Colloids and Surfaces B: Biointerfaces 170 (2018) 393–400

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

In vitro and in vivo tumor annihilation by near-infrared photothermal effect of a $NiFe_2O_4$/C nanocomposite

M. Gorgizadeh[a,b], N. Azarpira[c,b], N. Sattarahmady[a,b,*]

[a] Department of Medical Physics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[b] Nanomedicine and Nanobiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[c] Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

ARTICLE INFO

Keywords:
Photothermal therapy
Melanoma cancer
Diode laser
Ferrite
Carbon nanostructure

ABSTRACT

Nanothechnology-mediated photothermal therapy (PTT) is emerging as one of the inspiring alternative modality of cancer therapy that applies near-infrared radiation. High favorability of this approach is due to its minimum invasiveness, safety of non-targeted area, quick recovery, and capable simultaneous imaging. In this approach, photoabsorbing nanomaterials convert energy of infrared light to vibrational motion and generate heat. In the present study, a nanocomposite comprised nickel ferrite and carbon ($NiFe_2O_4$/C) was synthesized, characterized and introduced as a novel photoabsorbing agent in cancer phototherapy. $NiFe_2O_4$/C was characterized by field emission scanning electron microscopy, Fourier-transform infrared spectroscopy, and X-ray diffraction patterns. A diode laser of 808 nm with a power density of 1.0 W cm^{-2} was selected as the light source to evaluate the photothermal property of $NiFe_2O_4$/C toward cancer repression in C540 (B16/F10) cell line and melanoma bearing tumor model in male balb/c mice. Temperature enhancement ability of $NiFe_2O_4$/C confirmed its photoabsorbing property. While $NiFe_2O_4$/C had a concentration dependent cytotoxicity on C540 (B16/F10) cell line, PTT of $NiFe_2O_4$/C activated by laser irradiation showed its destroying effect on the C540 (B16/F10) cell line. On the other hand, histological analyses and tumor volume changes were performed for the in vivo PTT of $NiFe_2O_4$/C upon intratumoral injection. The results showed that after 24 h, PTT of the nanocomposite cured the tumor properly, whereas $NiFe_2O_4$/C injection or laser exposure alone had no treatment effect. Also, 5-day post-treating the melanoma bearing tumor model indicated that the level of necrosis significantly increased during this time in the PTT treated mouse. Therefore, PTT using $NiFe_2O_4$/C is proposed as a promising procedure for the melanoma cancer therapy.

1. Introduction

Cancer is a threatening disease worldwide. Today, finding an effective and impressive novel plan for cancer treatment is an important step for medical scientific community [1]. Therefore, the need for development of minimally invasive and more patient-friendly therapeutic strategies including radiation therapy, ultrasound therapy, cryotherapy, and phototherapy has increased [2]. In these routes, electromagnetic waves may change the conformation and function of proteins in thermal or non-thermal mechanisms [3,4].

Phototherapy is a therapeutic approach that includes photodynamic therapy (PDT) and photothermal therapy (PTT). In PDT, a photosensitizer transfers light energy to oxygen-containing molecules, and reactive oxygen species (ROS) or other free radicals are produced, and induce cell death. PTT is a tumor ablation therapy using thermal energy. The thermal ablation of malignant tissues at temperatures higher than 40 °C is a non-invasive alternative treatment for cancer [5,6]. In this therapeutic strategy, heat induces irreversible harms in the cell membranes and proteins [1]. The significant challenges in the thermal ablative plans are exclusive effect on malignant cells. Accidental damages of light energy affect both healthy and malignant tissues [1]. The efficiency and selectively of thermal ablation strategies are enhanced using proper sensitizing agents. Sensitizing agents absorb the radiation energy in the malignant site, and promise exclusive application. In PTT, nanomaterials such as gold nanoparticles (NPs), gold nanorods and carbon nanotubes have been used as sensitizers for absorption of near infrared (NIR) light, and dissipate light energy to vibrational energy, which result in heat generation in the local area and irreversible cell damages [7,8]. NIR radiation of wavelengths between 700–1100 nm is proper for the biological systems; while they have a good penetration into the organs, biological chromophores remain safe [9,10]. The efficiency of PTT is determined by the photonic properties of the

* Corresponding author at: Department of Medical Physics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
E-mail address: nsattar@sums.ac.ir (N. Sattarahmady).

https://doi.org/10.1016/j.colsurfb.2018.06.034
Received 2 April 2018; Received in revised form 31 May 2018; Accepted 17 June 2018
Available online 19 June 2018
0927-7765/

nanostructures of absorption and heat conversion efficiencies [11].

Carbon-based nanomaterials are conducting structures with high thermal and electrical conductivities, mechanical stiffness, photostability, and ability of photoconversion to heat in PTT [12]. So far, various carbon nanostructures have been applied as sensitizers in PTT. In this context, graphene-based nanomaterials, carbon nanotubes and carbon quantum dots exhibit desirable characteristics including chemical stability, dose-dependent toxicity, biocompatibility and eco-friendliness [13–15]. Carbon in combination with other NPs provides new and more efficient abilities with more structure stability and higher electrical conductivity [16]. Recently, different complex nanomateials contained magnetic NPs such as Fe_3O_4/SiO_2/graphene/CdTe/chitosan [17], Au-Fe_3O_4 [18], Ag_2S-Fe_3O_4 [19] and Arg/Fe_3O_4/albumin have been designed [20]. These structures are noteworthy because of the chances for dual applications in diagnostics and therapy. In some studies [21–23], plasmonic NPs with a magnetic core have been introduced with applications in imaging and tumor photothermal therapy. Magnetic iron oxide NPs have had different applications in medicine including diagnostics (as contrast agents in MRI), magnetic separation in biotechnological purposes, cell tracking, magnetic drug delivery, and hyperthermia [20,24–26]. Ferrite NPs with the formulation of AFe_2O_4 (A: Co, Ni, Zn, etc) have been also applied in medicine [27,28].

In this study, we synthesized a nanocomposite of nickel ferrite and carbon ($NiFe_2O_4$/C) for photothermal therapy in C540 (B16/F10) cell line and melanoma bearing tumor in balb/c mice by a continuous-wave diode laser of 808 nm as a light source. In vitro temperature increment, cell viability evaluation and histological analyses were performed. The nanocomposite presented a new and effective photothermal property for cancer treatment.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma (USA), Scharlau (Spain) or Merck (Germany), and employed without further purification. Deionized (DI) water was used throughout the study.

2.2. Synthesize of $NiFe_2O_4$/C

Dopamine was dissolved in a mixture of water:ethanol (5:1 v/v) with a concentration of 0.8 mg mL^{-1}. Then, $NiCl_2.6H_2O$ (0.4 mmol L^{-1}) and $FeSO_4.7H_2O$ (0.8 mmol L^{-1}) were added into the dopamine solution and stirred. Subsequently, the pH of the solution was adjusted to 8.5 using Tris buffer. The solution was mild stirred for three days at room temperature for the polymerization of dopamine. After that, the product was centrifuged and washed with DI water for five times and then dried. Finally, the sample was calcined at 600 °C at a heating rate of 2 °C min^{-1} for 2 h under an Ar atmosphere.

2.3. Characterization of $NiFe_2O_4$/C

UV-vis spectrum of $NiFe_2O_4$/C was recorded by a Rayleigh UV2601 double beam spectrophotometer. The size and surface morphology of $NiFe_2O_4$/C were evaluated using field emission scanning electron microscopy (FESEM) on a Zeiss, Sigma-IGMA/VP microscope (Germany).

The crystal structure of $NiFe_2O_4$/C was determined using X-ray diffraction (XRD) by a Philips X'Pert diffractometer (the Netherlands) equipped with a Cu/K_α radiation source (λ = 0.1540 nm) at a scanning rate of 1° min^{-1} in a 2θ range of 10 to 70°. Fourier transform infrared spectroscopy (FTIR) spectra were acquired by a Burker Tensor 27 (Germany) spectrometer. The samples were pelletized with KBr powder before measurements.

2.4. Photothermal activity of $NiFe_2O_4$/C upon laser irradiation

Light irradiation was administered by an 808-nm diode laser of Thorlabs (USA). $NiFe_2O_4$/C dispersions (100 and 1000 μg mL^{-1}) in autoclavable 1.5-mL vials were exposed to the diode laser. Output power density of laser was fixed at 1.0 W cm^{-2} by changing the spot size of the output mounting lens and the distance between the lens and the target. A thermoprobe of Lutron (Taiwan) with 0.01 °C accuracy was applied for measuring temperature increment of the $NiFe_2O_4$/C dispersions during laser radiation. Changes in the temperature of $NiFe_2O_4$/C dispersions were recorded after 5, 10 and 15 min of radiation. DI water was used as a control at the same conditions. Each measurement was repeated three times.

2.5. Cell line and animal preparations

Mouse malignant melanoma cell line C540 (B16/F10) was prepared from National Cell bank of Iran (NCBI) of Pasteur Institute (Iran). The cells were grown in Roswell Park Memorial Institute-1640 (RPMI) medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic (penicillin-streptomycin) in a humidified cell culture incubator at 37 °C, 5% CO_2.

Twelve male balb/c inbred mice (4-week old, body weight of ~20 g) were prepared from the center of comparative and experimental medicine, Shiraz University of Medical Sciences. The procedures were followed in accordance with the rules of the committee on the Ethics of Animal Experiments of Shiraz University of Medical Sciences. Maintenance cages of animals were fixed at a controlled temperature (24 ± 2 °C) and humidity (40–70%) with weekly floor exchange. They had free access to water and standard pelleted laboratory animal diets. A 12:12 light:dark cycle was followed in the mentioned animal vivarium.

2.6. Cytotoxicity of $NiFe_2O_4$/C

In vitro cytotoxicity of $NiFe_2O_4$/C was evaluated using the C540 (B16/F10) cells. 24 h-seeded cells at a density of 1.0×10^4 cells $well^{-1}$ were treated with different concentrations of $NiFe_2O_4$/C (10, 25, 50, 100, and 250 μg mL^{-1}) in 96-well culture plates for 24 h in dark. Then, the medium of the cells was substituted by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, 100 μL from a 0.5 mg mL^{-1} stock solution prepared in phosphate buffer saline, PBS) solution for 4 h at 37 °C. After centrifugation at 1800 rpm for 10 min, the culture supernatant was removed and 100 μL of dimethyl sulfoxide (DMSO) was added to dissolve the MTT formazan crystals. After centrifugation at 3500 rpm for 3 min, the MTT solution was removed from each well for recording the optical density (OD) of each well at 570 nm using a microplate reader of Biotek (USA). Wells containing no $NiFe_2O_4$/C were used as a control. Cell viability was expressed as the ratio of the 570-nm absorbance of the treated and control cells.

2.7. Cell viability measurement after laser exposure and photothermal effect

To determine the cell viability upon photothermal treatment, C540 (B16/F10) cells were seeded as described previously. 24 h after cell seeding, C540 (B16/F10) cells in 96-well culture plates (1.0×10^4 cells $well^{-1}$) were divided into four groups as follows: L-N-$_{cell}$ (no laser irradiation, no $NiFe_2O_4$/C impression, as a control), L^+N-$_{cell}$ (laser irradiation at 1.0 W cm^{-2} for 10 min, no $NiFe_2O_4$/C impression), L-N^+_{cell} (no laser irradiation, treated with $NiFe_2O_4$/C), and $L^+N^+_{cell}$ (laser irradiation at 1.0 W cm^{-2} for 10 min immediately after treatment with $NiFe_2O_4$/C). For N^+_{cell} groups, the cells medium was prepared containing $NiFe_2O_4$/C (10 and 50 μg mL^{-1}). For N-$_{cell}$ groups, DI water was used instead of $NiFe_2O_4$/C dispersion. For L^+_{cell} and L-$_{cell}$ groups, following 4 h of incubation (in the presence of $NiFe_2O_4$/C or DI water), the cells were irradiated with laser or kept at dark for 10 min, respectively. Then, the