The Combination of Photothermal Therapy and Chemotherapy using Alginate-Modified Iron Oxide-Gold Nanohybrids Carrying Cisplatin

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ABSTRACT

Background: Chemotherapy is typically the first-line treatment for the advanced stage of cancers. However, there are shortcomings with respect to conventional chemotherapy that limit therapeutic efficiency, including lack of tumor selectivity, systemic toxicity and drug resistance.

Objective: A multifunctional nanoplatform was build using of hydrogel co-loaded containing cisplatin and Iron oxide–gold core-shell nanoparticles. The Au shell comprises the light response and the iron core can be utilized as a negative contrast agent in nanocomplex.

Material and Methods: In this experimental study, KB cells derived from the epithelial cells located in the nasopharynx were exposed to different levels of concentration of hydrogel co-loaded with cisplatin and Iron oxide–gold core-shell nanoparticles. Afterwards, the cytotoxicity was determined using MTT assay.

Results: The cytotoxicity results showed that this nanoplatforms has potent to create higher cytotoxicity in KB cells than free cisplatin, so that Fe-Au@Alg and Fe-Au@Alg with cisplatin mixed with laser irradiation exhibited a significant reduction in cell viability after 5 min.

Conclusion: Hydrogel co-loaded with cisplatin and Iron oxide–gold core–shell nanoparticles are stable construct to combine chemo-photothermal therapy. Therefore, they can be used as a computed tomography-traceable nanocarrie, enabling us to monitor the delivery of therapeutics.

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Keywords

Chemo-Photothermal Therapy; Iron Oxide–Gold Core–Shell Nanoparticles; Cisplatin; Nanoparticles; KB Cells

Introduction

Chemotherapy is one of the first modalities to treat malignant growth (cancer). Absence of tumor selectivity, adequate drug concentration into the target tissues, serious side effects on cancer sufferers and several drugs resistance represent the main disadvantages of standard chemotherapy, resulting in poor healing effects. Recent advances in nanomedicine have brought new chances to lower the impediments of usual chemotherapy using nanoparticles exploiting [1].

There is evidence that nanoparticles used as vehicles to deliver drug

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are extended to the water solvency of drugs, lessen cytotoxicity, and enhanced drug distribution kinetics. It is possible therefore, to develop tumor-targeting drug delivery through improved permeability and retention (EPR) effect [2, 3].

As thermo-chemotherapy, Hyperthermia and chemotherapy mixture is a procedure to empower increment anticancer adequacy. The cytotoxic impact of numerous anticancer medications like Alkylating and platinum-based agents medications will be intense at the point blended with heat using different physiology-related components [4].

Regular hyperthermia methods might be attended with undesirable thermal harms to the encompassing solid tissues because of non-selective tumor heating [5, 6]. Subsequently, the design and utility of thermo-sensitive nanocarriers equipped for the concurrent conveyance of heat and drug have increased great consideration in nanomedicine throughout recent years [7, 8].

Highly tunable and facile synthesis, nontoxicity, biocompatibility, and ease of modification of surface mean that gold nanoparticles (AuNPs) are the exquisite nanocarriers to carry drug [9]. However, as a result of their remarkable thermo-physical properties, AuNPs are good absorbent of light to generate localized heat. Through this, tumor cells are photothermally ablated through excessive selectivity. The proper properties of AuNPs for drug delivery and the warmth technology functionality below laser light exposure mean that AuNPs can be used as a thermo-chemotherapy platform for many cancers. This creates synergistic anticancer effect [10-13].

A multifunctional nanoplatform of cisplatin and Iron oxide–gold core–shell nanoparticles (Fe_2O_3@Au) was developed co-loaded into alginate hydrogel network and potential uses for cancer diagnosis and therapy [5, 14]. This complex (Fe_2O_3@Au co-loaded into alginate hydrogel) has been result from intensive engineering to accelerate the clinical application of photothermal therapy in tumor therapy. The light-responsive part is comprised of the Au shell and creates a red-shift in the plasmon band that enables utilization of near-infrared (NIR) region (700-1100 nm laser) for nanocomplex photoactivation [15]. The iron core functions as a negative contrast agent in MRI [16]. The investigations demonstrated that this nanocomplex is suitable for mixing chemotherapy and radiotherapy [17]. Moreover, in another study, X-ray computed tomography (CT) imaging contrast was improved by Alginate hydrogel co-loaded with Cisplatin and AuNPs (ACA). In this way, we can trace the in vivo biodistribution and localization of this nanocomplex [17]. Considering the beneficial effects of combining chemotherapy and thermal therapy, this study aims to explore the potential of cisplatin and Iron oxide–gold core–shell nanoparticles (Fe_2O_3@Au) co-loaded into alginate hydrogel for combined thermo-chemotherapy of human nasopharyngeal tumor cells.

Material and Methods

Materials

In this experimental study, all experimental materials were utilized for culturing cells such as fetal bovine serum (FBS, Gibco®, USA), trypsin-ethylene diamine tetra acetic acid (EDTA), phosphate buffer saline, penicillin-streptomycin, and Roswell Park Memorial Institute (RPMI) cell culture medium. The materials were purchased from Sigma-Aldrich Company (USA). Tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O \geq 99.9\%$), ACS, and Alginic acid sodium salt utilized in the synthesis of nanoparticle were procured from Sigma- Aldrich (St. Louis, MO, USA).

Synthesis and Characterization of hydrogel co-loaded with cisplatin and Iron oxide–gold core–shell nanoparticles

The synthesis of Fe_2O_3@Au was explained

in our recent work [18]. A standard synthesis consists of dissolving 50 mg of hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4$) in 10 ml of deionized water. Afterwards, drops of 30 ml of 0.3% alginic acid sodium salt were added to create an Au^{3+}-alginic acid sodium salt mixture. The solution of cisplatin was then dropwise added when the final concentration was 50 ppm to the synthesized Fe_2O_3@Au /alginate hydrogel. At the same time, a magnetic stirrer stirred the mixture strongly at 800 rpm at ambient temperature [14].

To investigate the size and morphology of the synthesized nanocomplex, transmission electron microscopy (TEM; LEO 906; Zeiss) was performed. Dynamic light scattering (DLS) was used to weigh the hydrodynamic diameter of the sample using the Malvern Zetasizer Nano ZS-90 instrument. The Rayleigh UV-1601 instrument was used to record the optical properties of nanocomplex.

Cell Culture

Human Mouth Epidermal Carcinoma (KB) cell line was provided by the National Cell Bank of Iran (NCBI), Pasteur Institute of Iran. To culture the cells, RPMI-1640 medium supplemented with penicillin (100 µg/ml), 10% fetal bovine serum (FBS), streptomycin (100 units/ml) and L-glutamine (2 mm), at 37 °C with an atmosphere of 5% CO_2 was used. Cultures were propagated or harvested by trypsinizing with 500 µl of PBS containing 1 mm EDTA/0.25% trypsin (w/v).

Photothermal properties of hydrogel co-loaded with cisplatin and Iron oxide-gold core-shell nanoparticles

To investigate the photothermal effects of nanocmplex, the nanocomplex aqueous solutions were irradiated using a NIR laser sources (continuous-wave 808 nm, Nanobon Company, Tehran, Iran) with different Fe_2O_3@Au concentrations (25-150 µg/ml) in microcentrifuge tubes. The intensity was 1.4 W/cm^2 and the time period was set at 5 min. An infrared thermal imaging camera (Testo 875-1i, Germany) was used to check the temperature changes continuously. The laser supply was 1cm away from test sample. The focal point diameter of laser beam was 0.5 cm. For this reason, KB cells were seeded with a confluency of 5×10^6 cells per ml in 6-well plates (24 h at 37 °C) in a humidified 5% CO_2 atmosphere. After that, RPMI medium(comprised of a variety of Fe_2O_3@Au concentrations (20, 40 and 60 µg/ml)) was added into 96-well plates. In four hours, the cells were rinsed for three times using ice cold PBS followed by trypsinizing, centrifuging, and resuspending in medium. Then, real-time medium temperature during laser (1.4 W/cm^2, 5 min) irradiation was monitored.

Cell viability or cytotoxicity assay

To examine and compare the possible cytotoxicity of nanocomplexes, cells were first seeded into a 96-well plate exposed to cisplatin (0.5-50 µg/ml), alginate coated Fe_2O_3@Au (Fe-Au@Alg) (25-150 µg/ml of Fe_2O_3@Au) and nanocomplex (concentration per cisplatin: 2.5 µg/ ml, concentration per Fe_2O_3@Au: 20-40-60 µg/ml). To study the influence of laser irradiation on the proliferation cultures grown, plates were incubated at 25 °C for 4 h with Fe-Au@Alg and nanocomplex and then transfer to microtubes power density of laser irradiation was 1.4 W/cm^2 for 5 min. After 24 h of nanocomplex treatment, the cell cytotoxicity was performed using MTT assay following the standard instructions.

Statistical Methods

The collected data was analyzed using one-way ANOVA test in SPSS (v.11). With a confidence level of 95% and as a post hoc test, Tukey test was utilized to compare means of the treatment groups pairwise. The data are represented through mean±SD (P<0.05).

Results

Characterization of hydrogel co-loaded with cisplatin and Iron oxide-gold core-shell nanoparticles

Transmission electron microscopy (TEM) image analysis shows that nanocomplex has a magnetic core and alginate hydrogel matrix dispersed well (Figure 1a). The hydrodynamic diameter measurement by DLS was in 25-70 nm range and the average diameter of about 50 nm (Figure 1b). The UV-vis absorption spectrum of nanocomplex exhibits an absorption peak at 563 nm and a remarkably higher absorption at the near infrared region, that is consistent with the laser wavelength here (Figure 1c).

Photothermal Performance of hydrogel co-loaded with cisplatin and Iron oxide-gold core-shell nanoparticles

Temperature measurement of the nanocomplex shows laser irradiation dose-dependent and heat generation increase with various concentrations of Fe_2O_3@Au (Figure 2a). Pure water as control shows only a temperature change less than 4 °C, whereas with identical laser ir-

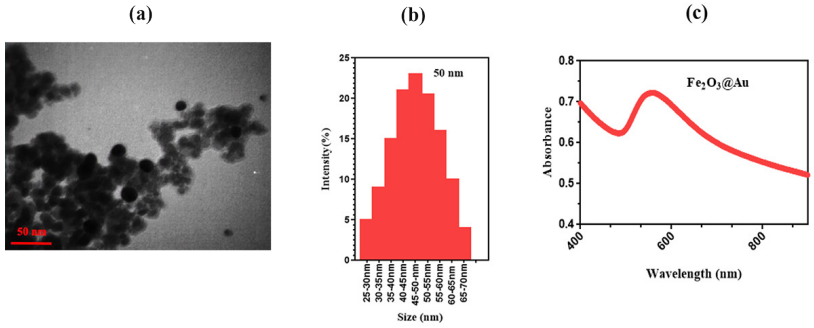

Figure 1: Characterization of hydrogel co-loaded with cisplatin and Iron oxide–gold core-shell nanoparticles. (a) Transmission electron microscopy (TEM) image of the nanocomplex illustrates Fe_2O_3@Au coated by alginate hydrogel. (b) Size distribution histogram of Fe_2O_3@Au, indicating the effective hydrodynamic size of the nanocomplex to be 50 nm (25-70 nm range). (c) UV-Vis spectra of the solution of Fe_2O_3@ shows a Plasmon band with the absorption peak at 563 nm.

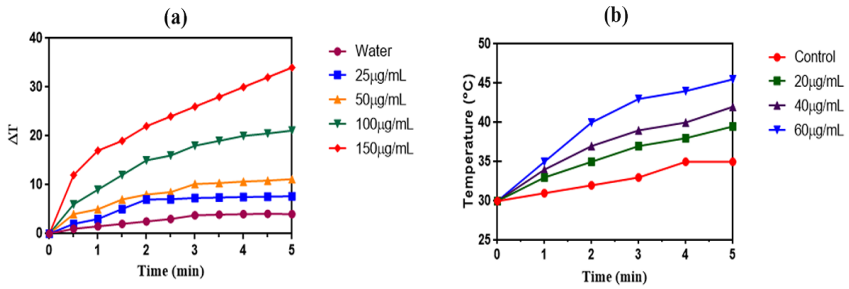

Figure 2: Photothermal Performance of hydrogel co-loaded with cisplatin and Iron oxide–gold core–shell nanoparticles. (a) Water temperature change and water Fe-Au@Alg contain cisplatin at different Fe_2O_3@Au concentrations under laser irradiation (808 nm, 1.4 W/cm^2) that depends on irradiation time. (b) Change in temperature of Human Mouth Epidermal Carcinoma (KB) cells treated with nanocomplex in advance at different Fe_2O_3@Au concentrations exposed to laser irradiation (808 nm, 1.4 W/cm^2) that depends on irradiation time.

radiation conditions nanocomplex suspension temperature increased significantly till 34.5 °C after 5 min at a concentration of 150 µg/ml of Fe_2O_3@Au. Furthermore, pretreated cells with different doses of nanocomplex compared to untreated cells displayed meaningful temperature enhancement. The cells pretreated with nanocomplex at the Fe_2O_3@Au concentration of 60 µg/ml were heated and the temperature increased by15 °C (Figure 2b). While without nanocomplex, the laser irradiation of the cells created a 5 °C temperature increase. The findings demonstrate that nanocomplex coverts light into heath proficiently. Therefore, it is appropriate for photothermal ablation.

Chemo-photothermal therapy of KB cell

Figure 3a shows the cytotoxicity effects of Fe_2O_3@Au nanoparticle on KB cell in the presence of alginate coating. As illustrated, at the same Fe_2O_3@Au nanoparticle concentration, the toxicity of Fe-Au@Alg is insignificantly lower than Fe_2O_3@Au. However, the effect of alginate coating to enhance the biocompatibility of Fe_2O_3@Au nanoparticle is not statistically significantly. When treated with Fe-Au@Alg, the viability of KB cells is concentration-dependent. The concentration of 150 µg/ml reduced the viability of KB cells by about 65%.

Chemotherapy efficiency of cisplatin loaded nanocomplex were plotted in Figure 3b, which shows the viability curves of KB cells in the presence of cisplatin in nanocomplex and its free structure. Apparently, under the same cisplatin concentration, hydrogel co-loaded with cisplatin and Iron oxide–gold core-shell nanoparticles can exert a more acute cytotoxic effect on KB cell in comparison with free cisplatin. At the same cisplatin concentration, cytotoxicity effects of cisplatin on noncomplex (Fe_2O_3@Au concentration of 30 µg/ml) was significantly less than cisplatin alone. For in-

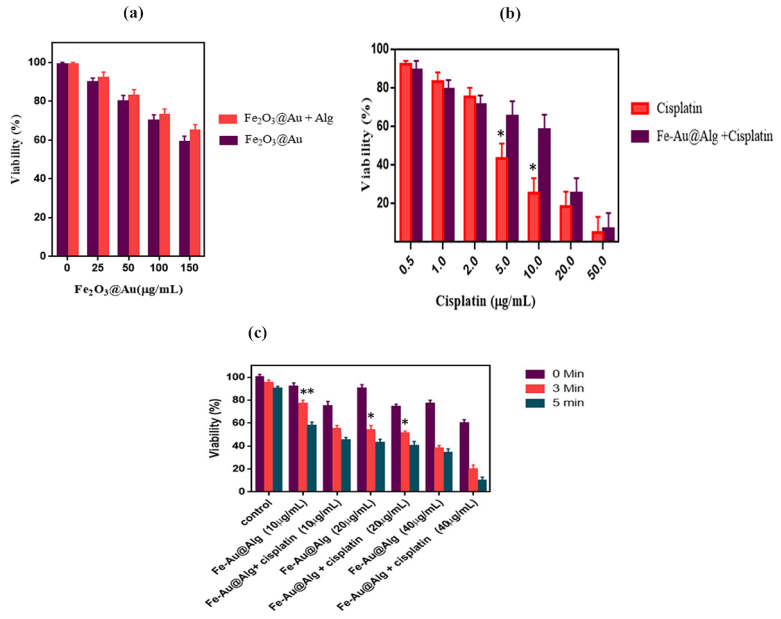

Figure 3: The cytotoxicity assay. (a) The viability of Human Mouth Epidermal Carcinoma (KB) cells following Fe_2O_3@Au nanoparticle and Fe-Au@Alg treatment with different Fe_2O_3@Au concentrations.(b) The viability of KB cells following cisplatin (0.5-50 µg/mL) and Fe-Au@Alg treatment along with cisplatin (per cisplatin: 0.5-50 µg/mL). (c) The viability of KB cells following Fe-Au@Alg (per Fe_2O_3@Au: 10, 20 and 40 µg/mL) and Fe-Au@Alg treatment with cisplatin (per Fe_2O_3@Au: 10, 20 and 40 µg/mL, per cisplatin: 2.5 µg/mL) along with laser irradiation (1.4 W/cm^2, 3 and 5 min) (* *P* value<0.05, ** *P* value<0.001).

stance, cisplatin (10 µg/ml) lowered the cell viability to 58%, although in nanocomplex with the equal cisplatin concentration, a notably lower cell viability of 25% was achieved.

To study the photothermal therapeutic effect of nanocomplex, viability of KB cell at different Fe-Au@Alg concentrations (10, 20 and 40 µg/ml) and Fe-Au@Alg with cisplatin after irradiation with the 808 nm laser (1.4 W/cm^2, 3 and 5 min) was measured. As seen in the Figure 3c, laser irradiation alone had no significant effect on cytotoxicity in KB cells, so that in 5 min after irradiation of laser, the viability was 90%. Fe-Au@Alg and Fe-Au@Alg with cisplatin mixed with laser irradiation exhibited a reduction in cell viability, so that cell viability significantly decreased with Fe-Au@Alg dose-dependent manner. Furthermore, the Figure 3c shows that at the same Fe-Au@Alg concentration, a significantly more cytotoxic was obtained for cells treated with Thermo-chemotherapy than the individual therapy approaches. Consequently, hydrogel co-loaded with cisplatin and Iron oxide–gold core–shell nanoparticles have the potential for integrating photothermal therapy and chemotherapy into one nano-platform appearing to generate a synergistic effect.

Discussion

The non-targeted drug delivery and related toxicity in healthy tissues, as a result of the unwanted accumulation of compounds to organs, decrease the efficacy and safety of chemotherapy. Adding drugs to nanocarriers is a promising complementary and alternative method to standard chemotherapy in order to enhance drug stability, targeted drug delivery, and decreased tissue toxicity. Given that chemotherapy efficiency can be intensified at elevated temperatures, designing the thermo-sensitive nanocarriers would be beneficial to combine chemotherapy and thermal therapy. To realize this goal, cisplatin and Iron oxide–gold core–shell nanoparticles co-loaded into alginate hydrogel network were used along with the synergistic interaction upon laser irradiation.

The findings showed that hydrogel co-loaded with cisplatin and Iron oxide–gold core–shell nanoparticles increased the killing efficiency of chemotherapy. Under the identical drug concentration, this complex significantly decreased cell viability compared to free cisplatin. Our results are consistent with findings from other studies that were reported at a concentration range of 0.5-50 µg/ml, cisplatin and alginate hydrogel co-loaded with cisplatin and AuNPs (ACA) with a concentration of 2.5 µg/ml (per cisplatin) and show similar cytotoxicity on KB human nasopharyngeal cells [14]. In this study, alginate gel based on seaweed-derived polysaccharides was used as a natural polysaccharide for surface modification of Fe_2O_3@Au. Alginate nanoparticles for drug delivery can enhance stability, bioavailability, biocompatibility, toxicity, solubility rates of poorly water-soluble drugs [19-22]. Moreover, several studies with X-rays have shown the potential of ACA as a CT imaging contrast agent given the high X-ray absorption capacity of AuNPs [23]. Previous researches observed that treating KB cells with ACA significantly increased CT [14].

Photothermal therapy of nanocomplex demonstrated thermo-responsive property of hydrogel co-loaded with cisplatin and Iron oxide–gold core–shell nanoparticles whereby it is able to absorb NIR laser light energy and convert it into heat energy. KB cells pre-treated with hydrogel co-loaded with cisplatin and Iron oxide–gold core–shell nanoparticles under laser irradiation displayed an enhanced temperature elevation in comparison with laser radiation alone. Thereby, the intracellular uptake of noncomplex was sufficient to create efficient heating.

MTT assay suggested that the combination of alginate hydrogel co-loaded with cisplatin and Iron oxide–gold core–shell nanoplatform and laser irradiation can result in superior therapeutic outcomes than the corresponding

monotherapies, including laser and nanocomplex. Fe_2O_3@ Au showed a red-shift in the surface plasmon band (absorption peak: 563 nm), making them a well-suited NIR photothermal. The magneticcore of Fe_2O_3@Au makes it possible for them to be targeted toward a desired location under external magnetic field and also be tracked through MRI.

The in vivo MRI analysis has shown that magnetic targeting is effective in concentrating nanoparticles into the tumor. So that, considerably greater temperature enhancement has been shown in tumor after NIR radiation, leading to complete eradication of local tumor with no recurrence after 28 days therapy [23].

Enhanced permeability and retention (EPR) effect is a well-known strategy that mediated by leaky vasculature and ineffective lymphatic drainage in the tumor, through the passive accumulation of nanoparticle. The previous study showed that the systemic injection of hybrid Fe_2O_3@Au can cause effective accumulation of the nanocomplex in tumor tissue [24].

The findings indicated that diverse treatment with nanocomplex regimens caused different changes in the viability of KB cells in comparison with the control group. The cytotoxicity of treated cells and concentration of nanocomplex demonstrated the correlation. Indeed, the higher concentrations of nanocomplex resulted in higher toxicity to tumor cells.

Conclusion

Considering the therapeutic advantages of combining heat and drug, a thermo-responsive nanocarrier was designed to combine photothermal therapy and chemotherapy. The clinical utility of photothermal therapy was extended using theranostic Fe_2O_3@ Au nanoparticles.

The developed nanocarrier significantly increased the effect of drug delivery through selective targeting of drugs. Irreversible damage to the cells was caused by the intracellular co-localization Fe_2O_3@ Au, laser irradiation, cisplatin, and the synergistic interactions. These findings indicate that the developed nanocarrier can be used as an efficient platform to heat and deliver drug to cancer cells effectively. Clinical entry dose of chemotherapy agents is changed and the high dose-associated side decreased.

Authors' Contribution

M. Soleimani conceived the idea. The introduction of the paper was written by A. Ghadimi and M. Soleimani. R. Mirjani gather the related literature and also help with the writing of the related works. The method implementation was carried out by A. Ghadimi. Results and Analysis were carried out by M. Soleimani and A. Ghadimi. The research work was proofread and supervised by M. Soleimani and R. Mirjani. All the authors read, modified, and approved the final version of the manuscript.

Ethical Approval

Not applicable, because this article does not contain any studies with human or animal subjects.Human Mouth Epidermal Carcinoma (KB) cell line was purchased from National Cell Bank of Iran (NCBI), Pasteur Institute of Iran (NCBI, C152).

Conflict of Interest

None

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