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Measurement of Enhanced Photothermal Effects of CuO-encapsulated Polymeric Nanospheres

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***Abstract*— Copper oxide nanoparticles (CuO-NPs) have the potential of serving as an anticancer theranostic agent with photothermal capabilities. In order to control their toxicity and release, the CuO NPs were encapsulated within polymeric nanospheres composed of poly(lactic-co-glycolic acid) (PLGA) core and polydopamine (PDA)/polyethylene glycol (PEG) shell. After the characterization of synthesized nanospheres, their photothermal response to different near-infrared laser sources (808 nm, 940 nm and 1064 nm) was assessed in terms of the measured temperature. Arrays of sub-millimetric fiber Bragg grating sensors were employed to achieve an optimal spatial resolution for resolving the temperature increase in samples embedding the nanospheres. The results have shown that the designed structure of CuO@PLGA/PDA/PEG nanospheres substantially augments the temperature elevation. A maximum of 30 °C temperature increase, in comparison with the control solution, was achieved for the 808 nm laser source. These results indicate that the designed structure of CuO@PLGA/PDA/PEG nanospheres is suitable for further applications towards chemo-photothermal therapy combined with diagnostic imaging for the treatment of cancer.**

Keywords*— *temperature measurement, photothermal therapy, nanoparticles, copper oxide nanoparticles, fiber optic sensors, nanotechnology

I. Introduction

Nanomedicine is a fascinating and fast-growing field, which applies the knowledge and tools of nanotechnology, such as biocompatible nanoparticles (NPs), to the prevention and treatment of diseases [1]. Photothermal therapy mediated with nano-agents is among the therapies developed by nanomedicine in the last two decades [2]. Among the large plethora of nanomaterials, copper oxide nanoparticles (CuO-NPs) in combination with a light-absorbing material, such as a biocompatible polydopamine (PDA) and polyethylene glycol as a well-known physiological stabilizer [3], [4], are investigated. CuO-NPs possess inherent multifunctional capabilities, serving as both contrast enhancing material for non-invasive imaging techniques (i.e., Ultrasound, Magnetic Resonance Imaging) [5] as well, as recently found, can cause a reduction in the size of cancer tumor [6].

Laser thermal therapy, on the other hand, has shown promising results as a novel minimally invasive therapeutic option [7], [8]. In this scenario, the accurate evaluation of the photothermal response of nanomaterials provides an important step towards the transition of the technique into clinical application. It can potentially prevent unintended collateral thermal damage through accurate spatial temperature measurements near the ablation point [2]. Most of the works evaluating the photothermal effects of NPs use traditional techniques based on superficial infrared (IR) thermography [9]. Although it offers contactless and non-destructive measurement capabilities, this approach can only measure surface temperatures. Other authors have used metallic thermocouples [10], which have the drawback of one single measurement point and temperature overestimation due to direct light absorption [11]. Thus, these techniques are not fully adequate to provide accurate measurement of the volumetric photothermal effect. These challenges can be solved by the use of quasi-distributed measurements provided by the fiber Bragg grating (FBG) sensors, which have already proved to be effective in the monitoring and control of minimally invasive thermal treatments [12]. FBG arrays, like most fiber optics sensors, are immune to electromagnetic interference, small (40 µm − 250 µm in diameter), and biocompatible. Under the point-of-view of measurement science, one of the most valuable properties of FBG array stems from the multiplexing capability, allowing quasi-distributed temperature measurement across the fiber [13], [14]. Moreover, FBGs can be inserted inside the sample embedding the NPs without interference with the laser light, providing more accurate temperature information than superficial and external IR thermography [15], [16].

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In this study, CuO-NPs encapsulated within poly(lactic-co-glycolic acid) (PLGA) nanospheres were prepared and characterized. Surface modification of the loaded nanospheres, through in-situ polymerization of dopamine and then PEGylation, yielded multifunctional nano-agents whose potential was evaluated for hyperthermal therapy by laser irradiation. The photothermal effects of the encapsulated CuO-NPs, triggered by near-infrared (NIR) laser sources, were assessed with FBG arrays, which provide quasi-distributed temperature measurements with mm spatial resolution.

II. Methods and Experimental Setup

A. Synthesis of CuO-NPs loaded Nanospheres

CuO-NPs (~7 nm), readily suspended in water, were encapsulated within PLGA nanospheres by using the double emulsion ($W_1/O/W_2$) method [17]. The suspension of bare CuO-NPs@PLGA nanospheres was mixed with dopamine hydrochloride in alkaline conditions of Tris buffer to obtain the photothermal shell. For further improvement of biocompatibility, poly(ethylene glycol) methyl ether thiol (PEG-SH) was added as an external shell yielding CuO-NPs@PLGA/PDA/PEG nanospheres. The detailed procedure is described in our previous work [18].

B. Structural Characterization

ZetaSizer (ZetaSizer Nano ZS, Malvern) was used to assess zeta potential and particle size distribution (polydispersity index, z average mean hydrodynamic diameter). FEI T12 G2 cryogenic transmission electron microscopy (cryo-TEM) was used to analyze the shape and size of the nanospheres. Each sample was measured five times without time delay.

C. Measurement of the photothermal effects

To evaluate the thermal effects of the CuO-NPs loaded nanospheres, with and without PDA/PEG coating, three samples were tested: (i) CuO-NPs@PLGA/PDA/PEG nanospheres, (ii) CuO@PLGA nanospheres, and (iii) deionized water as a control. For irradiation, laser diodes with different wavelengths (808 nm, 940 nm, and 1064 nm) and output power of 2.5 W guided by a multimode optical fiber were used. The laser was switched on 30 s after starting temperature monitoring, the duration of irradiation was 120 s, and the temperature was measured for 200 s. The one-dimensional temperature profiles were measured along the custom-made fiber optic sensors. Each fiber houses an array of 25 FBG inscribed in a polyimide-coated optical fiber with the femtosecond point-by-point writing technology that provides high-quality spectra characteristics and precise array geometry, suitable also for complex structures [14], [19], [20]. Each grating acts as a sensing point that has a 0.9 mm length and 0.1 mm edge-to-edge distance to the next grating. Temperature profiles along the sensors were obtained by analysis of the spectra reflected from the FBG arrays. Reflected spectra were measured by the interrogation unit (Micron Optics si255, wavelength range 1460 nm −1620 nm, wavelength accuracy 1 pm, wavelength stability 1 pm). A preliminary static calibration of the sensors, which allowed retrieving the temperature sensitivity coefficients of the FBGs, was performed with Giussani Quartz thermostatic calibrator in the temperature range 30 °C−140 °C, with a step of 10 °C. As a reference sensor, a PT100 thermistor (± 0.15 °C accuracy) was employed. A static sensitivity of $(7.43 \pm 0.01)\cdot 10^{-6}$ °C^{-1} was obtained for the two arrays.

Fig. 1 shows the experimental setup describing two fiber optic sensors positioned in parallel to the laser fiber at 2 mm distance, and the laser fiber tip was placed parallel to the middle of the FBG arrays. Both the laser fiber and the sensors were immersed in a 1.5 mL Eppendorf tube filled with distilled water (control) or NPs suspensions.

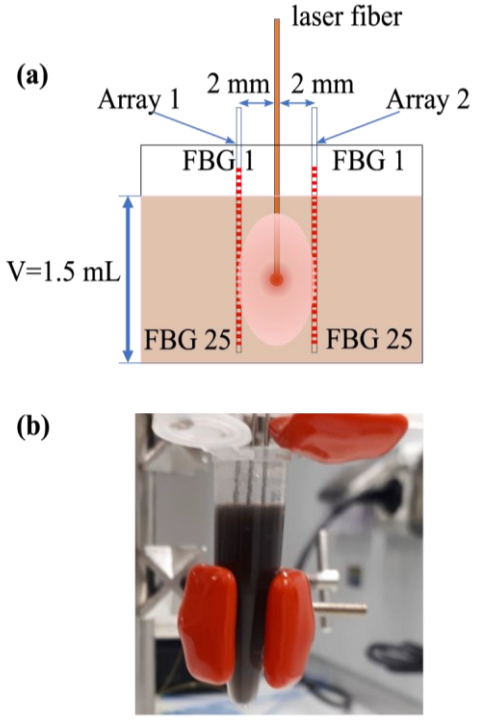

Fig. 1. Schematic of the experiment: (a) two fiber sensors (FBG arrays) are positioned at 2 mm distance from the laser fiber in the 1.5 ml Eppendorf filled with NPs; each fiber grating length is 0.9 mm, distance between gratings is 0.1 mm; (b) photo of the setup.

III. Results and Discussion

A. Characterization of CuO-NPs loaded PLGA Nanospheres

The CuO-NPs@PLGA/PDA/PEG nanospheres with spherical morphology were directly observed by cryo-TEM. As shown in Fig. 2, CuO-NPs of average size 7 nm were successfully encapsulated into the PLGA matrix.

The TEM clearly shows the core-shell structure with a PDA/PEG shell. An average hydrodynamic diameter of coated nanospheres was found to be around 290 nm (PDI=0.124) in comparison to uncoated nanospheres characterized with z-average of close to 240 nm (PDI=0.066). The size and size distribution of nanospheres increased after the addition of PDA/PEG coating (Fig. 3a). Zeta potential values were found to be 36.9±1.0 mV, -19.0±2.3 mV and -16.5±2.1 mV for CuO-NPs, CuO-NPs@PLGA nanospheres and CuO-NPs@PLGA/PDA/PEG nanospheres, respectively indicating good particle stability in aqueous solutions (Fig. 3b).

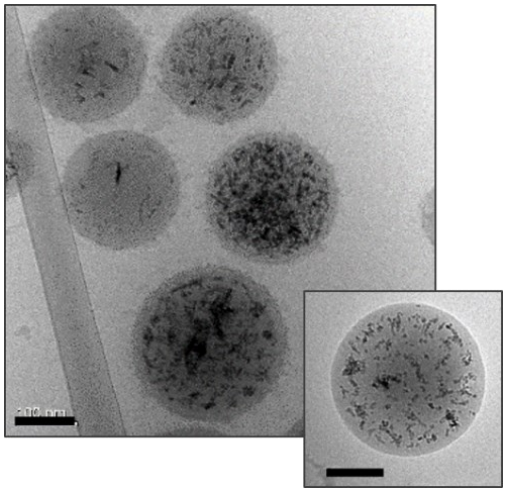

Fig. 2. Cryogenic-transmission electron microscopy images of coated-nanospheres showing thin rough film of thick PDA/PEG shell structure, and bare nanospheres characterized with a smooth surface (inset). Scale bars are 100 nm.

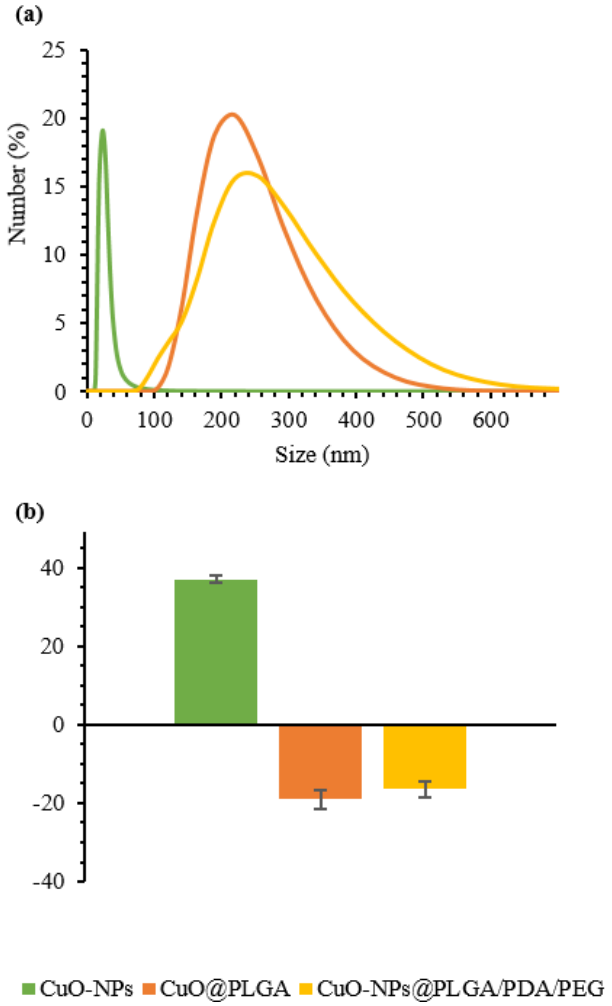

Fig. 3. (a) Representative hydrodynamic diameter histogram distribution profiles and (b) zeta potential of CuO-NPs, CuO-NPs@PLGA nanospheres and CuO-NPs@PLGA/PDA/PEG nanospheres. The results are presented as the mean ± standard deviation (n = 5).

B. Evaluation of Photothermal Effects

The temperature evolution in the samples at the three wavelengths measured by the two FBG arrays is shown in Fig. 4. Three tests for each sample were repeated under the same conditions. The values of the temperature measured by FBG experiencing the highest temperature increase are presented in Fig. 5 (left) as mean±standard deviation. The CuO-NPs@PLGA/PDA/PEG nanospheres significantly increased the absorption of laser energy in the samples, in correspondence of the three laser wavelengths in the NIR. In comparison with the water control, temperature increases of 30 °C, 16 °C and 17 °C were shown for 808 nm, 940 nm and 1064 nm, respectively. As far as it concerns the influence of the wavelength on the heating effect, the maximum temperature increase of 41.3±2.4 °C after 120 s of irradiation was shown with an 808 nm laser source. These results proved the augmented photothermal effect of the PDA shell when the samples were irradiated with the NIR-laser beam. Indeed, the black PDA coating of the proposed nano-agents is photothermal sensitive, and it is indicated to trigger temperature augmentation for hyperthermia therapy.

Additionally, these results showed that the increase of the laser wavelength decreases the energy absorption of PDA/PEG coating. Conversely, the composite of CuO-NPs@PLGA slightly increased the temperature during irradiation and showed a thermal response highly comparable with the one of water. As a consequence, the uncoated nanospheres do not represent a viable option for photothermal therapy since they show no significant thermal increase when compared to PDA/PEG-coated nanospheres. The peak temperature change for the aqueous control was always below 20 °C, and the highest value was reached during irradiation at 940 nm. This evidence is in accordance with water absorption properties [21].

Fig.5 (right) illustrates the time evolution of temperature profile along the FBG sensor for the test with the 808 nm laser irradiation. As it can be clearly seen, the sensor is able to measure not only peak temperature (Fig. 4 left), but also heat distribution in the Eppendorf. For all three samples, the temperature is higher in the direction of laser irradiation. The biggest heated area corresponds to the CuO-NPs@PLGA/PDA/PEG sample, while temperature maps for water and CuO-NPs@PLGA are approximately the same. The possibility to measure temperature profile evolution shows the importance of quasi-distributed properties of FBG sensors for proper evaluation of photothermal effects of nanoparticles.

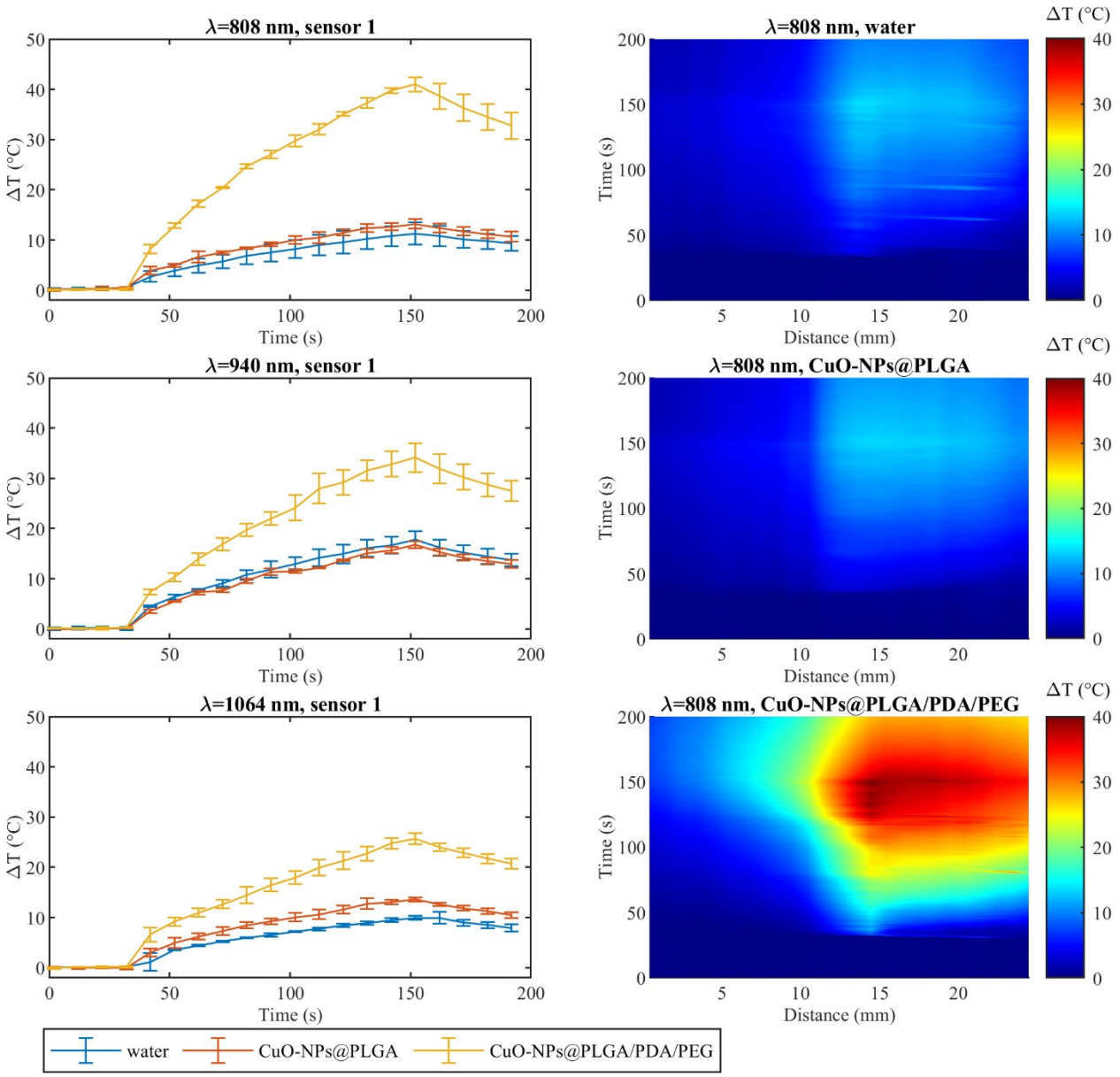

Fig. 4. (left) Peak temperature evolution measured by the two FBG arrays in water solutions including CuO-NPs@PLGA/PDA/PEG nanospheres (yellow curves), CuO@PLGA nanospheres (orange curves) and deionized water used as a control (blue curves). The results are presented as the mean ± standard deviation (n = 3); (right) Temperature profiles evolution in time for laser irradiation with 808 nm laser.

An analysis of the accuracy of the measurement setup is performed by evaluating the output of the two FBG arrays placed in symmetric configuration with respect to the laser fiber. Table I lists the two indicators which have been selected for this evaluation, i.e., the average difference between the maximum temperature evolution measured by the two symmetric arrays during the photothermal test, ΔT_{array} (expressed as an absolute value of the mean) and the root mean squared error (RMSE) of the same difference.

TABLE I. TEMPERATURE DIFFERENCE OF THE MAXIMUM TEMPERATURE EVOLUTION MEASURED BY THE TWO SYMMETRIC ARRAYS

Wavelength [nm]	Sample	ΔT_{array} [°C]	RMSE [°C]
808	CuO@PLGA/PDA/PEG nanospheres	0.02	0.37
	CuO@PLGA nanospheres	0.46	0.55
	water	0.17	0.26
940	CuO@PLGA/PDA/PEG nanospheres	0.06	0.62
	CuO@PLGA nanospheres	0.26	0.41
	water	0.13	0.27
1064	CuO@PLGA/PDA/PEG nanospheres	0.39	0.54
	CuO@PLGA nanospheres	0.09	0.34
	water	0.36	0.43

Table I shows that the temperature measured by the symmetric sensors is very similar in all tests. The mean difference along the irradiation time is always lower than 0.5 °C, which is an index of a good correspondence between the measurement provided by the two sensors, indicating the reliability of the experimental setup.

IV. Conclusions

In summary, multifunctional CuO-NPs@PLGA/PDA/PEG nanospheres were successfully synthesized, and a measurement setup has been developed to characterize the photothermal effect of these nano-agents. The nanospheres provide excellent thermal enhancement in correspondence of several wavelengths within the NIR range, with a particular peak temperature rise at 808 nm. These results encourage further study of nanospheres with embedded CuO-NPs towards offering a novel chemo-photothermal therapy approach that can be combined with imaging for an efficient treatment of cancer.

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