Recent Progress of Polymer Micelles used as Anti-cancer Multifunctional Nano-carriers

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Abstract: Polymer micelles, formed mostly by core-shell structures, are one kind of nano-carriers, which are used in the therapy of cancer through diverse methods. The shell is usually formed by polyethylene glycol (PEG) thus having the advantage of preparing amphiphilic and biocompatible drug-carriers. With different drugs as the core, multi-functions such as magnetic thermal, photothermal, targeting, contrast etc. were discovered to be used in the therapeutic system. Simultaneously, the loading and delivery of drugs can be adjusted under the control of polymer micelles, so that drug-releasing in vivo is enabled to be quantified, accompanied by the change of external conditions in the sake for reduction of cytotoxicity. Therefore, polymer micelles have crucial significance for the treatment of cancer. This essay introduces the latest research progress of polymer micelles to make simple guides for researchers who are getting touched with this field so as to broaden the scope and propose innovations.

1. Introduction
Cancer is a chronic disease, which has made vast damage on the health of people. Traditional chemotherapy is partly efficient on the cure of cancer, but it also brings massive side-effects, thus making adverse influences [1]. Nano-carriers have the ability to deliver the loading drugs [2] to the tumor through physicochemical navigation or enhanced permeability and retention (EPR) [3], therefore they can replace chemotherapy to play a therapeutic role in cancer medicine [9], simultaneously solving the problem of side-effects [10,14]. At present, many kinds of nano-carriers have been developed [11-13]. Moreover, multifunction of them can be searched besides the therapeutic function [15]. For example, some special nano-carriers can be used as contrast in PET-CT, or to assist hyperthermia or magnetic therapy [16]. This essay focuses on the introduction and performance of polymer micelles, in order to provide some thoughts to the choice of carriers.

2. Materials and Methods

2.1. PMAA-Graft-PEG
To beginning the research on PMAA-graft-PEG [6], the work published by Efstatia Voulgari and Aristides Bakandritsos etc. on Journal of Controlled Release in 2016 is described as follows.

This micelle was prepared by dissolving FeSO4ꞏ7H2O (0.72g) in distilled water (10ml) containing HCl (37%, 0.03ml). The solution of the P (MAA-g-EGMA) copolymer (0.15g, 30ml) was stirred (350rpm) at 60 °C, and after a period of time, ammonia (30%, 2ml) was added. Rhodamine-conjugated magnetic nanoparticle can be prepared by connecting N-(2-aminoethyl) rhodamine 6G-amidebis (trifluoroacetate) (Sigma Aldrich) with carboxyl groups on the polymer coating of nanoparticles.
Thinking about drug-loading, a 240 μg nano-carrier was uniformly mixed with a small amount of cis-Pt to form a sample of 1.2ml (0.016-0.2 μg drug/μg nano-carriers). The nano-assemblies and drugs mixed in the pure water were gently stirred overnight at room temperature. Cis-Pt loading capacity (Wt.%) of Mag102-6 increased with the increase of mass ratios between cis-Pt and medicine drugs delivery system (MDDS).

As for the releasing process of cis-Pt in vivo, it was simulated with phosphate buffered saline (PBS,0.1 M), with pH of 5.5,7.4 at 37 °C. The effect of AC magnetic field on the release of cis-Pt was studied by using Magne-Therm Nano-Therics device (f = 106.9 kHz, B = 25 mT). Alternating current magnetic fields are enabled at 240-250 min and 300-310 min from the beginning of drug release. The release rate is inversely proportional to the density of anion sites in the process of binding. In the presence of electrolytes (NaCl), the change of colloid stability with time was also evaluated by recording the change of Dh (Intensity-weighted hydrodynamic diameter). The sample (0.6 mg/mL, 100 μL) was cultured in 50% diluted human plasma at 37 °C for 24 hours, and the particles were washed and then suspended in 1 mL water.

At room temperature, Dh and zeta potential did not rise or decrease continuously within 5 months, indicating that the carrier could remain stable during this period (shown as Ref.6, Fig.2a,2b). Even if the ionic strength of NaCl in the medium reached 2m, there was no obvious increase in the Dh of Mag23-6, indicating no aggregation. After 5 hours of incubation, the Dh and zeta potential of Mag23-6 were significantly higher than those of Mag102-6, indicating that it had negatively aggregated or absorbed proteins in plasma. However, the Dh and zeta potential of Mag102-6 are still about the same as initial. The saturation magnetization (Ms) value of Unloaded nano-carriers can reach 57 Am2/kg. Taking the view of diamagnetic reaction, the value increased to 76 Am2/kg, which was close to bulk Fe3O4 (~ 90 Am2/kg), which was enough to prove the chemical stability of the product related to the magnetization and non-oxidation. After loading cis-Pt, the Ms of the carriers was increased to 63Am2/kg.

In order to evaporate the efficacy of therapy, the same kind of mice carrying HT-29 tumor cells were divided into five groups sequentially: no administration (control), saline (blank), cis-Pt (free drug, FD), cis-Pt loaded Mag102-6 (particulate drug, PD) and cis-Pt loaded Mag102-6 with external magnetic field gradient (particulate drug + magnetic field, PDMF). From the first operation on the 13th day to the sacrifice of mice on the 38th day, the tumor volume has been from large to small according to the order of groups. After dissecting and sampling, it was found that the volume of FD reached 900 mm3 while the PDMF was restricted under 500 mm3. Comparison to the control (>1300mm3) shows that both FD and PDMF are able to inhibit tumor growth, but this ability of PDMF is more obvious.

The weight of mice was measured and recorded during the experiment. Only the mice in the FD group suffered weight loss (close to 20% final reduction), which means that cis-Pt without carriers has adverse side-effects and is significantly harmful to the mice. By measuring the spleen index of mice, it was indicted that the PD and PDMF groups were identical to the blank group (about 3.5), while the FD group (about 1.5) was much lower. This phenomenon further supports the view that drug loaded-carriers can effectively reduce the cis-Pt toxicity.

The Pt-concentration (FD, PD and PDMF) in tumors and organs (blood, liver, spleen and kidney) was determined respectively by XRF. It was found that the concentration in tumors of both PD and PDMF is lower than FD, indicating that more effective cellar uptake had occurred with the help of Mag102-6. Pt-concentration in kidney has the same but more obvious distribution as in tumors. This phenomenon suggests that the cis-Pt in vivo was not extensively separated from MDDS, thus revealing a controlled release. In the PD groups, cis-Pt levels have higher values in both liver and spleen because drug-particles hardly penetrate the fenestrae of liver and spleen.

2.2. PEG-IR-780-C13

Then, the research on PEG-IR-780-C13 [7] published by Ahu Yuan and Xuefeng Qiu etc. on Biomaterials in 2015 is introduced as follows.

In the sake for preparing this micelle, acetonitrile (100ml) was used to dissolve 2,3,3-Trimethylindolenine (12 g 75 mmol) and 1-Bromohexadecane (22 g, 70 mmol), then the mixed solution
was agitated at reflux temperature for the whole day. This product (3g, 6.5mmol) and an intermediate (0.4g, 2.3mmol synthesis as Ref.4) were set to dissolve in ethanol (20ml). Then, 0.8 g Sodium-acetate was added and stirred at 70 °C for half a day. After evaporation and purification, obtained IR-780-C13 (50mg, 0.052mmol) was mixed and stirred in chloroform (50ml) with PEG2000-SH (120 mg, 0.060 mmol) and TEA (10mL, 0.072 mmol) at room temperature for 24h.

PEG-IR-780-C13 was dissolved in d-H2O and then self-assembled to amphiphilic micelles with hydrophobic IR-780-C13 and hydrophobic PEG, thus assembling core-shell structure in solution. Determined by ultrafiltration, the critical micelle concentration (CMC) of micelle was 4 μg/ml. In d-H2O, a wider and lower UV-vis absorption was observed than in DMF. These micelles shaped as spherical vesicle were in diameter of 50-150nm and mainly concentrated in 116nm, which was recombined the range requirement of EPR. Moreover, the zeta potential (8.5mv) and particle size enabled it to sustain great stability without any precipitation stored at 4°C after a month. To evaluate the photothermal effect, the laser (808 nm, 1 W/cm2) was used to irradiate PEG-IR-780-C13 micelles, IR-780-C13, free IR-780, and PBS for 6 min. No obvious warm was shown in PBS. IR-780-C13 and IR-780 exhibited an identical temperature augment (25.5-48 °C), certifying that the photothermal property of IR-780 was unaffected to the additional carbon chain alone. However, the heating range of PEG-IR-780-C13 micelles is the most obvious (25.7-66.5 °C), indicating that additional carbon chain with modification of PEG enabled it to improve the property.

Three-time laser irradiation cycles were settled to evaluate the repeated heat production efficiency, it was found that the peak of PEG-IR-780-C13 micelles was more than 60 °C in the first two times, even in the 3rd time it was still above 40 °C. Continued with laser irradiation on PEG-IR-780-C13, the peak of UV-vis absorption was not disappeared until the 9th time, while pure IR-780 insisted only to the 3rd time, indicating that the degradation of IR-780 can be alleviated by PEG-IR-780-C13.

PEG-IR-780-C13 micelles (30mg/kg) were injected into Balb/c mice bearing CT26 tumors to measure therapeutic efficacy. NIR fluorescence signal appeared in the tumor area 1h after injection. The micelles showed a tendency of enrichment in tumors with the passage of time, and obvious uptake was observed from 24h to 48h, which confirmed that PEG-IR-780-C13 micelles can accumulate in tumors. 24h after injection, these anesthetized mice were exposed to an 808 nm laser at 1 W/cm2 for 10 min. The average temperature of tumors increased from 33.6 °C to 52.1 °C within the first 5 min, and kept at about 52.5 °C in the remained 5 min. What’s more, there were no obvious burns on the skin, but the average volume of tumors decreased to lower than 10mm^3 from original 200mm^3 on day3. Above all, this conclusion that PEG-IR-780-C13 micelles have the ability to serve as a photothermal agent in vivo can be acquired. During the experimental period, no significant weight variations of mice were observed, so that preliminary judgment can be obtained that there was no obvious toxicity of PEG-IR-780-C13 micelles in vivo.

To further investigate toxicity, PEG-IR-780-C13 micelles (30mg/kg) were injected into healthy Balb/c mice which would be sacrificed 2 weeks later and their major organs would be sliced to observe. No obvious damage to major organs was noticed at the therapeutic dose of 30mg/kg. Even at 60 or 90mg/kg, there was still no damage. Then, selecting mice which were sacrificed on the 15th and 30th days for serum biochemistry assays respectively, no abnormality was found in the main functional indexes of both liver and kidney. Compared with the free IR-780, which was enough to kill mice in the dose of only 2mg/kg, it was known that PEG-IR-780-C13 micelles have no obvious toxicity and can effectively relief the toxicity of IR-780.

2.3. ICG-CPPDN/rGO
Finally, the research on ICG-CPPDN/rGO [8] published by Shazid Md. Sharker and Jung Eun Lee etc. on Biomaterials in 2015 gave the following description.

C-PPDN [5] (100mg) was dissolved in 1 mM tris buffer (8 ml, pH 8.5) mixed with aqueous suspension of graphene oxide (GO) plates (2 ml, 1mg/ml) firstly. The obtained solution was modulated to 8.5pH and stirred at 40-60°C for 24h. After centrifugation and free-drying, GO-polymer
powder-like material (CPPDN/rGO) was obtained. Then the CPPDN/rGO (100mg) was dissolved in d-H2O (8ml) and added to Indocyanine green (ICG,2ml,0.5mg/ml). After 10min stirring, the mixture was centrifuged and freeze-dried to prepare ICG-CPPDN/rGO.

The presence of ICG-CPPDN/rGO was confirmed by UV-vis absorption peak on 775nm wavelength and fluorescence emission following 780nm excitation. The loading content of ICG into CPPDN/rGO was measured as 13% by UV-vis, while the efficiency was 65.2%. Fluorescence emission was found to decrease continuously from acidic pH (5.0) and not maintain stability until physiological pH (7.4). The changes of zeta potential with pH (5.0-8.5) were measured to illustrate the colloidal stability. A stable positive potential was found in ICG-CPPDN/rGO regardless of pH (5.0-7.4). Conversely, sharp decreases were shown in both CPPDN and CPPDN/rGO at the same pH interval, thus indicating the formation of ICG-CPPDN/rGO once again. The zeta potential of all materials decreased at pH (7.4-8.5) because surface charge was potentiality stabilized by electrostatic interaction. Moreover, the values of zeta potential had been kept below 25mv, which was considered as the ceiling of nano-particles with high stability.

The pattern and shape of ICG-CPPDN/rGO were shown as sphere by AFM image with height profile. The diameter changes from 210nm and depth 62nm at pH 6.0 to 220nm and 65nm at pH 7.4, and the maximum size was 255nm to 295nm, which was considered as suitable cellular delivery for endocytosis.

To determine the heat generation ability of NIR light, the ICG-CPPDN/rGO nano-particles was dissolved in PBS at different pH (5.0-7.4) and irradiated (808nm, 2W/cm^2). The heating rate was found to decease with the increasing pH and the temperature after 5min irradiation had exceeded 70 °C at pH 5.0 while that was about 30°C at pH 7.4. In addition, the temperature was also found to rise with increasing concentration (0.02-0.2mg/ml) at a specific pH. Above all, it was certified that pH had an evident influence on the capacity and morphology of ICG-CPPDN/rGO. To evaluate photothermal therapy (PTT) efficiency in vivo, ICG-CPPDN/rGO and free ICG (both 30mg/kg) were injected into healthy Balb/c mice and Balb/c mice bearing tumors respectively. 2h after injection, these mice were exposed to laser irradiation (808 nm, 1 W/cm2) for 5min and the average surface temperature per minute was recorded. It was found that the temperature of the diseased mice with ICG-CPPDN/rGO had been raising to 65 °C rapidly, while that of the healthy mice only stayed at 42°C eventually. Simultaneously, sustaining and localized fluorescence signal was exhibited only on tumors.

As for free ICG, the temperatures of diseased and healthy mice reached to 55°C and 50°C. Besides tumors, fluorescence signal was also exhibited on corresponding normal sites of normal mice. Compared with free ICG, ICG-CPPDN/rGO produced a larger temperature difference and selective cytotoxic activity against tumors, indicating more effective damage on the tumor and milder protection on the normal tissue. From 5 min laser radiation, the changes of tumors on surface of mice to the 18th day were also observed. The irradiated mice injected only with PBS were used as the control group, and tumor tissues grew continuously to 300mm^3 on the 10th day. However, tumor size of mice treated with free ICG was retreated to 150mm^3. More effective inhibition was shown in mice treated with ICG-CPPDN/rGO, whose tumor size was 100mm^3 on the 10th day and was totally ablated on the 18th day.

In summary, the above results certified that ICG-CPPDN/rGO has profound potential for cancer therapy based on pH dependent NIR irradiated PTT.

3. Conclusion
We have eventually analyzed three kinds of polymer micelles (PMAA-Graft-PEG, PEG-IR-780-C13, and Responsive polymer-indocyanine) from the aspects of synthesis, characterization, drug-conjugation, therapeutic efficacy, multifunction and side-effects. It was certified that the delivery of cis-Pt loaded in PMAA-Graft-PEG could be effectively achieved in vivo with the effect of external magnetic field, thus leading to the increasement of efficacy and reduction of toxicity. Under the protection of PMAA-Graft-PEG micelle, cis-Pt could be stayed in vivo continuously and drug resistance of tumors could be partly prevented. Simultaneously, PEG-IR-780-C13 micelles were found to be photostable in an aqueous
condition and exhibit no obvious toxicity at therapeutic dose. High tumor accumulation was observed through fluorescent image because of EPR effect, all above indicating that PEG-IR-780-C13 could be used as diagnostic agent for PTT. Meanwhile, the thermographic images of ICG-CPPDN/rGO in cancerous and healthy mice were obtained in vivo, and the selective sensitivity of PTT preparation to tumor environment was confirmed. The tumors of these mice treated with ICG-CPPDN/rGO were totally disappeared on the 18th day in the lab-environment, indicating high potential for cancer therapy. Generally speaking, using polymer micelles as nano-carriers is crucial for the field of cancer treatment, whose excellent properties are helpful to solve the problems of pharmacology and pharmacokinetics in cancer. No doubt the research of polymer micelles is able to promote the process for cancer conquering.

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