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Understanding the Role of NIR Laser Power and Wavelength in Tuning the Photothermal Transduction Efficiency of Gold Nanosystems in Biomedical Applications

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Photothermal therapy (PTT) is an emerging, affordable alternative to conventional therapies for superficial tumors and topical microbial infections. Photothermal agents (PTA) play a crucial role to achieve PTT. Several nanoparticles have been widely used as PTA owing to their small size and tunability of absorbance in the near infrared region (NIR). When irradiated with the NIR laser, they generate localized heat, which results in localized ablation of cancer cells or microbes. Additionally, several organic or inorganic PTAs exhibit surface plasmon resonance (SPR), an important characteristic for achieving laser-induced hyperthermia. However, among the various nano PTAs, the metallic nanosystems exhibit comparatively better thermal raise properties. Among the metallic systems, gold is a superior, biocompatible, and well-studied system for PTT. However, the photothermal conversion efficacy of the PTAs greatly depend on the wavelength and the power of the laser source used. In this regard, this study is aimed at understanding the photothermal conversion efficacy of gold coated calcium peroxide nanoparticles when exposed to lasers of various wavelengths and power. The results showed that the photothermal conversion efficacy (PTE) of the particles irradiated with continuos laser was higher, when compared with the particles irradiated with low power pointer laser and pulsed laser.

Keywords: Photothermal transduction; Gold nanosystems; Photothermal agents; SPR

1 Introduction

Photothermal therapy (PTT) is an affordable alternative of temperature-augmented therapy for treating solid tumors and topical microbial infections. PTT takes advantage of photothermal agents (PTA), which convert the light energy into localized hyperthermia when excited by near-infrared radiations (NIR)[1]. PTT is advantageous over conventional therapies owing to their selectivity, accuracy, and minimally invasive nature. Mild hyperthermia is generally employed to kill the cancer cells, whereas high-temperature hyperthermia would aid in killing the microorganisms, especially drug-resistant bacteria[1,2].

Nanoparticles have been widely used in PTT. Nanoparticles either act as PTA or aid in ferrying the PTA moieties to the target site. They are classified into inorganic (gold, copper, iron, *etc*) and organic PTA (conjugated polymers, small molecule dye, *etc*). PTA nanoparticles exploited in PTT generate heat due to the surface plasmon resonance (SPR). The phenomenon in which the light absorbed by the nanoparticles causes the electrons of the PTA to resonate at the frequency of light, thus resulting in the generation of heat[3,4].

Among the different metallic PTAs, Gold nanoparticles (GNPs) have been widely employed for PTT owing to their unique properties. The surface plasmon resonance (SPR) phenomenon is particularly well-suited for noble metal nanoparticles, which improves their radioactive absorption and dispersal characteristics. Since PTT uses the SPR effect to generate heat through laser absorption, GNPs are promising materials for temperature-augmented PTT[5,6]. GNPs possess good biocompatibility, surface modification window, and high-yield scale-up possibility[7-9].

However, the photothermal conversion efficacy of the PTA is influenced by the wavelength and the power of the laser used. The power of the NIR laser usedplays a crucial role in determining the photothermal conversion efficacy of PTA. For instance, previous reports have shown the increased

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photothermal conversion efficacy of GNPs with the increased optical power of the NIR laser[6,7]. Similarly, the photothermal conversion efficacy of PTA is dependent on the wavelength of the NIR laser used. Zinoune *et al*., showed an increase in the photothermal conversion efficacy of GNPs with the shorter wavelength lasers[8].

In this perspective, this study is focused on understanding the photothermal conversion efficacy of novel gold coated calcium peroxide nanoparticles when irradiated with lasers of different power and wavelengths. For this purpose, low power (<150mW) pointer laser, a continuous 650mW laser and a femtosecond pulsed laser of 650mW (1kHz repetition rate, 35fs pulse duration) was used. The results indicated the superiority of continuous laser in increasing the photothermal conversion efficacy of the gold coated calcium peroxide nanoparticles, when compared with the other lasers used.

2 Experimental Section

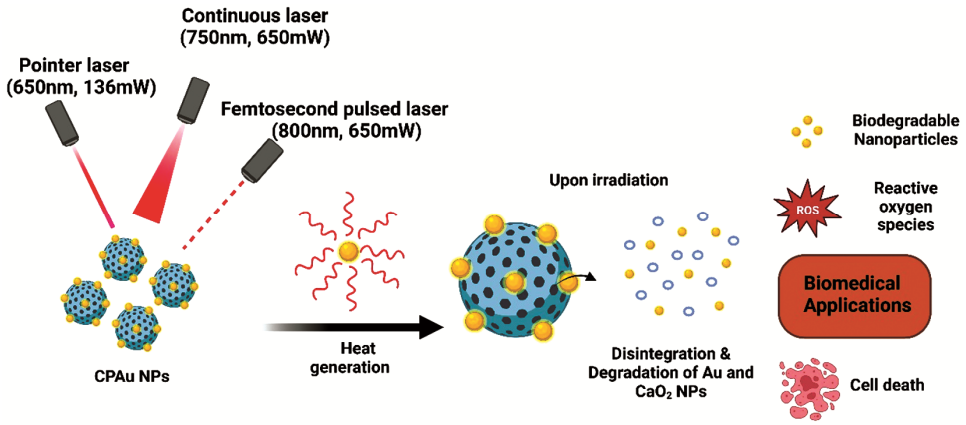
Calcium peroxide nanoparticles were prepared using previously reported methods. Gold was coated on the surface of CaO_2 NPs to tune its NIR absorbance through an *in-situ* reduction method. Briefly, CaO_2 and $HauCl_4.3H_2O$ were taken 1:1 ratio and 200µL of 20mM ascorbic acid was added. A visible color change was observed from white colored solution to dark blue colored solution. The developed nanoparticle, CPAu NPs was characterized for its UV-Vis absorbance, hydrodynamic diameter, and morphology using TEM analysis. The release of peroxide groups from the core CaO_2 NPs was tested using a simple colorimetric test using potassium permanganate ($KMNO_4$).

In the case of a lower power laser, a 136mW, 650nm wavelength pointer laser was used. Briefly, 100µL of the sample was irradiated using the pointer laser and the rise in temperature was recorded every 2 mins for 10 mins. The thermal images of the same were captured using an infrared thermal camera, and the temperature vs time graph was plotted. The thermal transduction efficiency was calculated as mentioned in the below section.

In the case of a continuous laser, a 650mW, 750nm wavelength laser was used, and the temperature rise was recorded every 1 min for 5 mins. The thermal images were captured and the temperature vs time graph was plotted. For calculating the photothermal thermal transduction efficiency, 100µL of the developed CPAu NPs and water (Milli-Q) were subjected to irradiation by 750nm NIR laser once per minute for a total of 10 mins. The initial and final temperature readings were recorded. The NIR laser was switched off upon reaching a stable temperature state, and the temperature drop was recorded. Similarly, the heating and cooling curves for water were recorded. The photothermal transduction efficiency was calculated using the formula:

$$\eta = \frac{hS(T_{max}-T_{surr})- Q_{Dis}}{I(1-10^{-A750})}$$

η - photothermal efficiency
h - sample cell's heat transfer coefficient
S - sample cell's surface area
T_{max} - maximum temperature reached by CPAu NPs with laser irradiation (750nm, 650mW continuous laser)
T_{surr} - surrounding ambient room temperature
Q_{Dis} - baseline energy of the heat generated from water and the sample cell
I - laser power (650mW)
A750 - absorbance of CPAu NPs at 750nm



Scheme 1 — Schematic depicting the role of NIR laser in CPAu NPs mediated photothermal therapy.

In the case of a 650mW femtosecond pulsed laser, 100µL of the sample was irradiated using 800nm laser, and the rise in temperature was recorded every 2 mins for 10 mins. An infrared thermal camera was used to capture the thermal images. Photothermal transduction efficiency was calculated using a similar procedure as adapted previously.

The biocompatibility of the synthesized CPAu NPs were tested in L929 cells. Briefly, $7x10^3$ cells were seeded, per well in a 96-well plate and after 24h, cells were treated with varying concentrations of CPAu NPs. The % cell viability was quantified using MTT assay post 24h of treatment.Laser-mediated cytotoxicity of CPAu and CPSAu NPs was evaluated in B16 melanoma cell line using 750nm continuous laser. Briefly, $1x10^4$ cells were seeded, per well in 96-well plate. Upon attachment, 100 µg/mL of CPAu and CPSAu NPs were added to the cells and were treated with and irradiated with 750nm laser for 5 mins. The cells treated with the nanoparticles without NIR laser irradiation were considered as respective controls. After 24h of laser treatment, the cell viability was quantified using MTT assay. DCFHDA assay was performed to evaluate the PTT mediated reactive oxygen species (ROS) generation. Briefly, 100 µg/mL of CPAu NPs was added to the cells and then 1µL of DCFDA was added, further irradiated with 750nm laser for 5 mins. The cells were then imaged under a fluorescence microscope.

3 Results

Gold coated calcium peroxide nanoparticles were synthesized using *insitu* reduction method. Briefly, 200µL of 20mM ascorbic acid was added to 1:1 mixture of CaO_2 NPs and $HAuCL_4.3H_2O$, resulting in an immediate color change of the solution to dark blue. The developed CPAu NPs exhibited a broad absorbance in the range of 600-800nm (Fig. 1(b)). TEM images revealed uniform nanoparticles of 60-70nm size, concordant with the number weighted hydrodynamic diameter of 60nm (Fig. 1(c,d) respectively)). A simple colorimetric test using $KMnO_4$ was performed to evaluate the release of peroxide groups from the core CaO_2 NPs. As seen in Fig. 1(e), the $KMnO_4$ solution completely gets decolorized upon interaction with the peroxide groups released from CaO_2 NPs and CPAu NPs.

Further, the synthesized nanoparticles were irradiated with the lower power laser (136mW, 650nm). A gradual increase in temperature upto 43 °C upon 10 mins of irradiation was observed as seen in Fig. 2(a,b). The temperature rise and cooling curve fitting of CPAu NPs were obtained (Fig. 2(c-e)) to calculate the photothermal transduction efficiency. Upon calculation, the PTE was found to be 13.6%, the lower PTE could be attributed to the low power of the pointer laser.

Furthermore, the synthesized nanoparticles were irradiated with the continuous laser (650mW, 750nm). A time-dependent increase in temperature till 65 ˚C was observed upon 5 mins of irradiation as seen in Fig. 3(a,b). The temperature rise and cooling curve fitting of CPAu NPs irradiated with 750nm laser were obtained (Fig. 3(c-e)) to calculate the photothermal transduction efficiency. Upon calculation, the PTE was found to be 53.11%. The relatively higher PTE

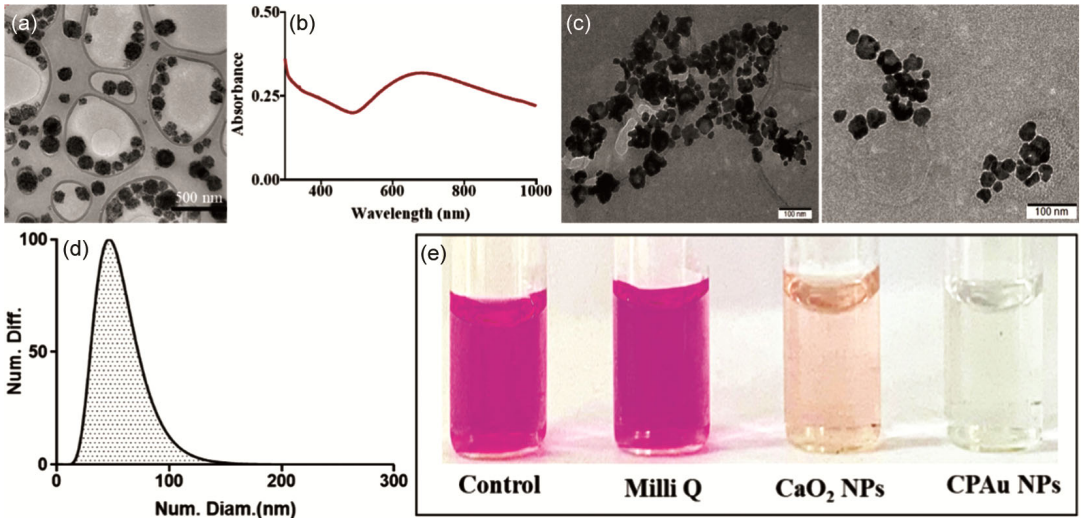


Fig. 1 — (a) TEM imaging of CaO_2 NPs, (b) UV-Vis absorbance, (c) TEM images of CPAu NPs and(d) Hydrodynamic diameter of CPAu NPs. (e) Colorimetric analysis of KMnO4 treated with CaO_2 NPs and CPAu NPs.

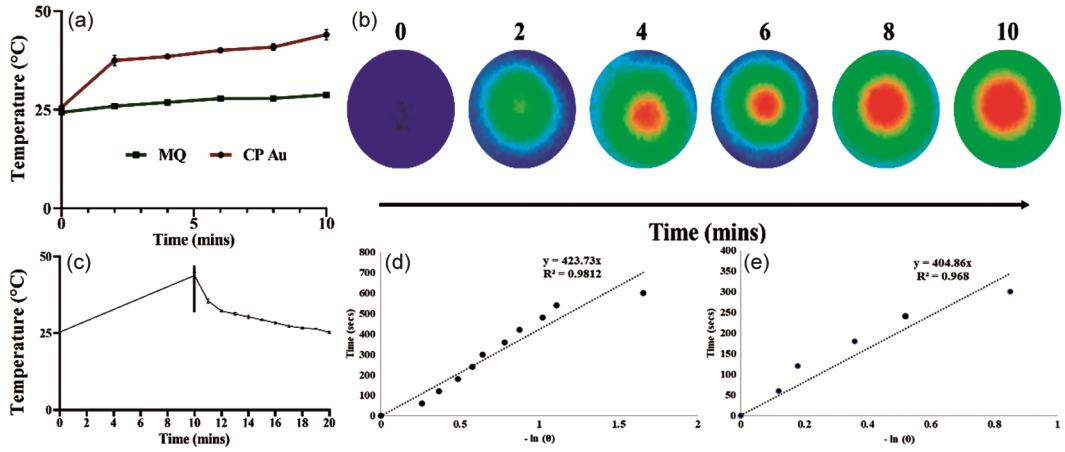


Fig. 2 — (a) Temperature vs time graph, (b) corresponding thermal images, (c&d) Temperature rise and cooling curve fitting for CPAu NPs, and (e) cooling curve fitting for MilliQ water irradiated using 650nm, 136mW pointer laser.

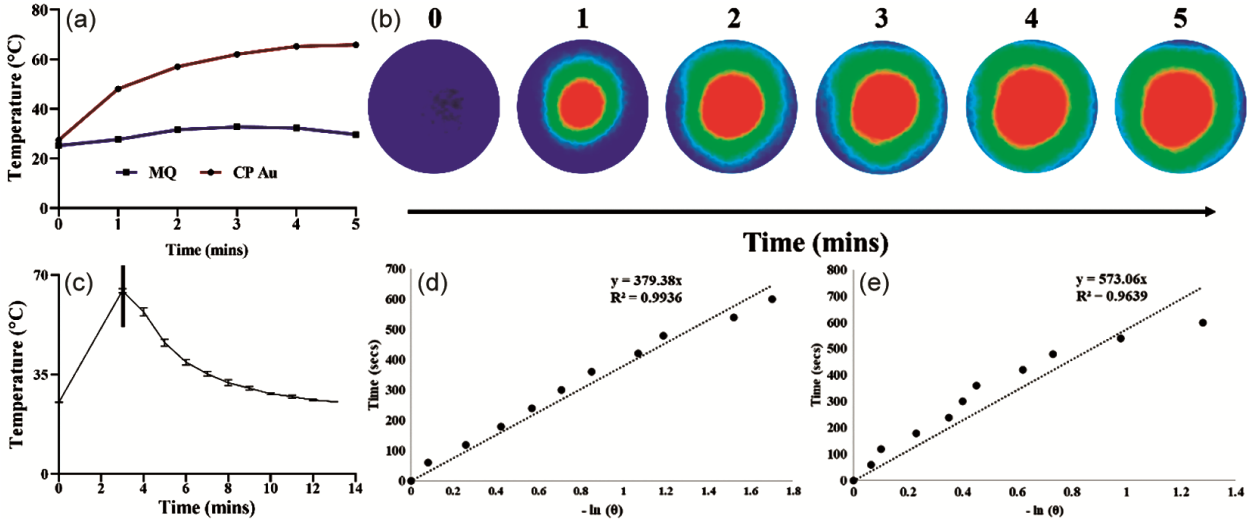


Fig. 3 — (a) Temperature vs time graph, (b) corresponding thermal images, (c&d) Temperature rise and cooling curve fitting for CPAu NPs, and (e) cooling curve fitting for MilliQ water irradiated using 750nm, 650mW continuous laser.

could be as a result of using higher power laser (650mW). This is consistent with the previous reports stating the role of laser power in determining the PTE (6,7).

The synthesized nanoparticles were irradiated with a femtosecond pulsed laser (800nm, 650mW, 1kHz repetition rate, 35fs pulse duration). It was observed that there was a time dependent temperature rise to 47 ˚C upon 10 mins of irradiation as seen in Fig. 4(a,b). The temperature rise and cooling curve fitting of CPAu NPs irradiated with 800nm laser were obtained (Fig. 4(c,e)) to calculate the photothermal transduction efficiency. Upon calculation, the PTE was found to be 45.46%.

Table 1 summarizes the different types of NIR laser source used, it's wavelength and power of the NIR laser and the corresponding photothermal transduction efficiency of CPAu NPs.

The biocompatibility of the CPAu NPs was tested in L929 cells. As seen in Fig. 5(a), the synthesized NPs did not exhibit any significant toxicity upto 100 µg/mL. The NIR laser mediated cytotoxicity was evaluated in B16 melanoma cell line. Briefly, the cells were exposed to 100µg/mL of CPAuNPs and irradiated with 750nm laser for 5 mins. Upon 24h of laser irradiation, the cell viability was quantified using MTT assay. As seen in Fig. 5(b), there was significant reduction in the viability of cells treated with CPAu (38% viability), upon laser irradiation compared to the respective control groups. ROS generated upon various external stimuli, is one of the basic phenomena leading to several cell death

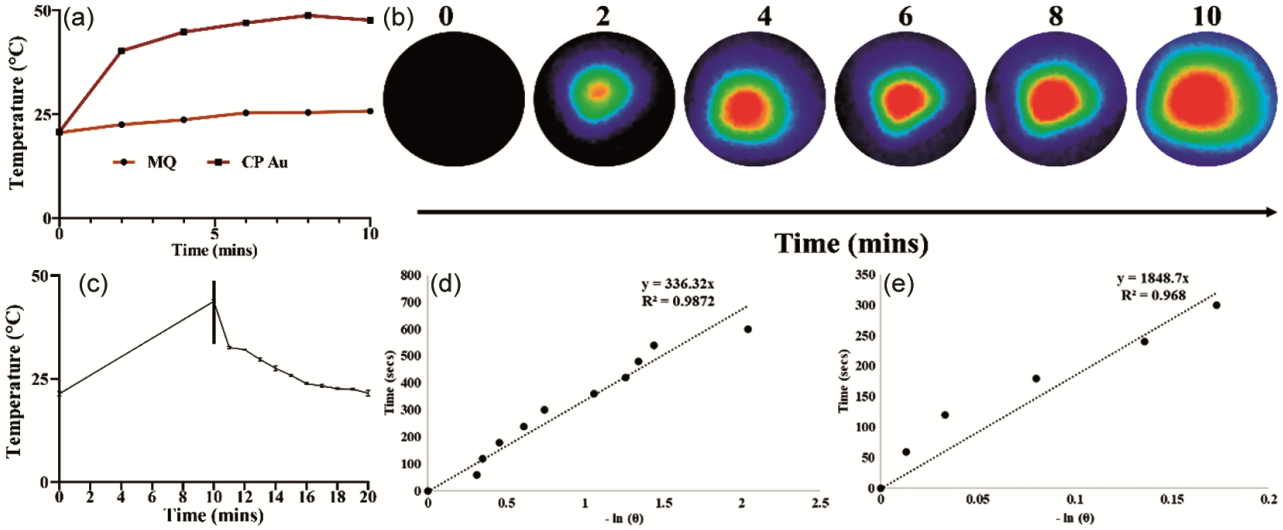


Fig. 4 — (a) Temperature vs time graph, (b) corresponding thermal images, (c&d) Temperature rise and cooling curve fitting for CPAu NPs, and (e) cooling curve fitting for MilliQ water irradiated using 800nm, 650mW femtosecond pulsed laser.

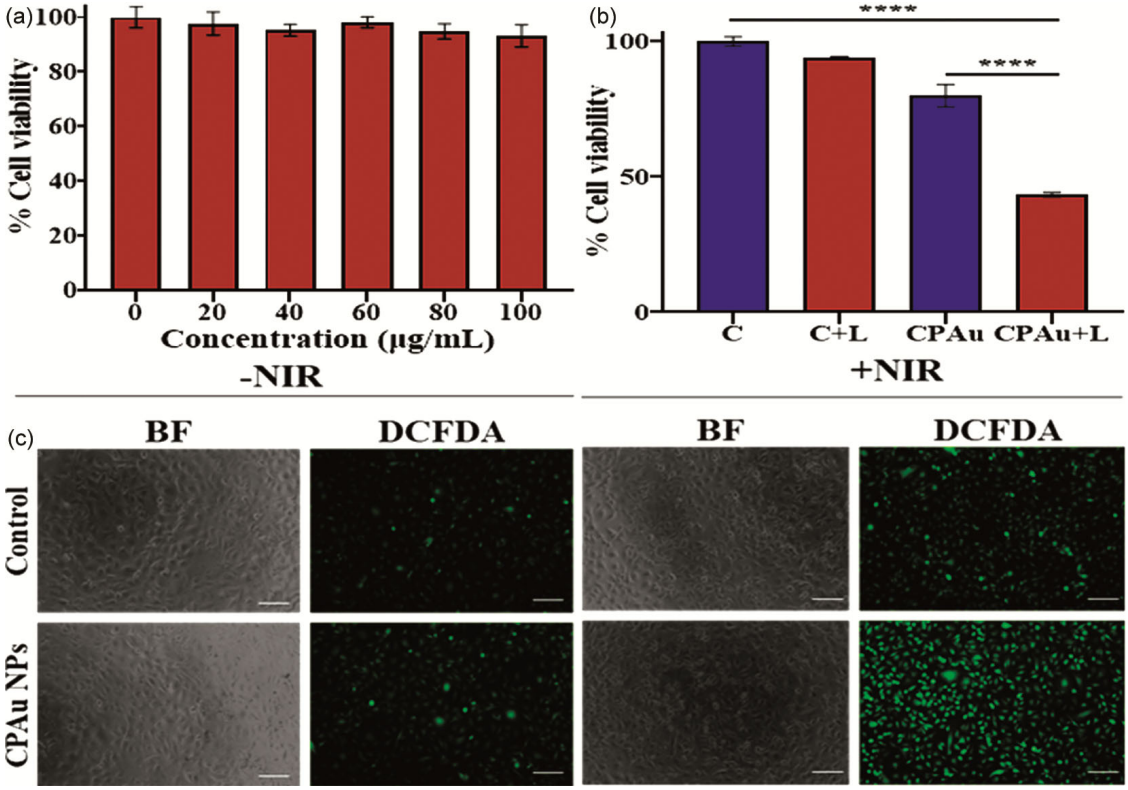


Fig. 5 — (a) Biocompatibility of CPAu NPs in L929 cells, (b) PTT mediated cytotoxicity of CPAu NPs and (c) PTT mediated ROS generation in B16 cells.

Table 1 — Summary of the NIR laser source, wavelength, power and corresponding photothermal transduction efficiency of CPAu NPs.

NIR Laser source	Wavelength	Power	Photothermal transduction efficiency
Low power pointer laser	650nm	136mW	13.6%
Continuous laser	750nm	650mW	53.11%
Femtosecond pulsed laser	800nm	650mW	45.46%

pathways. PTT mediated ROS generation was estimated using DCFDA assay. Briefly, the cells treated with CPAuNPs, were exposed to DCFHDA stain and subjected to 750nm NIR laser irradiation. The cells were then imaged under the fluorescence microscope. As seen in Fig. 5(c), there was an increase in the ROS generation upon laser irradiation in CPAu NPs treated group, compared to their respective controls. From the above studies, it is evident that the developed CPAu NPs can be potentially used as an efficient photothermal agent in biomedical applications.

4 Conclusion

Gold coated calcium peroxide nanoparticles (CPAu NPs) were subjected to NIR lasers of different wavelengths and power. It was observed with particles showered with low power pointer laser for 10 minutes showed a very less photothermal transduction efficacy of around 13.6%. Whereas particles showered with continuous laser and pulsed laser exhibited a transduction efficacy of 53.11% and 45.46% respectively. Which might be attributed to their higher power. This is consistent with the previous report of continuous laser being a better source for localized PTT. This study revealed the potential of the developed nanoparticles to act as an efficient photothermal agent.

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