Scientific paper

Polymer Nanoparticles Containing 2,4,6-triiodophenol: A Potential Contrast Medium for Medical Imaging

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Abstract

Contrast agents have been utilized for x-ray imaging to visualize blood vessels. Triiodobenzene derivatives are known contrast agents yet have not been formulated in a nanoparticle form for the purpose of enhancing the contrast of cancer tissues. In this study, experiments to encapsulate 2,4,6-triiodophenol in a polymer matrix were designed. Spherical NPs made of PLA, PLGA, PLA-TPGS, PLGA-TPGS and TPGS-FOL, were synthesized and characterized. Using the oil-in-water single-emulsion technique, the effect of several experimental parameters such as sonication power, ratio of 2,4,6-triiodophenol/polymer, type and concentration of emulsifier, and polymer type has been studied. A good morphology of polymer NPs with entrapped 2,4,6-triiodophenol was successfully obtained however the encapsulated iodine was in the range of 5 to 26%.

Keywords: X-ray contrast, polymer nanoparticles, x-ray imaging, biodegradable copolymer, 2,4,6-triiodophenol, iodine encapsulation

1. Introduction

Iodine-containing dyes are routinely used in interventional radiology. The triiodobenzene moiety is the main pharmacophore in several contrast agents used in x-ray imaging such as diatrizoate,[1] iohexol[1,2] and iodixanol.[3] These contrast agents have been formulated in water soluble media to image blood vessels during angiography procedures and not for targeting cancer tissues. Tumor tissue is detectable only if there is a distinct difference in the tissue density and its size from the density and size of the surrounding normal tissues. X-ray contrast agents have been encapsulated in liposomes,[4,5] PEG-based micelles[6] and polymer-stabilized nanoparticulate suspensions.[7]

Nanoparticles (NPs) have been extensively investigated in biomedical and biotechnological areas, especially in drug delivery systems.[8] NPs conjugated with contrast agents have been formulated for many imaging modalities especially for mammography imaging. A few reports in literature have been devoted to the use of nanoparticle formulations to enhance the contrast of x-ray imaging. Gold NPs achieved better contrast using lower x-ray dose compared to iodine when mice xenograft model was imaged using mammography machine.[9] A number of different polymers have been utilized in the preparation of biodegradable NPs and drug delivery agents, such as poly(lactide) (PLA),[10] poly(lactide-*co*-glycolide) (PLGA),[11] poly(lactide)-D-α-tocopheryl polyethylene glycol 1000 succinate copolymer (PLA-TPGS),[12] poly(lactide-*co*-glycolide)-vitamin E TPGS copolymer (PLGA-TPGS),[13] and folate-decorated PLGA-vitamin E TPGS (TPGS-FOL).[14]

In this study, we aim to encapsulate 2,4,6-triiodophenol as a potential contrast agent in polymeric based NPs following the oil-in-water single-emulsion technique.[15] Our hypothesis is that significantly higher enhancement of tumor contrast can be achieved using iodine agent-loaded NPs of biodegradable polymer conjugated to targeting agent such as folate. Moreover, the biodegradable polymer encapsula-

tion of the contrast agents would allow more prolonged uptake time of the NPs by the tumor cells and hence better chance of optimal imaging and detecting of cancer tissues.

2. Experimental

2. 1. Materials

The polymers: PLA and PLGA were purchased from Sigma-Aldrich. The PLA used in this study is with inherent viscosity ~0.5 dl/g; the PLGA is with molecular weight range 40,000–75,000, and DL-lactide:glycolide ratio of 50:50. The surfactants used in the emulsification process are vitamin E TPGS and poly(vinyl alcohol) (PVA, MW range 31,000–50,000, 98–99% hydrolyzed), and both were obtained from Sigma-Aldrich. Also, these chemicals and reagents were procured from Sigma-Aldrich: 2,4,6-triiodophenol, stannous octoate, N,N-dicyclohexylcarbodiimide (DCC), 4-dimethylamino pyridine (DMAP), ethylene diamine, glutaric acid, folic acid, and pyridine. N-hydroxysuccinimide (NHS) was procured from Fluka. All solvents used in this study: dichloromethane (DCM), dimethyl sulfoxide DMSO (anhydrous), N,N-dimethylformamide (DMF), acetonitrile, ethyl acetate and toluene were HPLC grade. All chemicals were used as received without further purification. Dialysis membrane (MWCO less than 1000) was purchased from SERVA. Ultra-pure water was prepared using Milli-Q (Milford, MA) System.

2. 2. Synthesis of PLA-TPGS

The copolymer was synthesized by ring-opening polymerization of lactide monomer with TPGS (ratio of 88:12, respectively) in toluene in presence of stannous octoate as a catalyst.[12] Weighted amounts of lactide, TPGS and 0.5 wt% stannous octoate were reacted at 145 °C for 24 h in a sealed glass ampoule under vacuum. The product was dissolved in DCM and then precipitated in cold methanol. The copolymer was recovered as a white solid after heating at 45 °C under vacuum for 2 days.

2. 3. Synthesis of PLGA-TPGS

The copolymer was synthesized by ring-opening polymerization mechanism with the presence of lactide, glycolide and TPGS (ratio of 60:27:13, respectively) in toluene.[14] Lactide, glycolide, TPGS and 0.5 wt% stannous octoate were reacted at 145 °C for 24 h in a sealed glass ampoule under vacuum. The product was dissolved in DCM and then precipitated in cold methanol. The copolymer was recovered as a white solid after heating at 45 °C under vacuum for 2 days.

2. 4. Synthesis of TPGS-FOL

The synthesis was started by the preparation of TPGS-NHS and FOL-NH_2 as it was reported.[14] TPGS-NHS was prepared by reacting TPGS, glutaric acid and DCC (1/1/1 in stoichiometric molar ratio) in DMSO under nitrogen atmosphere at room temperature for 24 h. The product was filtered to remove N,N-dicyclohexylurea (DCU) and then dialyzed against DMSO for 24 h to remove excess DCC and against water for 24 h to remove DMSO. After that, the resulted product was freeze-dried, and then reacted with NHS in the presence of DCC for 6 h at 50 °C (carbonated TPGS/NHS/DCC = 1/2/2 in stoichiometric molar ratio). FOL-NH_2 was prepared by reacting FOL with DCC and NHS in DMSO at stoichiometric molar ratio of FOL/DCC/NHS = 1/1.2/2 for 6 h at 50 °C. The activated folate was then reacted with excess ethylene diamine in the presence of pyridine as a catalyst. FOL-NH_2 was precipitated out by adding excess acetonitrile, followed by vacuum filtration. Finally, TPGS-NHS and FOL-NH_2 were allowed to react in DMSO at a molar ratio of 1:2 under nitrogen atmosphere for 2 days at room temperature. The resulted product was dialyzed against DMSO and water for 24 h each. The product was then collected after freeze-drying as a yellow solid.

2. 5. Characterization of the Synthesized Copolymers and Conjugate

The chemical structure of the synthesized PLA-TPGS and PLGA-TPGS copolymers, and TPGS-FOL conjugate was characterized by NMR. The ^{1}H NMR spectra were obtained on a JEOL LA 500 NMR spectrometer operating at a frequency of 500 MHz. ^{13}C NMR spectra were obtained at the frequency of 125 MHz with ^{1}H broadband decoupling at 298 K. The spectral conditions were: 32k data points, 0.967 s acquisition time, 1.00 pulse delay and 45° pulse angle.

2. 6. Preparation of Polymer NPs Containing 2,4,6-triiodophenol

2,4,6-triiodophenol-loaded NPs of biodegradable polymers reported in this research were prepared by the oil-in-water single-emulsion method. The formulation process involves adding dropwise a dichloromethane solution of 2,4,6-triiodophenol and PLA, PLGA, PLA-TPGS, PLGA-TPGS or the TPGS-Fol conjugate to a magnetic-stirred aqueous phase containing PVA or TPGS. The mixture, while cooled in an ice bath, was then emulsified using a probe sonicator (Sonics, Vibra-Cell, VCX750) with controlled energy. The resulting oil-in-water emulsion was then subjected to the following: (i) direct analysis of the emulsion, (ii) recovering the NPs by centrifugation (5810R, Eppendorf, 5,000 or 10,000 rpm, 10 min, 25 °C) followed by washing with ultra-pure water to remove excessive emulsifier, and (iii) suspending the obtained nanoparticle in ultra-pure water which is then lyophilized using freeze dryer (Christ, Alpha1-4 LD plus, Martin Christ, Germany) to get NPs powder.

2. 7. Characterization of Polymer NPs Containing 2,4,6-triiodophenol

The surface morphology of the NPs was imaged by a field emission scanning electron microscopy (FESEM, LYRA 3 Dual Beam, Tescan) integrated with Energy dispersive X-ray spectrometry (Oxford Instrument) operated at 30 kV acceleration voltage. Samples for SEM were prepared from either NPs suspension or dried NPs powder. To prepare samples from the particle suspension, a drop of the suspension was dripped onto the copper tape placed on the surface of the sample stub and dried. The stub was coated with a gold layer by an automatic fine gold coater for 120 s. The same procedure was applied when the dry powder samples were used, for which a double-sided sticky tape was used in place of the copper tape, and no drying was required before coating. The Energy Dispersive x-ray (EDX) spectrum data were taken from same machine, Tescan Lyra-3. The chemical and elemental analysis of the NPs was carried using the EDX. A 20 kV x-ray source was used to excite the sample and the emitted characteristic x-ray was analyzed. Quantitative analysis allows the identification of the chemical constituents of the sample as well as the %weight or atomic composition of the sample.

3. Results and Discussion

3. 1. Characterization of the Synthesized Copolymers and Conjugate

The structure of the synthesized PLA-TPGS copolymer was checked by ^{1}H NMR spectroscopy as shown in

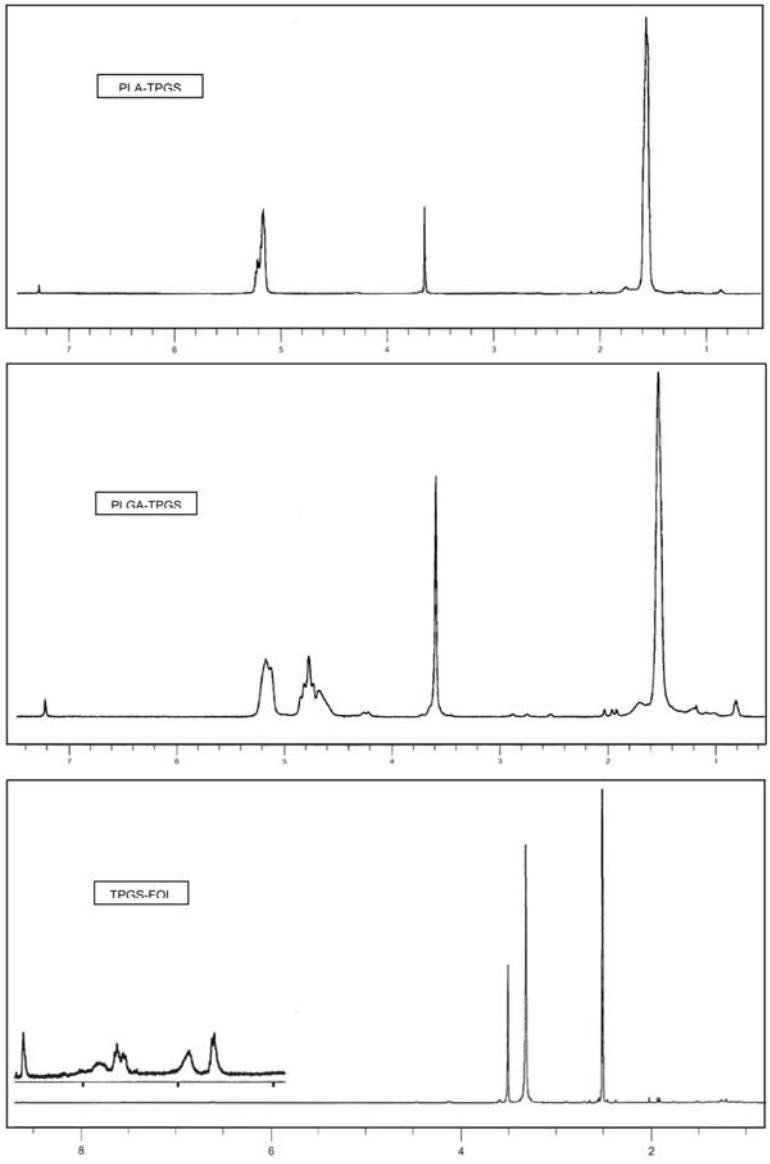

Figure 1. ^{1}H NMR spectra of PLA-TPGS and PLGA-TPGS copolymers, and TPGS-FOL conjugate.

Figure 1 ($CDCl_3$, 500 MHz, ppm) δ 5.15 (–CH protons of PLA), 3.63 (–CH_2 protons of PEO part of TPGS) and 1.54 (–CH_3 protons of PLA); and ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.9, 169.7, 169.5, 70.8, 69.5, 69.3, 17.0 ppm. Lactide monomer peak at 5.10 ppm was not detected. The precipitation process in treatment of the copolymer can remove the TPGS and lactide monomer thoroughly. The ratio between the peak areas at 5.15 and 3.63 ppm was found similar to the molar ratio of the reactants. The structure of the synthesized PLGA-TPGS copolymer was verified by ^1H NMR spectroscopy ($CDCl_3$, 500 MHz, ppm) δ 5.20 (–CH protons of PLA), 4.78 (–CH protons of PGA), 3.62 (–CH_2 protons of PEO part of TPGS) and 1.55 (–CH_3 protons of PLA); and ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.7, 169.6, 166.7, 70.8, 69.4, 69.3, 61.1, 17.0 ppm. The ratio among the peak areas at 5.20, 4.87 and 3.62 ppm as shown in Figure 1 was taken to determine the component ratio which was found similar to the molar ratio of the reactants. The TPGS-FOL conjugate was synthesized by coupling reaction of TPGS-NHS and FOL-NH_2. TPGS-NHS was synthesized using glutaric acid as a linker between TPGS and NHS. The hydroxyl group of TPGS was reacted with the carboxylic group of glutaric acid to give an ester. Another carboxylic group in the structure of glutaric acid reacted with NHS to yield TPGS-NHS. FOL was aminated by DCC/NHS chemistry in presence of pyridine as a catalyst. Due to the higher reactivity, γ-carboxylic group will mainly be transformed rather than the α-carboxylic group of folate. The structure of the synthesized TPGS-FOL conjugate was identified as shown in Figure 1 by ^1H NMR spectroscopy ($CDCl_3$, 500 MHz, ppm) δ 8.60 (pyridine-H), 8.10 (aliphatic amide-H), 7.61–6.62 (Ar-H), 2.30 (γ-CH_2 of glutamic acid) and 2.10–1.85 (β-CH_2 of glutamic acid).

3. 2. Effect of Preparation Variables on Characteristics of Polymer NPs Containing 2,4,6-Triiodophenol

By using the oil-in-water single-emulsion technique, several parameters were evaluated in order to achieve optimal preparation conditions including sonication power, type and concentration of emulsifier, ratio of 2,4,6-triiodophenol to polymer and polymer type employed in the preparation of NPs. Only one parameter was changed in each set of experiments. The influence of those parameters on the content of the contrast agent, particle size and morphology of the NPs was investigated (Table 1). The preparation procedure gave spherical particles in all reported experiments (according to SEM analysis, Figure 2).

Sonication was a fundamental step in the preparation of the polymer NPs. To study the influence of the sonication power on NPs morphology, size distribution and % iodine content, the power was set at 25W (Entry 1) and 80W (Entry 2) as shown in Table 1. From the results obtained, it can be concluded that increasing the sonication power leads to a larger size of NPs but the % iodine content was not affected.

In order to study the effect of emulsifier content on the NPs properties, some batches were prepared using an aqueous phase consisting of PVA at different concentrations. It was observed that there was an increase of % iodine content when the PVA concentration in the aqueous phase was increased from 0.2 to 0.3% (w/v) when PLA and PLA-TPGS were used as polymers (Table 1, Entries 3 and 4). When PLGA and PLGA-TPGS were utilized in the preparation of NPs containing 2,4,6-triiodophenol, increasing the PVA concentration from 0.1 to 0.3% (w/v) not only increased the % iodine content, but the spherical particles diameter was also decreased (Table 1, Entries 6 and 7). The presence of TPGS in the polymer content might also reduce the size of the NPs produced. This phenomenon can be expected from the stabilizing function of an emulsifier.[16]

The incorporation of 2,4,6-triiodophenol into polymer NPs was examined. Maintaining a constant initial mass of PLA-TPGS copolymer, the mass of the 2,4,6-triiodophenol used was varied between 20 and 100% in relation to polymer mass. It was noticed that increasing the loading of 2,4,6-triiodophenol increases the NPs diameter as shown in Table 1, Entries 4 and 5. Using a greater amount of the iodo-compound (300–400% w/w) resulted

Table 1. Preparation of polymer NPs containing 2,4,6-triiodophenol using various polymers.

Entry	Polymer	Ratio of triiodophenol/ polymer	Emulsifier	Sonication Power (W)	Particle Size (nm)[a]	Iodine Content (%)[b]
1	PLA	200% w/w	TPGS (0.03% w/v)	Probe 25W	100–200 and 500–700 nm	4.5
2	PLA	200% w/w	TPGS (0.03% w/v)	Probe 80W	100–300 nm and 2 μm	4
3	PLA	20% w/w	PVA (0.2% w/v)	Probe 25W	20–40 nm	1
4	PLA-TPGS	20% w/w	PVA (0.3% w/v)	Probe 25W	20–40 nm	4.5
5	PLA-TPGS	100% w/w	PVA (0.3% w/v)	Probe 25W	20–40 nm and 1 μm	3
6	PLGA	20% w/w	PVA (0.1% w/v)	Probe 25W	200–500 nm and 2–3 μm	7
7	PLGA-TPGS	20% w/w	PVA (0.3% w/v)	Probe 25W	20–30 nm	18
8	TPGS-FOL	200% w/w	TPGS (0.03% w/v)	Probe 25W	200–300 nm	26

[a] Measured by SEM. [b] Measured by EDX.

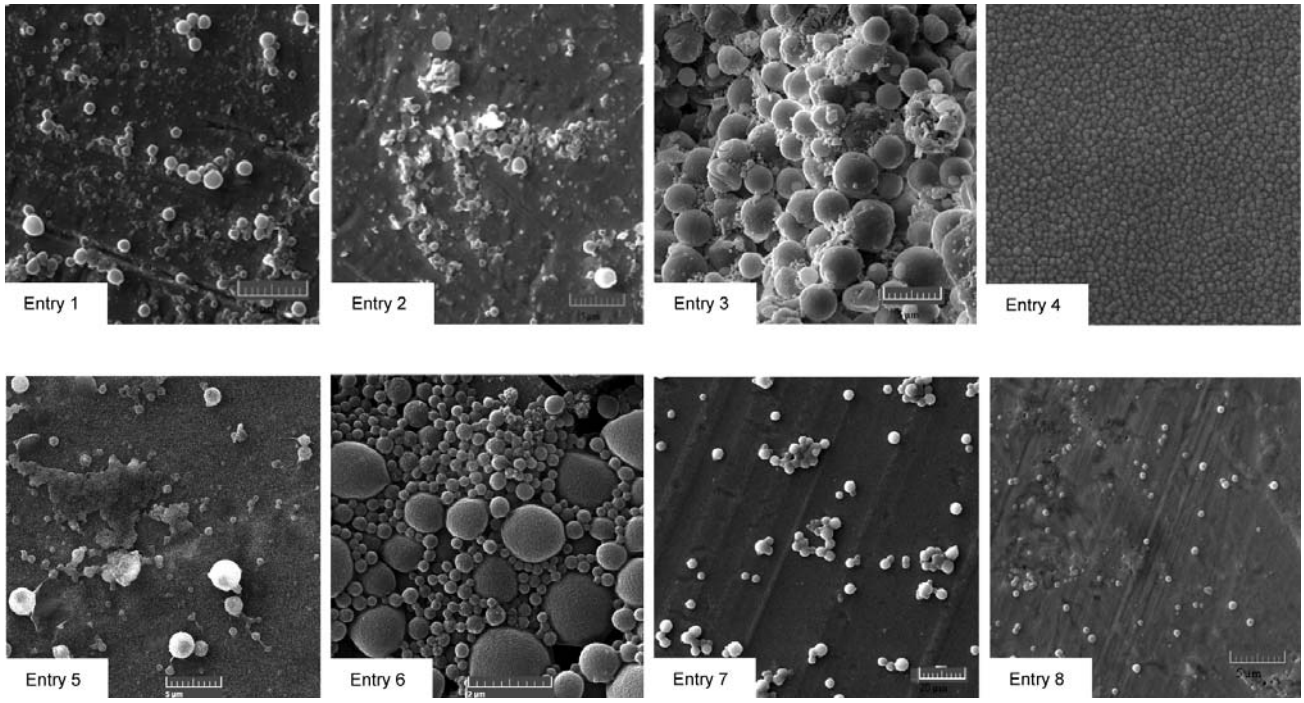

Figure 2. SEM images of the polymer nanoparticles containing 2,4,6-triiodophenol.

in the formation of a rod-like structures and the disappearance of the spherical NPs. A greater amount of the iodostructure results in a more viscous dispersed phase. This leads to a harder dispersion of the aqueous and organic phases, and in turn larger particles are formed.[17] It is important to mention that a spherical shape for the polymer NPs was maintained and the % iodine content was also found comparable (3–4.5%). It was quiet surprising that the % iodine content was increased when PLGA-TPGS (Table 1, Entry 7) was used instead of PLA-TPGS (Table 1, Entry 4) in the preparation of the NPs containing 2,4,6-triiodophenol. The intermolecular forces between the polar groups of both the phenol moiety and the copolymer seem to favor the entrapment of 2,4,6-triiodophenol in the polymer NPs.

Our attention was then turned to the use of TPGS-FOL conjugate as polymer. Folate decorated NPs of biodegradable polymers have been found to increase the cellular uptake and cell cytotoxicity of the formulated anticancer drugs. Feng's group reported that TPGS-FOL conjugate can be used for targeted chemotherapy.[14] In Table 1, Entry 8 shows that utilizing TPGS-FOL conjugate as polymer not only produced spherical and uniformly distributed NPs, but the iodine content was also increased (26%).

4. Conclusion

The oil-in-water single-emulsion technique allowed the preparation of spherical NPs containing 2,4,6-triiodophenol that can be used to enhance the x-ray contrast of cancer cells. Several preparative parameters such as sonication power, type and concentration of emulsifier, ratio of 2,4,6-triiodophenol to polymer and polymer type employed in the formation of NPs were studied. The effect of those factors on the content of the contrast agent, particle size and morphology of the NPs was investigated. A spherical polymer NPs containing 2,4,6-triiodophenol were prepared using polymers: PLA, PLGA, PLA-TPGS, PLGA-TPGS and TPGS-FOL and the % iodine content was in the range of 5 to 26%. We are currently trying to develop more reliable emulsification system to achieve higher encapsulation efficiency and better polymer NPs morphology.

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Povzetek

Kontrastna sredstva se v medicini uporabljajo tudi pri rentgenskih preiskavah ožilja. Derivati trijodobenzena so kontrastna sredstva, vendar jih do sedaj niso uporabljali v obliki nanodelcev za preiskave rakavih tkiv. V sklopu te študije smo pripravili eksperimente za enkapsulacijo 2, 4, 6 – trijodofenola v polimerni matrici. Sintetizirali in karakterizirali smo sferične nanodelce pripravljene s polimerovPLA, PLGA, PLA-TPGS, PLGA-TPGS in TPGS-FOL. Uporabljali smo emulzijo olja v vodi in preučevali vplive različnih eksperimentalnih parametrov:moč sonfikatorja, razmerje 2, 4, 6 – trijodofenol/polimer, vrsto in koncentracijo emulgatorja in vrsto polimera. Uspeli smo pripraviti morfološko ustrezne nanodelce z ujetim 2, 4, 6 - trijodofenolom, z deležem ujetega joda med 5 do 26 %.