In Situ Fabrication of Intelligent Photothermal Indocyanine Green–Alginate Hydrogel for Localized Tumor Ablation

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Supporting Information

ABSTRACT: Simplifying synthesis and administration process, improving photothermal agents’ accumulation in tumors, and ensuring excellent biocompatibility and biodegrability are keys to promoting the clinical application of photothermal therapy. However, current photothermal agents have great difficulties in meeting the requirements of clinic drugs from synthesis to administration. Herein, we reported the in situ formation of a Ca²⁺/Mg²⁺ stimuli-responsive ICG–alginate hydrogel in vivo for localized tumor photothermal therapy. An ICG–alginate hydrogel can form by the simple introduction of Ca²⁺/Mg²⁺ into ICG–alginate solution in vitro, and the widely distributed divalent cations in organization in vivo enabled the in situ fabrication of the ICG–alginate hydrogel without the leakage of any agents by simple injection of ICG–alginate solution into the body of mice. The as-prepared ICG–alginate hydrogel not only owns good photothermal therapy efficacy and excellent biocompatibility but also exhibits strong ICG fixation ability, greatly benefiting the high photothermal agents’ accumulation and minimizing the potential side effects induced by the diffusion of ICG to surrounding tissues. The in situ-fabricated ICG–alginate hydrogel was applied successfully in highly efficient PTT in vivo without obvious side effects. Besides, the precursor of the hydrogel, ICG and alginate, can be stored in a stable solid form, and only simple mixing and noninvasive injection are needed to achieve PTT in vivo. The proposed in situ gelation strategy using biocompatible components lays down a simple and mild way for the fabrication of high-performance PTT agents with the superiors in the aspects of synthesis, storage, transportation, and clinic administration.

KEYWORDS: ICG, alginate, hydrogel, photothermal therapy, tumor

1. INTRODUCTION

Photothermal therapy (PTT), which converts near-infrared (NIR) light energy to heat based on PTT agents, is an attractively noninvasive approach with the advantages of high selectivity, powerful tumor ablation, and minimal damage to normal tissues.¹,² Current PTT agents mainly include nanostructures,²,³ hydrogels,³–¹⁸ and organic dyes¹⁹–²¹ with strong NIR absorption. Up to now, various nanoparticles, such as gold nanostructures,²²,²³ nanocarbons,²⁴–²⁵ transition metal sulfide/oxide nanoparticles,²⁶–²⁸ and organic nanoparticles,²⁹–³⁰ hydrogels containing various PTT agents,³–¹⁵ and others (phosphorene,³⁹ antimonene,⁴⁰–⁴² borophene,⁴³ and MXene⁴⁴), have been widely applied for PTT. Despite the high therapeutic efficacy, their inherent nonbiodegradable components and potential long-term toxicity of the nanoparticles hindered further clinical translation.²,¹² Organic dyes with definite structure and physicochemical properties are another type of PPT agents; however, the great safety concerns make most organic dyes difficult in clinic applications, apart from indocyanine green (ICG).¹⁹–²¹

ICG with intense NIR-absorbing and fluorescence properties is an ideal theranostic agent approved by the FDA because of its low toxicity. ICG is widely used in clinic for fluorescence imaging, cardiac output analysis, plasma volume, and liver function assessment. Recently, ICG and ICG-loaded nanostructures have been explored for PTT in vivo.⁴⁵–⁵⁰ However, most intravenously injected ICG-based PTT agents suffer from limited ICG accumulation in tumors, and potential side effects resulted from unspecific distribution in various organs,¹⁹–²¹,⁴⁵–⁵⁰ although intratumorally injected ICG-based agents undergo the obstacle of the strong pressure in a solid tumor, leading to leakage of ICG along the path of the needle and limiting the injected dose of agents. Ideally, theranostic...
PTT agents should be guided by the following criteria: (1) ease of preparation, storage, transportation, and administration; (2) high accumulation capability in tumors; (3) excellent biocompatibility and biodegradability; (4) cost and time effectiveness. Therefore, it is of great significance to develop novel strategies to fabricate an ideal PTT agent using ICG as the only FDA-approved dye.

Sodium alginate, a kind of polysaccharide carbohydrate extracted from brown seaweed, is widely applied in clinic and food industry with their inherent biodegradability and biocompatibility. Sodium alginate consists of guluronic (G) and mannuronic (M) acid regions, and divalent cations such as Ca$^{2+}$/Mg$^{2+}$ prefer to bind the G-blocks in the linear copolymer to form the structure of “egg box”, thereby obtaining a hydrogel in an ultrasimple way. Alginate hydrogels with superior biocompatibility and biodegradability are widely used in medical treatment because of their structural similarity with extracellular matrixes of the living tissues, such as the carrier of drugs, cell transplantation, and tissue engineering compared to other hydrogels. The body fluid circulation is a process of dynamic balance, and the ions in the body are constantly updated but relatively constant, which provides great opportunities for in situ formation of Ca$^{2+}$/Mg$^{2+}$-responsive alginate hydrogel in vivo. Thus, the integration of ICG and alginate hydrogel with unique advantages makes it possible to build a localized ICG depot for improving ICG accumulation in tumors and reducing the side effects induced by the leakage of ICG.

Herein, we show the in situ fabrication of a photothermal ICG–alginate hydrogel for localized tumor ablation in an ultrafacile way (Scheme 1). The precursor of the ICG–alginate hydrogel was synthesized by simply mixing ICG and alginate at room temperature. The introduction of Ca$^{2+}$/Mg$^{2+}$ enables the transformation of ICG–alginate from solution to hydrogel easily, and ICG distributes in the hydrogel homogeneously. In vivo gelation study confirmed that the ICG–alginate hydrogel could generate quickly as soon as the ICG–alginate solution was injected into the body of mice. The in situ fabrication process of the hydrogel can avoid the leakage of ICG along the path of the needle, improve the accumulation of ICG in tumors, and minimize the diffusion of ICG to surrounding tissues. The ICG–alginate solution exhibits intense NIR absorption and fluorescence as same as free ICG, and the formed ICG–alginate hydrogel owns an excellent photothermal heating ability. In vitro and in vivo toxicity assessment demonstrated the favorable biocompatibility of the hydrogel due to the inherent biosafety of ICG and alginate. The in situ fabricated ICG–alginate hydrogel was applied in localized PTT in vitro and in vivo successfully, and the fluorescence of ICG enables the monitoring of the distribution of ICG accurately. Besides, ICG and alginate can be stored as a stable solid form for a long time, and only the simple mixing in water and noninvasive injection are needed to perform high-efficient localized PPT of tumors, greatly benefiting the preparation, storage, transportation, and potential clinic usage.

2. RESULTS AND DISCUSSION

2.1. Synthesis of ICG–Alginate Hydrogel in Vitro. The viscosity of ICG–alginate hydrogel highly depends on the concentrations of alginate and divalent cations. Considering the constant concentration of Ca$^{2+}$/Mg$^{2+}$ in vivo, various concentrations of alginate solutions were employed to tune the viscosity of the hydrogel (Figure S1). High viscosity benefits the loading of ICG in the ICG–alginate hydrogel but is adverse to syringeability. Therefore, 20 mg/mL of alginate as the optimum concentration was chosen to ensure the high ICG loading efficiency and good syringeability. The ICG–alginate...
The hydrogel was synthesized via a simple mixing method. Briefly, 35 mg of alginate and 7.5 mg of ICG were mixed in 1.75 mL of H2O and stirred for 30 min. The ICG–alginate solution is homogeneous and stable, exhibiting excellent syringeability compared to hydrogels. To access the gelation properties in vitro, the well-dispersed ICG–alginate solution (60 μL, 4.28 mg mL−1 of ICG and 20 mg mL−1 of alginate) was injected into 8 mL of Ca2+/Mg2+ solution in a meter glass. The concentrations of Ca2+ and Mg2+ were fixed to be 1.8 and 1.5 mM to mimic the extracellular microenvironment in the living tissue.17 The ICG–alginate hydrogel immediately formed while ICG–alginate aqueous solution was being injected into the Ca2+/Mg2+ solution (Figure 1). We further investigated the ICG loading capacity of the ICG–alginate hydrogel, which could reach as high as 10 mg/mL of ICG in the hydrogel (Figure S2). The formed uniform hydrogel enables encapsulation of ICG and overcomes the burst release of ICG efficiently (Figure S3).

2.2. Characterization of ICG–Alginate Solution. The UV–Vis–NIR absorption and fluorescence of the ICG–alginate solution were similar to that of the ICG solution, indicating negligible interaction between ICG and alginate (Figure 2A–D). The fluorescence spectra of the ICG–alginate solution showed a maximum emission at 813 nm, benefiting in vivo fluorescence imaging with high tissue penetration depth. The strong NIR absorption peak at 808 nm linearly increased with the concentrations of ICG in the ICG–alginate solution with a fixed alginate concentration, which demonstrated the high photothermal potential with an 808 nm laser. The UV–Vis–NIR absorption and fluorescence spectra of the ICG–alginate hydrogel indicated that the formed hydrogel also exhibited a strong NIR absorption and an intense fluorescence emission due to effective encapsulation of ICG in the hydrogel (Figure S4). SEM images showed the morphology of a lyophilized ICG–alginate hydrogel (Figure S5).

2.3. Photothermal Performance of ICG–Alginate Hydrogel. To evaluate the photothermal performance of the ICG–alginate hydrogel, an ICG–alginate gel prepared by adding different volumes of ICG–alginate solution to Ca2+/Mg2+ solution was irradiated with an 808 nm laser for 7 min at a power density of 0.9 W cm−2. Figure 3 revealed that the increased temperature of the ICG–alginate hydrogel varied from 12.4 to 21.4 °C with the volumes of the ICG–alginate solution changed from 10 to 100 μL. In contrast, the temperature of Ca2+/Mg2+ solution pure water was only raised to 5.8 °C. Besides, IR thermal photos were taken to monitor the temperature change of the solution during the irradiation process, further demonstrating the favorable photothermal performance of the ICG–alginate hydrogel (Figure 3). We found that 660 or 980 nm laser irradiation could also lead to a photothermal heating effect of the ICG–alginate hydrogel. However, 660 nm laser irradiation suffers from lower photothermal heating efficacy compared to 808 nm laser irradiation, and 980 nm laser illumination results in a strong undesired photothermal heating of pure water due to the intense absorption of water at 980 nm.70 Therefore, 808 nm laser irradiation is the most suitable light source for photothermal heating of the ICG–alginate hydrogel (Figures S6 and S7).

2.4. Cytotoxicity of ICG–Alginate Hydrogel. The cytotoxicity of the ICG–alginate hydrogel was evaluated by a standard MTT assay with 4T1 cells. The cells were treated with ICG–alginate solution or preformation ICG–alginate hydrogel containing different concentrations of ICG. Figure 4A indicated that the cell viability remained above 85% even when the concentration of ICG reached 1 mg mL−1 in ICG–alginate solution, and the cell viability remained above 82% even when the concentration of ICG reached 500 mg L−1 in the preformation ICG–alginate hydrogel (Figure S8), demonstrating the excellent biocompatibility of in situ formation and preformation of the ICG–alginate hydrogel and its great potential for biological applications.
2.5. Photothermal Therapy in Vitro. We then investigated the PTT capability of the ICG−alginate hydrogel in vitro. 4T1 cells treated with ICG−alginate gel containing 200 mg L\(^{-1}\) of ICG were irradiated with an 808 nm laser at a power density of 0.3 or 1 W cm\(^{-2}\) for 5 min. The viabilities of 4T1 cells treated with the hydrogel and laser irradiation were only 5.20% and 4.84% at the laser power densities of 0.3 and 1 W cm\(^{-2}\), respectively (Figure 4B), whereas the cells without any treatments and treated with the hydrogel or laser irradiation alone all showed a negligible cell death. Moreover, calcein-AM/PI dual staining was performed to show the cell viability directly. The fluorescence images indicated that few live cells could be seen after the combined treatments of the ICG−alginate hydrogel and laser irradiation (Figure 4C). In contrast, a majority of cells were alive with other treatments. These results indicated that the ICG−alginate hydrogel possessed an excellent photothermal therapy ability.

2.6. In Situ Fabrication of ICG−Alginate Hydrogel in Vivo. We reasonably assume that the injection of ICG−alginate solution into the living organism will lead to the formation of an ICG−alginate hydrogel due to the presence of appropriate concentration of Ca\(^{2+}\)/Mg\(^{2+}\) in biological fluids. Like many PTT agent solutions, ICG solution leaked along the path of the needle from the pinhole because of the enormous pressure in the solid tumor when free ICG was injected directly into tumors. However, there was not any leakage of solution from the injection sites after the administration of ICG−alginate solution due to the in situ formation of an ICG−alginate hydrogel in vivo (Figure 5A). To evaluate the feasibility of in situ fabrication of the ICG−alginate hydrogel in vivo, four Kunming mice were subcutaneously injected with 60 μL of ICG−alginate solution (4.28 mg mL\(^{-1}\) of ICG and 20 mg mL\(^{-1}\) of alginate) on one side and 60 μL of ICG solution (4.28 mg mL\(^{-1}\)) on the other side as the control group. Moreover, the state of injected solutions was monitored by ex vivo imaging at different time points. Figure 5B indicated the formation of ICG−alginate hydrogel, and the green color of ICG was distributed into the hydrogel, avoiding the self-aggregation of ICG and favoring the uniform heating triggered by laser illumination. Both ICG solution and ICG−alginate hydrogel were gradually metabolized along with the time, whereas the metabolism rate of the ICG−alginate hydrogel was obviously slower than the bare ICG solution. These results demonstrated that the ICG−alginate hydrogel could be easily in situ-formed by the injection of ICG−alginate hydrogel without any leakage, proving a stable ICG depot for PTT in vivo.

2.7. Fluorescence Imaging of ICG−Alginate Hydrogel in Vivo. To investigate the ICG fixation capability of the ICG−alginate hydrogel, fluorescence imaging of mice was performed after the administration of ICG and ICG−alginate solution (Figure 5C). Two groups of Kunming mice were subcutaneously administrated with 60 μL of ICG−alginate solution (4.28 mg mL\(^{-1}\) of ICG and 20 mg mL\(^{-1}\) of alginate) and 60 μL of ICG solution (4.28 mg mL\(^{-1}\)), respectively. Fluorescence images indicated that free ICG was distributed...
and ICG solution in vivo. were injected subcutaneously with ICG−alginate solution for 0.5 h (4.28 mg mL\(^{-1}\) of ICG and 20 mg mL\(^{-1}\) of alginate). (B) Photos showing the metabolism of ICG fluid and ICG−alginate hydrogel in vivo. (C) Dynamic fluorescence imaging of ICG−alginate hydrogel and ICG solution in vivo.

Figure 5. (A) Pictures of 4T1-bearing mice were recorded after injecting 60 μL of ICG solution or ICG−alginate solution for 0.5 h (4.28 mg mL\(^{-1}\) of ICG and 20 mg mL\(^{-1}\) of alginate). (B) Photos showing the metabolism of ICG fluid and ICG−alginate hydrogel in vivo. (C) Dynamic fluorescence imaging of ICG−alginate hydrogel and ICG solution in vivo.

Fabricated ICG−alginate hydrogel was employed in vivo PTT. In summary, we proposed an in situ gelation strategy to synthesize an ICG−alginate hydrogel in vivo for PTT, minimizing the side effects induced by the fast leakage of PTT agents.

2.8. Toxicity of ICG−Alginate Hydrogel in Vivo. Before the in vivo PTT application, the in vivo toxicity of ICG−alginate was evaluated via body weight change monitoring and histopathological analysis (Figure 6A). The Kunming mice were injected subcutaneously with ICG−alginate solution (4.28 mg mL\(^{-1}\) of ICG and 20 mg mL\(^{-1}\) of alginate) or 60 μL of PBS as control, and the mice treated with the hydrogel showed a similar increase tendency in weight in comparison with the control group (Figure 6B). Hematoxylin−eosin staining analysis of the main organs (liver, heart, spleen, kidney, and lung) revealed that there were no obvious histopathological lesions for mice in both experimental and control groups. Under physiological condition, ion replacement and the elution of the cross-linking calcium cation resulted in osmotic swelling of Ca−alginate hydrogel, leading to increased pore size and destabilization and rupture of the hydrogel. Alginate is also a biodegradable polymer because the backbone of alginate can be degraded via hydrolysis of the glycosidic bonds. Therefore, the proposed hydrogel can be biodegraded in vivo on the basis of these mechanisms, ensuring the biosafety of the hydrogel.\(^{31−34}\) The in vivo toxicity investigation demonstrated the excellent biocompatibility of the ICG−alginate hydrogel in vivo.

2.9. Photothermal Therapy in Vivo Using in Situ-Fabricated ICG−Alginate Hydrogel. Inspired by the excellent performance of photothermal ability and biocompatibility, the ICG−alginate hydrogel was employed in vivo PTT.

Throughout the body quickly due to the continuous body fluid circulation. In contrast, the ICG in the ICG−alginate hydrogel penetrated to the bloodstream and surrounding tissues slowly due to the strong fixation capability of the hydrogel. The high ICG accumulation capacity of the in situ-formed hydrogel showed a great potential in improving the PTT efficacy and minimizing the side effects induced by the fast leakage of PTT agents.

2.8. Toxicity of ICG−Alginate Hydrogel in Vivo. Before the in vivo PTT application, the in vivo toxicity of ICG−alginate was evaluated via body weight change monitoring and histopathological analysis (Figure 6A). The Kunming mice were injected subcutaneously with ICG−alginate solution (4.28 mg mL\(^{-1}\) of ICG and 20 mg mL\(^{-1}\) of alginate) or 60 μL of PBS as control, and the mice treated with the hydrogel showed a similar increase tendency in weight in comparison with the control group (Figure 6B). Hematoxylin−eosin staining analysis of the main organs (liver, heart, spleen, kidney, and lung) revealed that there were no obvious histopathological lesions for mice in both experimental and control groups. Under physiological condition, ion replacement and the elution of the cross-linking calcium cation resulted in osmotic swelling of Ca−alginate hydrogel, leading to increased pore size and destabilization and rupture of the hydrogel. Alginate is also a biodegradable polymer because the backbone of alginate can be degraded via hydrolysis of the glycosidic bonds. Therefore, the proposed hydrogel can be biodegraded in vivo on the basis of these mechanisms, ensuring the biosafety of the hydrogel.\(^{31−34}\) The in vivo toxicity investigation demonstrated the excellent biocompatibility of the ICG−alginate hydrogel in vivo.

2.9. Photothermal Therapy in Vivo Using in Situ-Fabricated ICG−Alginate Hydrogel. Inspired by the excellent performance of photothermal ability and biocompatibility, the ICG−alginate hydrogel was employed in vivo PTT.

The 4T1 tumor-bearing mice were injected with 60 μL of PBS or ICG−alginate (0, 0.1, 1, and 4.28 mg mL\(^{-1}\) of ICG and 20 mg mL\(^{-1}\) of alginate) solution intratumorally, followed by the 808 nm laser irradiation at a power density of 0.7 W cm\(^{-2}\) for 15 min. The temperature of tumors was monitored by an IR thermal camera during the irradiation process. To achieve obvious PTT efficacy and minimize the administration dose, ICG−alginate solution containing 4.28 mg mL\(^{-1}\) of ICG was chosen as the optimum PTT agent for tumor ablation (Figures S9 and S10). Figure 6C indicated that the temperature of tumors in mice injected with ICG−alginate solution (4.28 mg mL\(^{-1}\) of ICG and 20 mg mL\(^{-1}\) of alginate) exhibited a remarkable increase, whereas the mice in the control group only had a much slower temperature. Furthermore, the sizes of tumors were measured with a vernier caliper every day after various treatments (Figure 6D). In addition, the photos of tumors were taken every day to observe the tumor change visually for 15 days. Figure 6E showed that the tumors of mice treated with PBS and laser irradiation kept growing all the time, whereas the tumors of mice treated with ICG−alginate solution almost disappeared in a week. These results obviously demonstrated that an ICG−alginate hydrogel with strong ICG fixation capability could serve as an excellent photothermal agent with outstanding biocompatibility.

3. CONCLUSIONS

In summary, we proposed an in situ gelation strategy to synthesize an ICG−alginate hydrogel in vivo for PTT, uniting the advantages of ultrasimple synthesis and administration procedures, excellent biocompatibility, avoidance of the leakage from injection sites, improvement of the accumulation in tumors, and minimizing of side effects induced by the metabolization from tumors. The ICG−alginate hydrogel can
be generated by adding Ca\(^{2+}\)/Mg\(^{2+}\) into the ICG–alginate solution in vitro or simply injecting the ICG–alginate solution into organisms without any leakage of any agents. The strong ICG-fixed ability of the hydrogel was demonstrated in vitro and in vivo, greatly benefiting the high accumulation of ICG in tumors and minimizing the side effects from the fast metabolism. The toxicity studies in vitro and in vivo confirmed that its favorable biocompatibility derived from the safety of ICG and alginate. The in situ-fabricated ICG–alginate hydrogel with outstanding photothermal performance was applied in tumor PTT in vitro and in vivo successfully. Moreover, the convenient storage of ICG and alginate in a solid form and simple synthesis and administration process make the in situ-formed ICG–alginate hydrogel promising in PTT in vivo. We will investigate the clinic transformation of the ICG–alginate hydrogel with unique superiorities in the future. The in situ fabrication of the photothermal hydrogel using biocompatible components lays down a promising way to revolutionize PTT toward clinic transformation from synthesis of administration.

ASSOCIATED CONTENT

Supporting Information
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Notes
The authors declare no competing financial interest.

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