acted as in situ adjuvant for cancer treatment. Curcumin encapsulated Au-Lipos nanoparticles (Au-Lipos Cur NPs), irradiated with 780 nm laser (650 mW) were proficient to generate heat

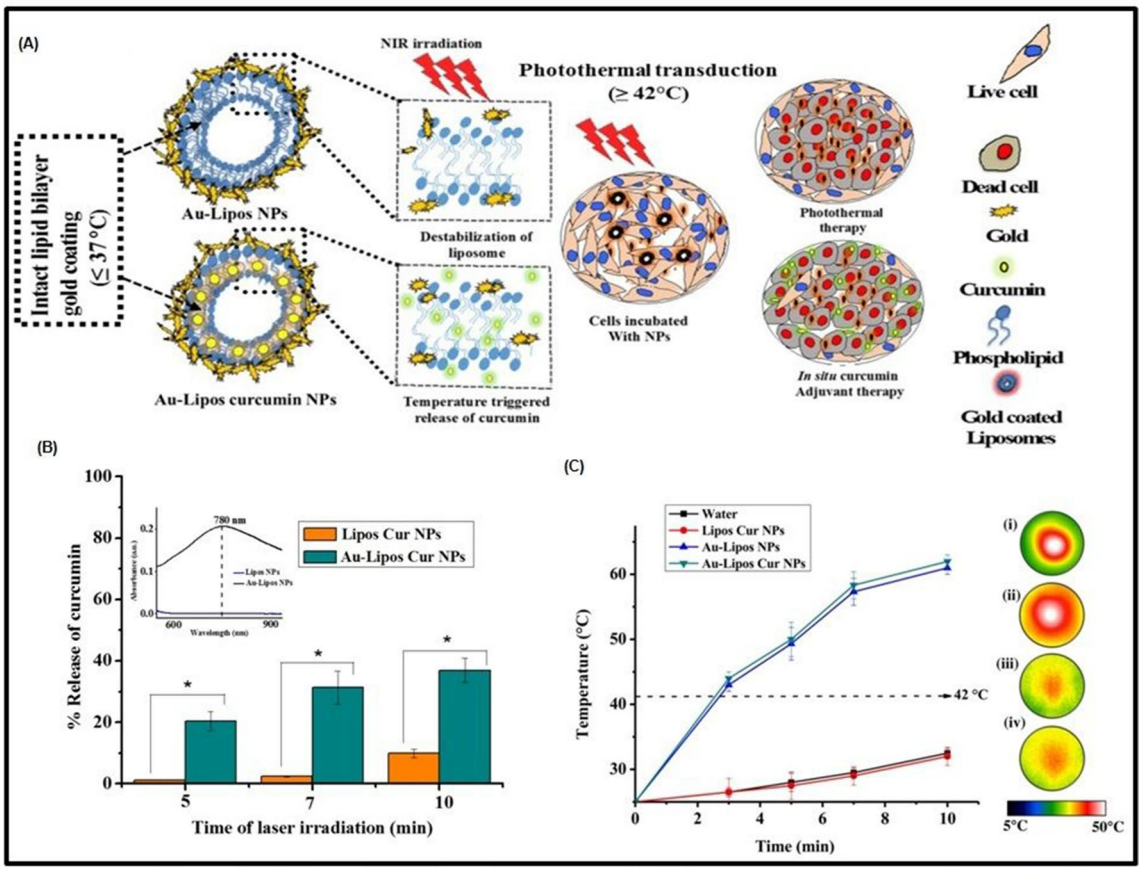


Fig. 2 **a** Schematic illustration showing in situ adjuvant effect and photothermal triggered release of curcumin (**b**) (i) NIR light-triggered the release of curcumin by Au-Liposome and Liposome NPs (ii) Photothermal transduction efficacy of Au-Lipos NPs. Reprinted with permission, Copyright 2011 Elsevier, Singh et al. (2018)

beyond 42 °C within 3 min of laser irradiation facilitating release of curcumin (Fig. 2b, c) (Singh et al. 2018).

We also studied the dynamics of the drug after its release with PTT. Curcumin was entrapped in the form of nanocrystals in Au Lipos NPs. These nanocrystals come together to form curcumin microcrystals (CMCs) after NIR light irradiation (Fig. 3a, b). The transition of nano to microcrystals exhibited sustained release of drugs for the prolonged time duration (> 10 days). Curcumin showed its effect as an adjuvant for inhibiting the regrowth of cancer cells that escape PTT. Western blot analysis showed the downregulation of HSP 70 and SLUG protein expression in cancer cells at different temperatures (Fig. 3c) in the presence of curcumin. We observed the localized release of curcumin in B16 skin cancer tumor model enabled regression in tumor volume consequential in the augmentation of PTT (Fig. 3d–f) (Alvi et al. 2019). Liver enzymes and biochemical parameters such as aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were estimated in animals treated with Au-Lipos Cur NPs and the experimental data indicated a minimal toxicity with maximal tumor volume regression (Alvi et al. 2019).

According to Hisato Konoeda et al., gold nanoparticles (GNPs) are highly tunable and can be very helpful for drug delivery. Their group studied clearance, acute toxicity, and organ distribution of GNP based drug delivery systems. Experiments performed in rats administered with GNP/peptide hybrid showed rapid clearance from the blood and frequent accumulation in the liver and spleen tissue. GNP/peptide hybrid showed no toxicity with dosing ratio up to 16 folds of therapeutic dose. The TEM imaging of GNP-peptide hybrids showed an average size of 20 nm. Their studies emphasized the significance of testing the pharmacokinetics and demonstrated the impact of surface coating over the behavior and biodistribution of Au NPs to different organs (Hisato et al. 2020). The size, surface charge, shape, and surface coating of Au NPs has a very important role in their activity (Ghosh et al. 2008). Lipos Au NPs are hybrid nanosystems with multimodal nature, which disintegrate after laser irradiation resulting in 5.5 nm gold seeds that can be easily cleared from the body. The heat generated by Lipos Au NPs led to thermal ablation resulting in the death of cancer cells. After laser irradiation, Lipos Au NPs disintegrated into gold seeds of size less than 5.5 nm which were capable of getting cleared through the renal system and hepatobiliary route as well. We were the first group to report the clearance

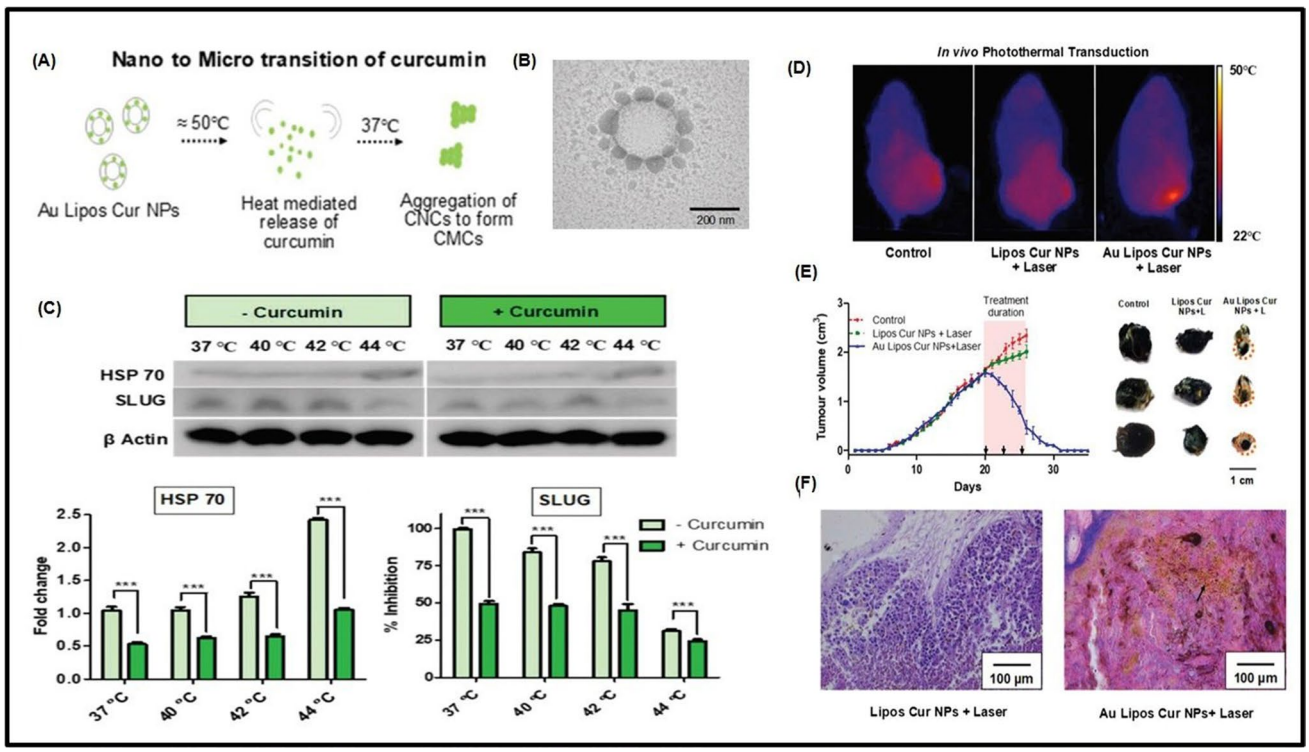


Fig. 3 **a** Schematic illustration of NIR laser mediated transition of curcumin nanocrystals (CNCs) to curcumin microcrystals CMCs. **b** TEM imaging of CNCs surrounding liposomes in Lipos Cur NPs. **c** Western blot analysis of HSP 70 and SLUG protein expression in cancer cells at different temperature. **d** In vivo photothermal imaging of Au Lipos Cur NPs. **e** Tumor volume regression and images of the dissected tumor (dashed red circle shows the occurrence of remnant melanoma over inverted skin). **f** H & E staining of the tumor sections. Reproduced by permission of The Royal Society of Chemistry, Alvi et al. (2019). (Color figure online)

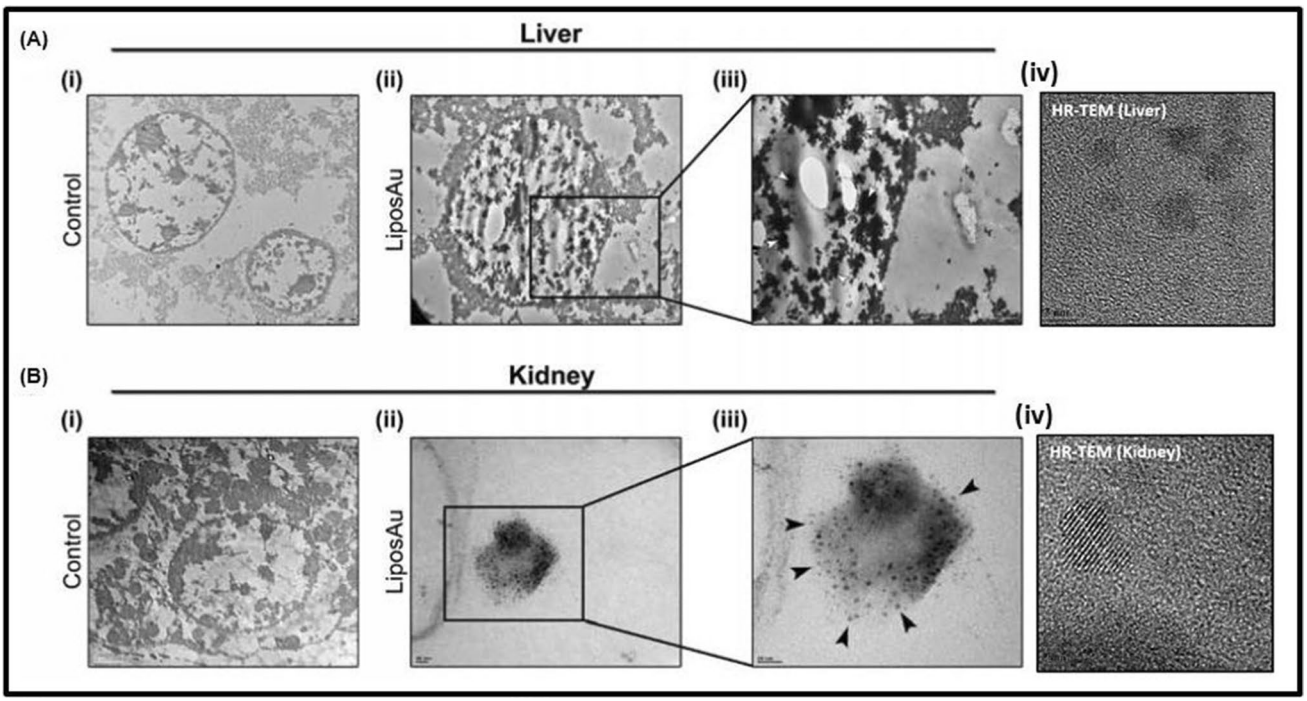


Fig. 4 **a** Liver tissue TEM images (i) control hepatocytes (ii) and (iii) Lipos Au NPs in aggregated state in liver tissue (iv) HR TEM image of liver tissue after 14 days. **b** kidney tissue TEM images (i) control kidney tissue (ii) and (iii) Lipos Au NPs in its dissociated state in kidney tissue (iv) HR TEM image of kidney tissue after 14 days. Reprinted with permission from Rengan et al. (2015, Copyright (2020) American Chemical Society)

of these gold nanoparticles in vivo. Lipos Au NPs showed the aggregation and disintegration in the liver (Fig. 4a) and kidney (Fig. 4b) respectively. For biodegradability and clearance studies, experiments were performed in small animal model. The HR TEM images of liver and kidney tissues were examined after 14 days of Lipos Au NPs injection. Lipos Au NPs were observed to be in their aggregated state in liver tissue and found to be dissociated in kidney tissue after 14 days. These results confirm the clearance of Lipos Au NPs without causing any significant toxicity (Fig. 4) (Rengan et al. 2015).

We report a polymeric nanosystem: Poly D,L lactic-co-glycolic acid (PLGA) encapsulated with chlorophyll rich biomolecular fraction (CfAc) isolated from the plant *Anthocephalus cadamba* (PC NPs) and a NIR dye (IR-780) (PCIR NPs) (Fig. 5a, b). CfAc has inherent anti-cancer properties

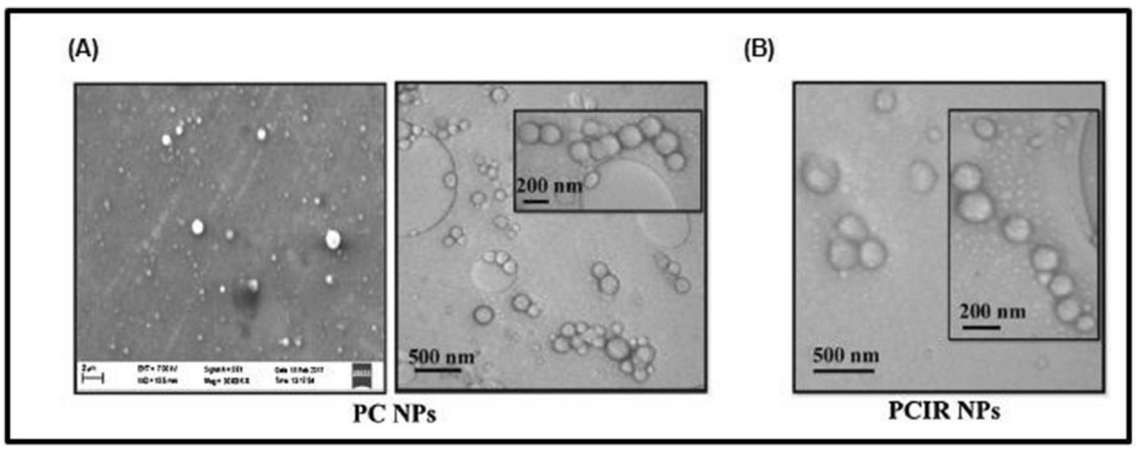


Fig. 5 **a** SEM (left) and TEM (right) PC NPs images. **b** TEM image of PCIR NPs. Reprinted with permission, Copyright 2011 Elsevier, Pemmaraju et al. (2018)

and red fluorescence which was used to comprehend the drug release. Cellular uptake studies confirmed the enhanced uptake of hydrophobic CfAc with the aid of PLGA nanoformulation. The photo stability of IR-780 was also observed to increase when incorporated within PLGA nanosystem which resulted in enhanced photothermal transduction efficiency. The red fluorescence of chlorophyll was used to understand the triggered release by NIR light irradiation. CfAc exhibited selective cytotoxicity towards cancerous cells as compared to normal cells due to the generation of selective ROS that led to specific tumor cell death (Pemmaraju et al. 2018).

In addition to a polymeric nanosystem, we reported the encapsulation of NIR light-sensitive dye, IR780 with CfAc in liposomes (CIR NLPs) that induced ROS mediated autophagic cell death. The degradation of IR780 can be observed with decrease in its absorbance at 800 nm (Fig. 6a), while the release of anti-cancer agent could be observed with increase in fluorescence at 680 nm with NIR light irradiation (Fig. 6b). CIR NLPs showed the release of CfAc via degradation of IR780 dye after NIR light-exposure in-vitro in MCF-7 cells (Fig. 6c). The increase in CfAc's red fluorescence with NIR light irradiation was observed from the microscopy images and quantified data of CfAc and IR780 fluorescence in cells (Fig. 6d, e).

The inherent red fluorescence of CfAc NLPs was observed under UV light, but the presence of IR780 masked the CfAc's fluorescence in CIR NLPs (Fig. 7a). The hydrodynamic diameter and zeta potential of CIR NLPs were ~ 106 nm and ~ 11 mV respectively (Fig. 7b). CIR NLPs were found to be uniform and spherical, when imaged by transmission electron microscope (TEM). Upon NIR light irradiation, photothermal heat was generated, destabilizing the liposomes and facilitating the release of anti-cancer agent CfAc. The released CfAc increased intracellular ROS triggering the autophagic mediated cell death in cancer cells. The selective ROS generation by CfAc led to autophagic cell death in cancerous cells which was validated by presence of autophagosomes *in-vitro* (Fig. 7c). CIR NLPs were observed to show their effect only after activated by NIR light. The interplay of ROS, autophagy and viability with CIR NLPs in the presence of NIR light was shown in (Fig. 7d, e). CIR NLPs upon NIR

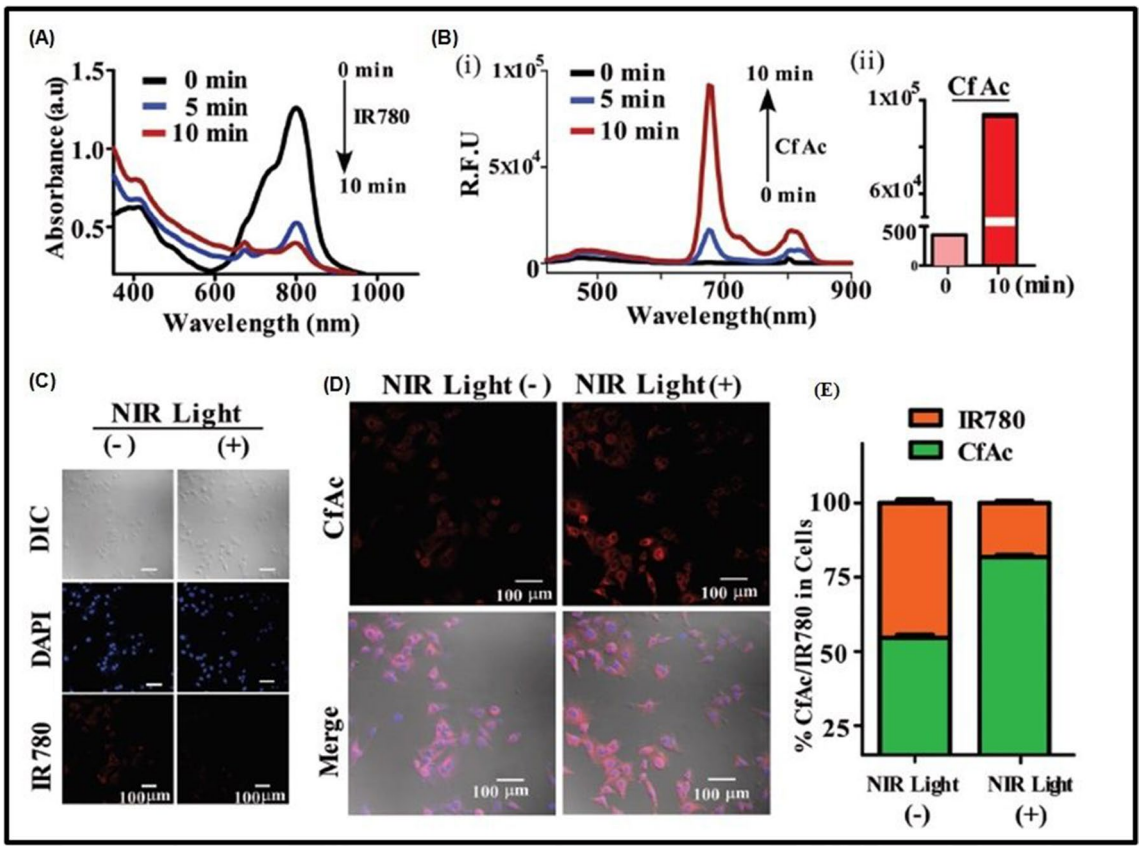


Fig. 6 Release study of CfAc from CIR NLPs (**a**) Absorbance spectra of CIR NLPs (**b**) (i) Fluorescence spectra of CIR NLPs showing the release of CfAc at the different time interval after NIR light exposure (ii) Quantification for the release of CfAc. **c** Confocal microscopy images of CIR NLPs treated MCF-7 cells. **d** Cellular images of CfAc's fluorescence after NIR treatment. **e** Quantification of CfAc and IR780 in cells. Reproduced by permission of The Royal Society of Chemistry, Appidi et al. (2020)

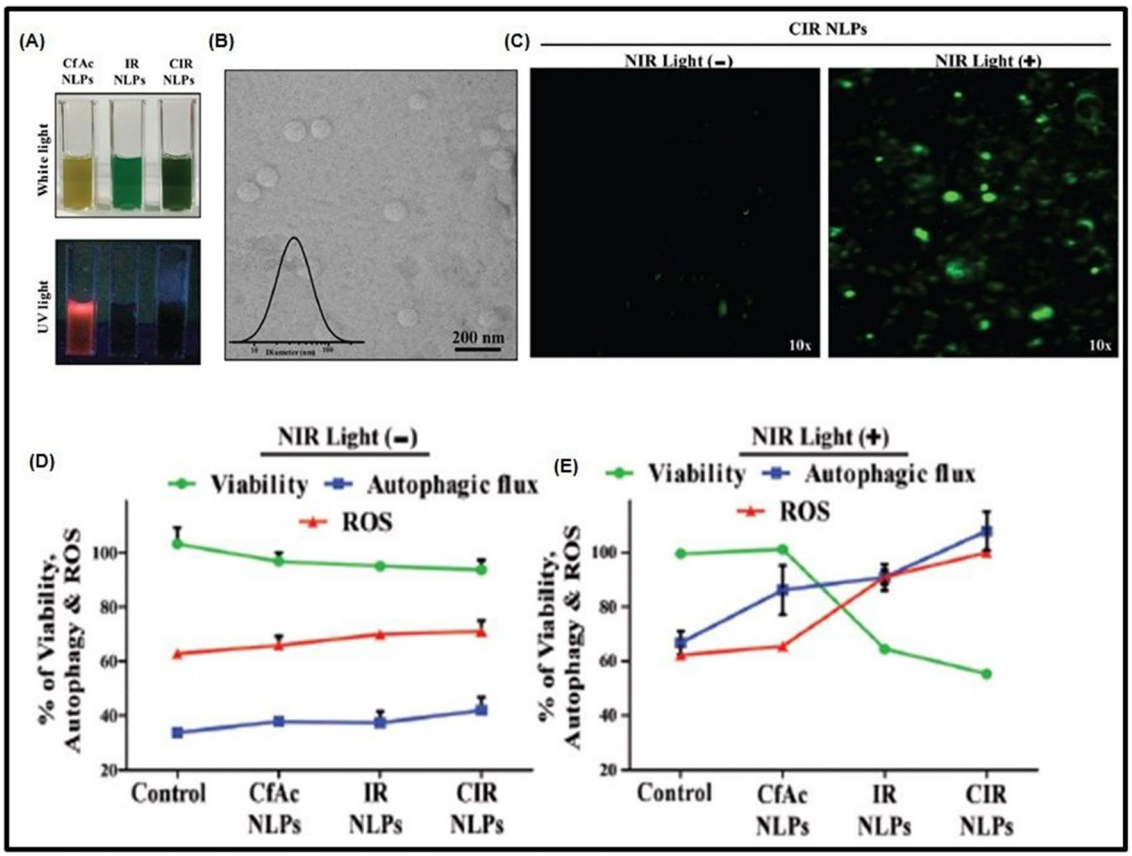


Fig. 7 **a** NLPs under white and UV light (CfAc nanoliposomes showing red color fluorescence). **b** TEM image of CIR NLPs (inset shows the DLS of CIR NLPs). **c** Images of IR, CfAc, and CIR NLPs in the presence and absence of NIR light. Changes in the viability, ROS level and autophagic flux in the MCF-7 cell line with different NLPs in (**d**) absence of NIR light (**e**) presence of NIR light. Reproduced by permission of The Royal Society of Chemistry, Appidi et al. (2020)

light irradiation showed increased ROS triggering rise in autophagic flux sequentially leading to cell death.

The autophagic cell death was also confirmed by the increase in lipidation of microtubule-associated protein light chain 3 (LC3-II), an autophagic cell protein marker. CfAc associated ROS generation enhanced lipidation of LC3-II that resulted in selective cancer cell death. We are the first group to report specific and localized autophagic cancer cell death and tumor volume regression via bioactive phyto-fraction and NIR sensitive dye under in vitro *&* in vivo conditions (Appidi et al. 2020).

Combinative approach: PTT and anti-cancer drugs

Various structures of plasmonic gold nanostructures were developed over the years, but their application in photothermal therapy was limited by the toxicity of the precursors used for achieving specific shape and size. To establish the use of gold nano rods for photothermal therapy, limited by the toxicity of CTAB, we developed a novel liposome-gold nanorod hybrid loaded with DOX and conjugated with folate receptors. The photo-chemo therapeutic efficacy of the nanomaterial was evaluated against breast cancer cell lines MDA-MB 231. The DOX-loaded and PEG-FA functionalized nanohybrids with NIR exposure of 5 min. (i.e. targeted chemo-photothermal therapy) rendered more than 90% cell death which is more when compared to individual treatment groups. These nanohybrids also showed good contrast in CT imaging and hence a multifunctional nanosystem for theranostic cancer application has been developed and demonstrated (Chauhan et al. 2017b). Lin et al. reported a pH-responsive theranostic NPs for drug delivery as well as CT imaging. DOX encapsulated micelle were prepared by the combination of b-cyclodextrin-{poly (lactide)-poly(2-(dimethylamino) ethyl methacrylate)-poly [oligo(2-ethyl-2-oxazoline) methacrylate]}21and [b-CD (PLAPDMAEMA-PEtOxMA)21]. These micelle templates were used for in situ synthesis of AuNPs. This nanosystem showed ~ 61% encapsulation efficiency of DOX, which were more effective in acidic tumor environment due to the pH-sensitive nature

of micelles. This nanosystem exhibited both therapeutic as well as diagnostic properties. In vivo experiments showed significant tumor volume regression with CT imaging in xenografted HepG2 tumor model. (Lin et al. 2017).

Photothermal therapy, conjugated with the anti-cancer agents will definitely improve the therapeutic efficacy and minimize the tumor recurrence. The development of multifunctional, biodegradable nanosystems that can release potent hydrophobic drugs in tumor micro-environment upon laser irradiation is still a challenge. To address this challenge, we have loaded Lipos Au NPs with curcumin which served as an *in- situ* adjuvant for photothermal therapy. The photothermal transduction efficiency of curcumin loaded nanoparticles caused irreversible cellular damage. Liposome curcumin nanoparticles were synthesized by thin film hydration method. The encapsulation efficiency and loading content of curcumin in Au-Lipos NPs was found to be ~70% and ~12% respectively. Free curcumin under aqueous condition degraded, while curcumin encapsulated in Lipos Cur NPs were well dispersed and did not show any degradation. The dose escalation was confirmed by the uptake studies in B16 cells (~ 14 times more uptake by Au-Lipos Cur NPs as compared to free curcumin). The fluorescence studies of encapsulated curcumin in Au-Lipos Cur NPs exhibited ~ fourfold increase in the emission intensity when compared to free curcumin. These rationales helped in the dose volume selection (Singh et al. 2018). The generated photothermal heat led to the destabilization of liposomal assembly, enhancing the release of entrapped curcumin. Au Lipos curcumin nanoparticles showed significant cellular uptake and increased cytotoxicity in B16 (melanoma) cancer cell lines. The theranostics stability of free curcumin, Lipos Cur NPs, and Au-Lipos Cur NPs were examined by measuring absorbance (420 nm) at definite time intervals. Free curcumin showed ~80% degradation in aqueous media (PBS, pH 7.4, 37 °C) within 1 h while curcumin encapsulated within Au Lipos NPs was stable up to 8 h without any transformation. The storage stability of Au-Lipos Cur NPs was monitored for 15 days at 4 °C. It showed no significant difference either in absorbance or fluorescence spectra up to 15 days, confirming the stability under storage conditions (Singh et al. 2018).

Photothermal therapy in combination with anti-cancerous plant fractions could also be used as combinative approach for cancer theranostics. Chlorophyll-rich derivatives with therapeutic benefits have an added advantage of imaging. We explored a relatively new approach of loading a hydrophobic chlorophyll rich anti-cancer fraction (CfAc) into polymeric PLGA NPs and made it photothermally active using IR780, an NIR dye. The combinational therapy of PTT along with CfAc showed synergistic cytotoxicity in skin cancer cells. To confirm the photo-stability of PCIR NPs, absorbance spectra of IR-780 were monitored at regular time intervals for definite period. PCIR NPs encapsulated IR 780 exhibited higher photo-stability when compared to free dye upto 72 h. The absorbance spectra confirmed the stability of PCIR NPs, irrespective of their storage in either darkness or day-light. Encapsulation within PLGA NPs was responsible for preventing interaction of IR780 with their surroundings resulting in enhanced photo stability. (Pemmaraju et al. 2018).

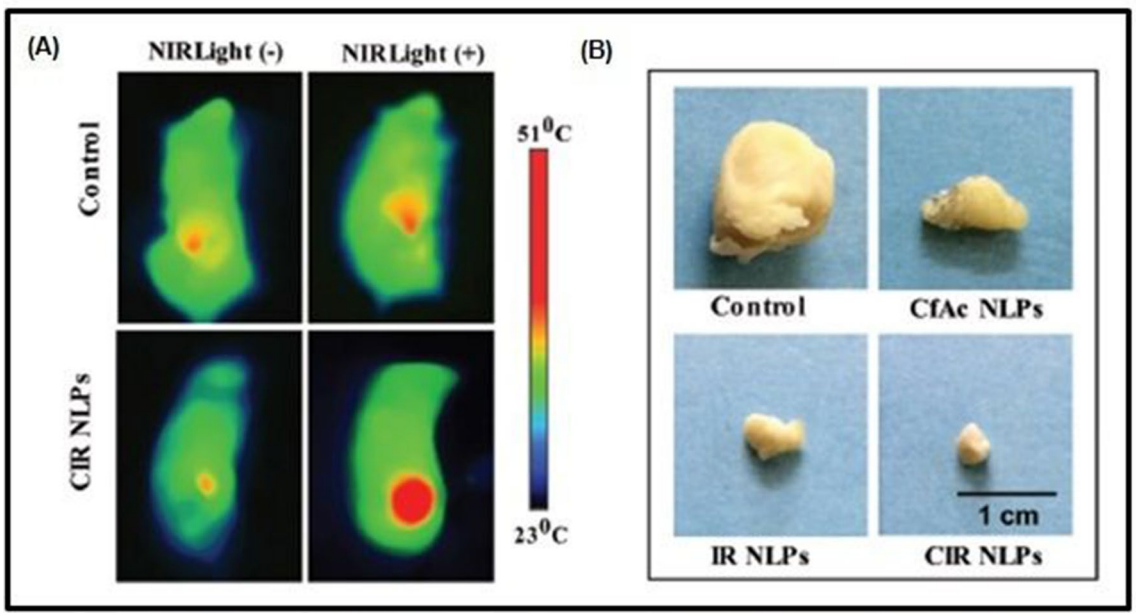


Fig. 8 **a** Thermal images of CIR NLPs administered animals and **b** images of the dissected tumor. Reproduced by permission of The Royal Society of Chemistry, Appidi et al. (2020)

A similar combination with liposome (CIR NLPs), when irradiated by NIR light, showed localized synergistic cancer cell death under both in vitro as well as in vivo conditions. Through a combined effect of heat and CfAc, CIR NLPs showed improved anti-tumorigenic potential in an orthotopic mouse model (Fig. 8a, b) (Appidi et al. 2020).

Conclusion

Although there are many existing nanosystems to improve the bioavailability and therapeutic efficacy of drugs & dyes, the translational potential of these nanosystems depend majorly on their degradation and disintegration. The byproducts of the nanosystem should be cleared from the body either by hepatobiliary or renal route. The multifunctional photothermally active nanosystems discussed in this paper meet all the requirements of an ideal drug delivery and therapeutic agents. These systems are biocompatible and can undergo degradation or disintegrate into smaller units that can be cleared from the body. Lipos Au nanosystem degraded and further disintegrated into gold seeds (size < 5–6 nm) which were cleared by hepatobiliary and renal route under in vivo condition. The fluorescent PEG coated gold nanoshells can be applied for both tracking and therapeutic purposes. Development and optimization of PTT with low dose X-ray based radiotherapy in tumor tissues while keeping the surrounding tissues and organs safe are the foremost challenges. Most of the existing in vivo data are either diagnostic or therapeutic NPs but not in combination. The combination of real time monitoring allows us to validate the usefulness of this novel treatment approach. Specific targeting at the cellular level and genetic level, molecular imaging approaches are essential and the biodistribution profiles may vary from small animal to humans. The combinative approach using anticancer agent and photothermally active dyes showed synergistic therapeutic effect and can be used for localized tumor treatment. These biocompatible and biodegradable nanosystems have greater scope for clinical translation owing to their multifunctional properties.

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