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Crosslinked Dextran Gel Microspheres with Computed Tomography Angiography and Drug Release Function

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For computed tomography (CT) angiography and drug release application, a kind of novel dextran hydrogel microspheres were prepared. β-cyclodextrin (β-CD) grafted poly(methyl vinyl ether-alt-maleic acid) (PMVE-alt-MA-g-β-CD) and succinic acid modified dextran (Dex-SA) were first prepared, and then PMVE-alt-MA-g-β-CD was further used as the cross-linking agent to cross link Dex-SA for the formation of dextran hydrogel microspheres by using an inverse suspension polymerization method for the potential interventional embolization. The average diameter of the dextran hydrogel microspheres was 35 μm with 90% ranging from 20 μm to 50 μm. The obtained microspheres showed a rather high swelling rate and loading capacity of drug doxorubicin hydrochloride with content of 9.2 wt%. The results of *in vitro* experiments showed that about 35.5% of the total amount of the encapsulated doxorubicin hydrochloride can be released after 4 h at 37 °C. The microspheres had a good mechanical stiffness with Young's modulus of about 20.0 kPa. Iodine molecules (I_2) can be incorporated within the cavity of grafted cyclodextrin only through simply soaking in I_2 aqueous solution, and these I_2-loaded microspheres can preliminarily realize the function of CT angiography. This kind of dextran hydrogel microspheres with good biocompatibility would be a promising embolization material.

Keywords: β-Cyclodextrin, Dextran Hydrogel, Microspheres, CT Angiography, Embolization Agent.

1. INTRODUCTION

Hepatic artery embolization is often mentioned as the 'intervention,' which is to puncture the femoral artery first, under X-ray monitor, put the hepatic artery catheter into celiac artery or hepatic artery to realize angiography, determine the arterial blood supply of the liver cancer, then superselect catheterization to the hepatic artery or its branches, followed by the injection of chemotherapy drugs and embolic agents.[1, 2]

Hepatic artery embolization microsphere is a kind of new treating tool for middle-late liver cancer. Through using the selective artery intubation, the drug-containing microspheres were inputted to target tissue to block tumorous blood-supply arteries and slowly release drugs, so as to improve the local concentration of effective chemotherapeutic drugs and reduce the side effect of the body.[3, 4] The biggest characteristic of microsphere embolism is to break the old pure embolism which is the pattern of the lesion at blocking blood supply, and embolization combined with chemotherapy. It can reduce the blood flow to tumors and release drugs; At the same time, blocking the blood supply of tumor can cause cancer ischemic necrosis.[5–7]

Dextran microspheres not only has the common characteristics of biocompatibility, such as non-toxicity and degradation of metabolites *in vivo*, but also it possesses the convenience of preparation, advantages in wide material sources and low cost. At the same time, especially it won't have the antigenicity of protein material in the body after the application.[8–10] As a permanent peripheral inspection agent, dextran microspheres with uniform particle size and smooth surface can produce a uniform level of micro artery embolism, and can performance better than polystyrene microspheres, polyvinyl alcohol (PVA) microspheres or

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gelatin sponge.[11–13] Dextran microspheres can be kept for a long time in the suspension liquid, and there are no propensity of connection block and aggregation.[14,15]

The expansion of dextran microspheres in physiological saline is about 30%∼50%, *in vitro* it will continue to swell to twice its original size.[16] This is not enough, so we attempted to use the cross-linking agent poly(methyl vinyl ether-alt-maleic acid) to crosslink dextran. Poly(methyl vinyl ether-alt-maleic anhydride) is FDA approved polymer with advantages of biodegradability and low toxicity, and its physical and chemical properties is like polymer polyvinylpyrrolidone (PVP), which is a handful of synthetic compounds which are non-toxic and harmless to human or animals.[17,18] These products have good chemical stability, low toxicity, biocompatibility, cohesion.[19] So the new crosslinked dextran microspheres will be expected to have a very large expansion. β-cyclodextrin (β-CD) is non-toxic, commercially available compounds with low price, and their structures are rigid and well defined.[20,21] Most importantly, β-CD possesses a hydrophobic cavity that can bind various inorganic molecules in both aqueous solution and the solid state. In present work, β-CD was loaded into dextran microspheres to pack I_2 molecules as a contrast media in micro-CT.[22–24] The prepared dextran microspheres are expected to have lower cost, good biocompatibility and certain mechanical properties, nonfriability, capability of carrying anticancer drugs, and clinical imaging effect for embolization.[25–33]

2. MATERIALS AND METHODS

2.1. Materials

Poly(methyl vinyl ether-alt-maleic anhydride) (PVME-alt-MAH) (M_w = 1,080,000, M_n = 311,000, MDW = 3.47), Dextran 40 K (M_w = 40,000), iodine were purchased from Aladdin (Shanghai, China); β-cyclodextrin (β-CD), dimethyl formamide (DMF), *n*-hexane, and doxorubicin hydrochloride (Dox) were provided by Sinopharm Chemical Reagent Co., Ltd. Other reagents are commercially available.

2.2. Preparation of Modified Dextran

Under nitrogen atmosphere, 4.000 g dextran 40 K and 0.500 g catalyst 4-dimethylaminopyridine (DMAP) were dissolved in 20 mL dimethyl sulfoxide (DMSO), then mixed with 0.500 g succinic anhydride for 48 h at room temperature, then the clarified solution was washed by anhydrous ethanol for three times, the white precipitate was obtained by vacuum filtration, and then vacuum dried.[34–37]

2.3. Preparation of Dextran Microspheres

As shown in Figure 1, 1.560 g PVME-alt-MAH and 1.135 g β-CD were dissolved in 20 mL dry dimethyl formamide (DMF) under 80 °C to react for 8 h to decorate β-CD into the anhydride chains.[38] After the reaction, DMF was removed with a rotary evaporator to obtain

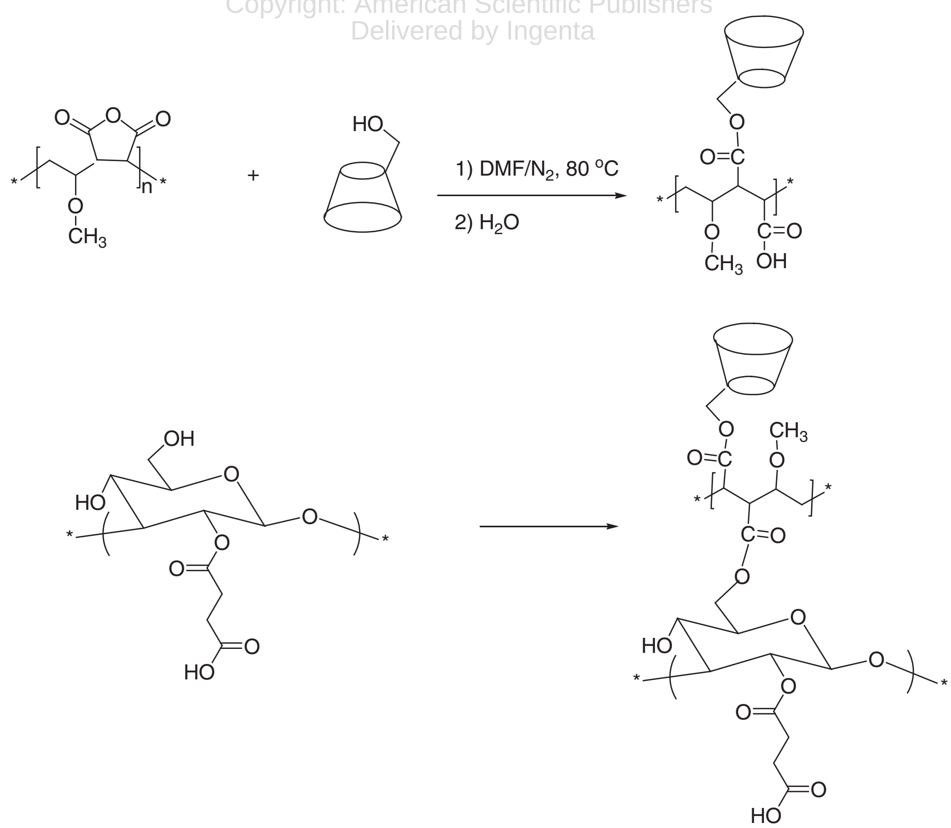


Figure 1. β-CD grafting of PVME-alt-MA and crosslinking with Dex-SA.

dry powder, 0.300 g powder was taken and dissolved in 10 mL of deionized water, 0.346 g modified dextran was then added. The above aqueous solution was used as the water phase, and 50 mL dimethyl silicone oil was used as the oil phase. The water phase was slowly dropped to the oil phase at 80 °C under mechanical stirring at 600 rpm, after 24 h reaction, the beaker was stewed for about 12 h, the upper clear silicone oil was abandoned, then a certain amount of *n*-hexane was added to clean the rest of the silicone oil for three times, then microspheres was dried under vacuum.[39] At last, the cross-linked dextran microspheres were obtained (Scheme 1).

2.4. Analysis of ATR-FTIR Spectra

The attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra were obtained using a Nicolet 5700 spectrometer (Thermo, U.S.A.) with a Wilks model 10 ATR accessory at an angle of 45° using a KRS-5 crystal. Spectra were recorded at 4 cm^{-1} resolution between 4000 cm^{-1} and 400 cm^{-1} and were the sum of 256 individual scans.

2.5. Morphological and Size of Microspheres

The morphology of microspheres was observed by using the optical microscope and scanning electron microscope (LEO, 1530VP, Germany). Microsphere size and size distribution was determined by fractionation using standard test sieves.

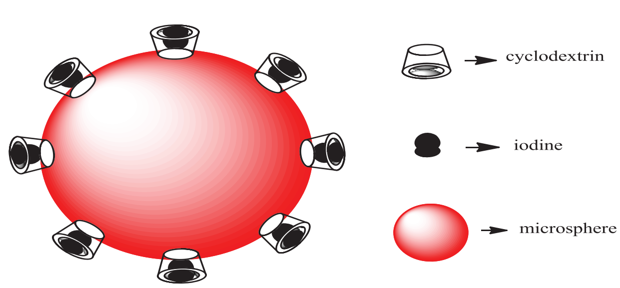
2.6. Swelling Degree of Microspheres

The volume expansion of microspheres was determined at equilibrium, after having placed the microspheres in water. The ratio of volume of the swollen beads (V_S) to the dried volume (V_D), measured by placing the microspheres in a graduated cylinder (12 mm i.d.), was defined as the swelling factor (q).

$$q = \frac{V_S}{V_D}$$

2.7. *β*-CD Cross-Linking Degree

The degree of cross-linking was estimated by a novel approach evaluating the amount of iodine retained by the



Scheme 1. Cross-linked dextran hydrogel microsphere loaded by iodine molecules by inverse suspension polymerization.

microspheres: briefly, 100 mg microspheres were soaked in 10 mL of 0.1 N solution of iodine in 10% (w/v) potassium iodide solution, and kept for 48 h under gentle stirring. After 48 h the equilibrium was reached and 5 mL of the iodine solution were taken up and assayed for the iodine content by titration with 0.1 N $Na_2S_2O_3$, in the presence of starch (1%, w/v) as the indicator.[40]

2.8. Internal Structure of Microspheres

SEM measurements of this kind of microspheres samples were performed to investigate their interior morphology. A certain amount of microspheres were first swollen to equilibrium in distilled water at room temperature, quickly frozen in liquid nitrogen thereafter, and further freeze-dried in a Freezone 6 freeze drier (LABCONCO CORPORATION, USA) under vacuum at −42 °C for at least 48 h until all the solvent was sublimed, after that, was put in the freeze dryer. After freeze-drying, the microspheres was sectioned in the middle, the observation of plane structure was performed by SEM.

2.9. Loading of Doxorubicin Hydrochloride

Doxorubicin hydrochloride was loaded into microspheres by drying and then the successive soaking procedures. One drying method, lyophilization, was employed to the microspheres. Dried microspheres were soaked in 0.4 mol · L^{-1} doxorubicin hydrochloride water solution for 24 h and the excess of solution was removed.[41] Doxorubicin hydrochloride-loaded microspheres were vacuumized at 45 °C till all the solvent in the microspheres was evaporated off, which caused doxorubicin hydrochloride loaded within the microspheres to crystallize. Doxorubicin hydrochloride-loaded microspheres were then rehydrated with a minimal amount of deionized water. Hydrated doxorubicin hydrochloride-loaded microspheres were obtained after removing the supernatant.

2.10. Drug Loading Level in Microspheres

The level of drug loading in microspheres was determined by spectrophotometric method.[42] 1 mL of hydrated doxorubicin hydrochloride-loaded microspheres was put in 1,000 mL of phosphate buffer solution (PBS, pH 7.4), which was stirred for 5 h to ensure a complete extraction of doxorubicin hydrochloride from the microspheres. The solution was filtered and assayed at the maximum absorption wavelength (480 nm) of doxorubicin hydrochloride by a UV spectrophotometer (UV-3600, Shimazdu). The blank microspheres were processed in the same way and no absorption was found at 480 nm. The concentration of doxorubicin hydrochloride was determined by comparing with a standard curve. Doxorubicin hydrochloride loaded in microspheres was expressed as the mass (mg) of doxorubicin hydrochloride-loaded in a unit volume (mL) of hydrated microspheres.

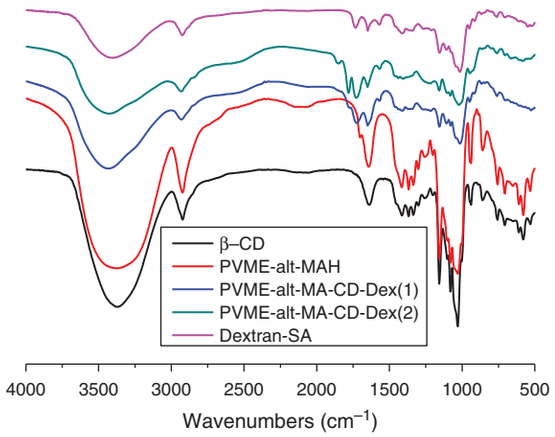


Figure 2. ATR-FTIR spectra of β-CD, PMVE-alt-MAH, Dex-SA and cross-linked dextran.

3. RESULTS AND DISCUSSION

3.1. Preparation and Characterization of Microspheres

Spherical microparticles were successfully synthesized by chemical cross-linking reaction of modified dextran and PVME-alt-MA-g-β-CD. Figure 2 shows the ATR-FTIR spectra of three samples. According to the previous report,[43] the absorption band of ester carbonyl is 1730 cm^{-1} while the absorption band of carbonyl in the carboxylic acid group is at 1708 cm^{-1} and at 1020 cm^{-1}, there is a β-CD characteristic peak, and these results indicates that the esterification reaction did occur with the addition of cross linker modified dextran. The IR measurement results are consistent with that reported previously.

The morphology of microspheres was observed by using optical and scanning electron microscopy (SEM). Microsphere size and size distribution was determined by fractionation using standard test sieves. From Figure 3, it was found that the diameter of the dextran microspheres was about 30 μm, the distribution of size is rather uniform, and the surface is very smooth. Figure 4 shows that there are full of holes inside the microspheres through the cut surface, so it proved that the microspheres were highly absorbent. Compared with other embolism microspheres, new crosslinked dextran microspheres were able to absorb a lot of water in the blood environment to fully fill the different diameter of the hepatic artery.

3.2. Inclusion Properties of Microspheres

After fully water swollen, the diameter of the microspheres changed from 30 μm to 100 μm, thus the volume was about 100 times than ever. 0.050 g of microspheres were added to 2 mL ultrapure water, after swollen fully and were centrifuged, the weight of the microspheres was 0.152 g finally, so we can calculate the swelling degree is about 300% ($q = 300\%$). Different diameter arteries could be blocked by the swelled microspheres. The elastic modulus of the swelled microspheres by the universal compression instrument was about 30,000 Pa, which could withstand the impact of the flowing blood. And this is very important in the practical application.

3.3. Grafting Degree of β-CD

The affinity of iodine with β-CD is a well-known phenomenon which has been studied extensively, β-CD cavity can accommodate iodine molecules were proved here. We adopt the way of reverse titration to measure the amount of β-CD carried by 100 mg microspheres. According to the calculation, the content of β-CD in microspheres is 52 μmol/g.

3.4. Micro-CT Radiography

It was reported in clinical hepatic artery embolism chemotherapy that the patients were femoral artery punctured under X-ray monitor, with the hepatic artery catheterization, followed by injection of chemotherapy drugs and embolic agents.[44] Surgeons need the CT to guide where was the embolism microspheres, so it is extremely necessary that the microspheres must have imaging effect. In this paper, we mixed the dextran microspheres and iodine in *n*-hexane and vibrated for 24 h, then centrifuged to get the microspheres. We took 0.200 g precipitation, added 2 mL ultrapure water, as the experimental group; at the same time, 0.200 g microspheres which were not soaked in the iodine solution, also added 2 mL ultrapure

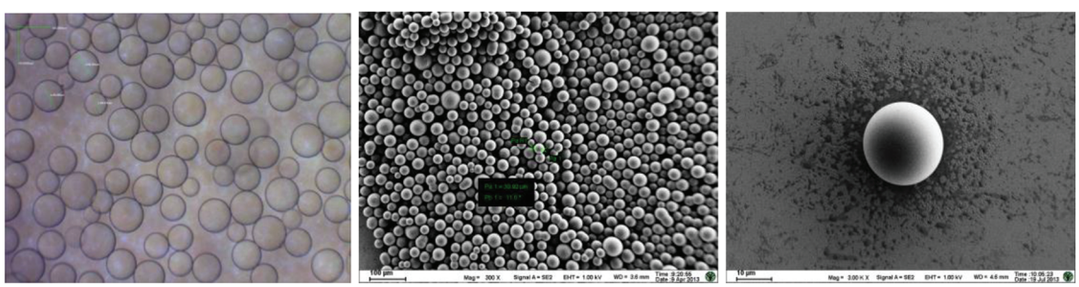


Figure 3. The optical image, SEM images of the microspheres (from the left to the right: optical image, SEM image, and a single microsphere SEM image).

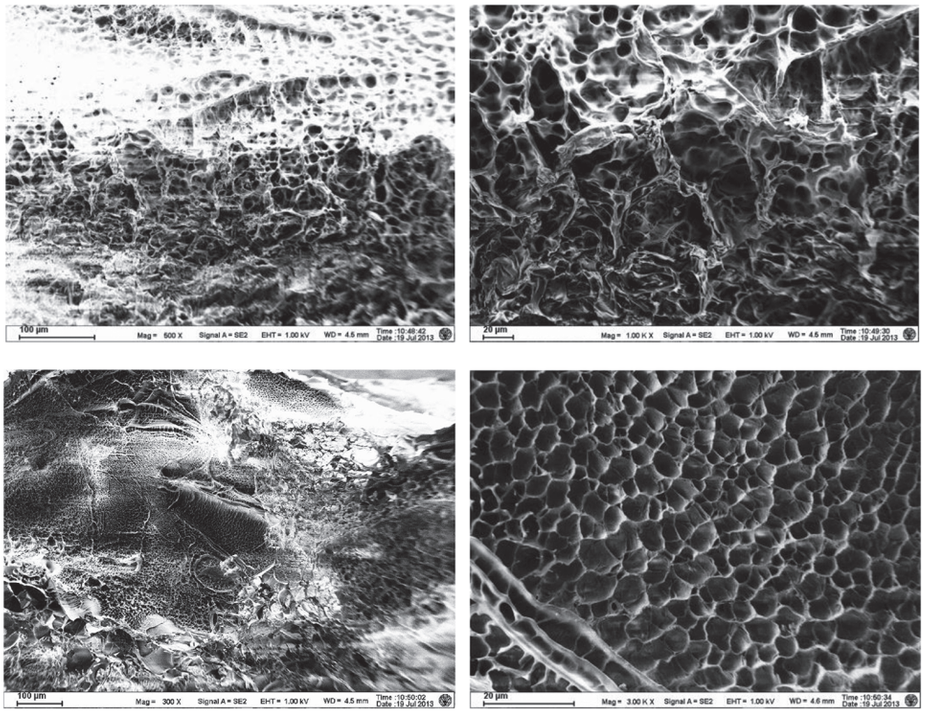


Figure 4. SEM images of the internal structure of microspheres by freeze-drying slice. Scale bars are 100 μm and 20 μm, respectively.

Figure 5. Micro-CT radiography of the microspheres. The left is the microspheres soaked in the *n*-hexane solution; the right is the microspheres soaked in the iodine *n*-hexane solution.

water, as the control group. Through the micro CT observation (Fig. 5), we found the microspheres soaked in the iodine solution had higher grey value then which was not. So this also indirectly reflects that the β-CD was modified on the microspheres.

3.5. Drug Release

The embolism blocked the blood transporting nutrients and oxygen, under this condition, the tumor was more sensitive to the chemotherapy drugs, at the same time, under the stimulus of chemotherapy drugs, the tolerance of tumor cells on hypoxia and ischemia was lower.[44] So in this paper we wanted to study the capacity of microspheres for the drug absorption and drug release. Doxorubicin hydrochloride can inhibit the cancer cell from synthesizing the nucleic acid; and has a broad spectrum anti-tumor drug for a variety of tumor cells. We performed the release test

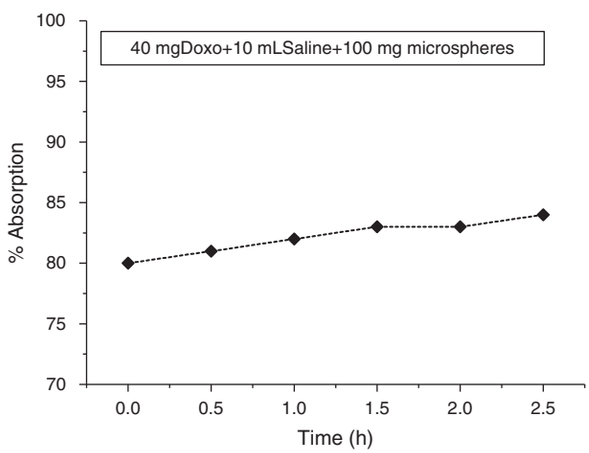


Figure 6. Loading of 100 mg of doxorubicin hydrochloride in 25 mg of microspheres.

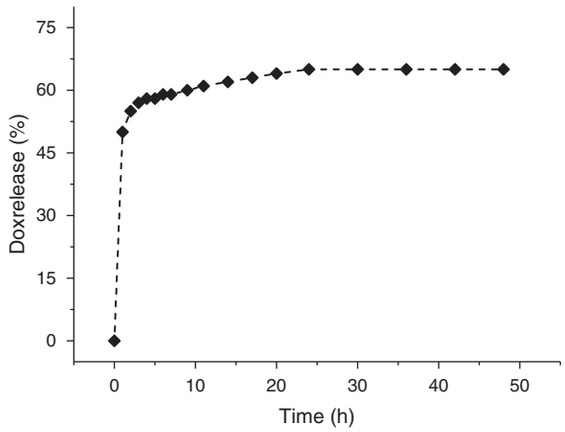


Figure 7. Cumulative release curve of doxorubicin hydrochloride from the microspheres *in vitro*.

of Dox in dextran microsphere. From Figure 6 we found that 25 mg cross-linked microspheres absorbed about 80% of the doxorubicin hydrochloride within 3 hours. The loading efficiency was far more than those which were not cross-linked. And from Figure 7, we could see that the cumulative release of doxorubicin hydrochloride from the microspheres *in vitro* dumped in the first two hours, and then slowly released in the next time. In the end, the drug release rate reached about 60% which can satisfy the needs of clinical medication.

4. CONCLUSION

In conclusion, PMVE-alt-MA cross-linked dextran gel microspheres were prepared by inverse suspension polymerization. It has a good swelling property which can adapt to all kinds of hepatic artery blood vessels, ensure the embolism of the cancerous part. It has a good mechanical property that can withstand the impact of the blood without broken. In addition, cross-linked dextran microspheres have the function of radiography, doctors can better track the location of the embolism in clinic treatment. The drug release test shows that the cross-linked dextran microspheres can be used as a drug carrier, and realize the slow release. The character of the cross-linked microspheres *in vitro* appears potential application for embolization. And, more study *in vivo* should be followed.

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