Preparation and Characterization of Magnetic Targeted Drug Controlled-Release Hydrogel Microspheres

Jie Chen,[1] Liming Yang,[1] Yinfeng Liu,[2] Guowei Ding,[3] Yong Pei,[1] Jian Li,[1] Guofei Hua,[2] Jian Huang[3]*

[1]Department of Chemical Engineering and Technology, Shanghai University (Jiading Campus), Shanghai 201800, China
[2]Department of Polymer Materials, Shanghai University (Jiading Campus), Shanghai 201800, China
[3]Tongji Hospital, Tongji University, Shanghai 200065, China

Summary: Magnetic targeted drug controlled release hydrogel microspheres were prepared by a radiation technique. Ferric oxide granules (size around 50 nm) were used as the core for magnetic target. The PVP ferrogels (ferromagnetic nanoparticles in hydrogel microsphere) were obtained by irradiating an emulsion of Poly (N-vinylpyrrolidone) PVP/ferromagnetic granule with cobalt 60 γ–ray. The morphology of the PVP ferrogel was studied by both optical and electronic microscopy, respectively. A broad-spectrum anticancer drug, Bleomycin A5 Hydrochloride (BLM), was immobilized in the ferrogel and the release property of the drug in vitro was studied. The function of targeting and anti-cancer was studied on the New Zealand White rabbits, based on the implantation of experimental VX2 squamous cell carcinoma in the auricles of the rabbits.
Keywords: Bleomycin; hydrogel; magnetic particle; microsphere; PVP; VX2

Introduction

It is well known that the systemic toxicity is usually a serious problem for the anticancer drugs. Controlled drug delivery system is supposed available for keeping the drug concentration in blood with lower toxicity but higher effectiveness. In recent years, microspheres have been proposed for the treatment of many diseases needing a constant drug concentration in the blood or drug targeting to specific cells or organs [1, 2]. Ferrogel, a chemically cross-linked polymer network swollen by a ferrofluid, has been successfully carried out [3]. Ferrogel is a new material with magnetic nanocrystals, embedded in a flexible polymer network, that provide high magneto-elasticity. This property makes the material applicable as a drug carrier. Ideally, such materials bear on their surface or in their bulk a pharmaceutical drug that can be driven to the target organ

 DOI: 10.1002/masy.200550706

and released there [4-7]. Experiments performed in vivo on the toxicity of magnetite and ferrogel have demonstrated that both have low toxicity and little adverse effect [7,8].

In this work, a ferrogel consisting of a magnetic core and a polyvinyl pyrrolidone (PVP) hydrogel shell was prepared by irradiating the emulsion of ferromagnetic/PVP aqueous solution mixture in n-heptanes. An anticancer drug, Bleomycin A5 Hydrochloride (BLM), was immobilized into the ferrogel by a swelling method. Experiments in vitro and in vivo were performed.

Experimental

Materials

Bleomycin A5 Hydrochloride (BLM) for perfusion was purchased from Bolai Pharmaceutical Company, LTD (China). Polyvinyl pyrrolidone (PVP K-30 was reagent grade) was purchased from BASF (Germay). Other chemicals used were of analytical grade, and purchased from Shanghai Reagent Company. They were used without further treatment. New Zealand White rabbits (2.5 ~3.0Kg) were purchased from the Experimental Animal Center of Tongji University.

Preparation and Characterization of the Ferro-Magnetic Nano-Particles

The preparation and characterization of the ferro-magnetic nano-particles were described in our previously published paper [8].

Synthesis of Ferrogel

The magnetic hydrogel microsphere was synthesized by irradiating an emulsion of PVP/(ferro-magnetic nano-particles) with γ -ray. n-Heptane was used as continuous phase. Span-80 5% (w/w related to n-heptane) was used as emulsification agent. The water phase was a mixture of 10% w/w PVP and about 20% w/w ferric oxide. The reversed emulsion was prepared by dropping the water phase into the oil phase, with high-speed stirring. Stirring continued for half an hour after the dropping. The emulsion was irradiated by Co-60 γ -ray, with a dose of about 25kGy in a moving state. After the irradiation, the oil face was removed by washing with acetone, ethanol and water, respectively. Finally the swelled magnetic hydrogel microsphere was lyophilized.

Morphology

The morphometrics of both the magnetic ferric oxide particles and the ferrogel were observed by an optical microscopy (BEX-60 system, JVC) and a transmission electron microscope TEM (H800 HITACH, Japan).

Determination of Compositions

The fraction of PVP and ferric oxide of the ferrogel was determined by TG-DTA/DSC apparatus (STA409 PC, NETCH, Germany). 10 mg of the dried ferrogel sample was placed in an aluminum oxide pan and heated at a rate of 6℃/min, from 20℃ to 400℃. A nitrogen purge through the sample chamber was implemented to obtain a more uniform and stable thermal environment. The un-immobilized ferric oxide was removed by soaking the sample in a 10% aqueous solution of HCl before the determination.

In Vitro Release

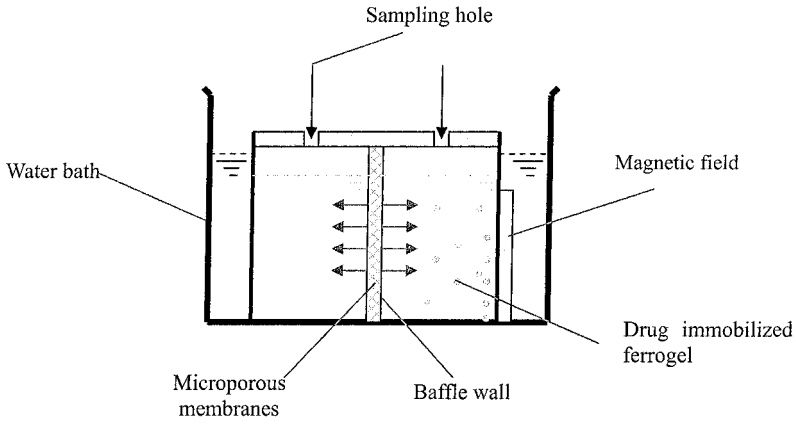


Fig. 1. Schematic diagram of the device used for drug release test.

An anticancer drug, Bleomycin A5 Hydrochloride (BLM), was used for evaluating the drug release properties of the ferrogel. 0.05g dried ferrogel was pre-swelled in 1 mL drug solution with a certain concentration (1 mg/mL, 3 mg/mL or 5 mg/mL), and was sonicated for 0.5 h. After 12 h, the drug was supposed to have been immobilized. The drug containing ferrogel was dispersed by adding 1 mL normal saline and then injected into 50

mL normal saline in a test device (as shown in Fig. 1) within a water bath at 37(±1)℃. Every 5 min 5 mL solution in the device was changed with normal saline. The drug release property was evaluated by determining the drug concentration in the device every 5 minutes. For the comparison, same amount of drug was injected in the 50 mL normal saline in the test device directly and the same procedure was repeated. The drug concentration was determined by recording the absorbance in a by UV–visible spectrophotometer (Angelent 100, USA) at the wavelength of 291, according to the standard curve of the drug concentration related to the absorbance at the same condition.

Animal Examination

Rabbits were divided into five groups, depending on the type of treatment, according to the literature of Dr. Van Es [11]. Group 1 received an i.a. infusion of BLM alone, without the immobilization, at the dose of 1.0 mg. Group 2 received an i.a. infusion of ferrogel (ferromagnetic hydrogel) microspheres (50 mg) alone, with the magnetic field of 0.5 T. Group 3 received an infusion of the drug-immobilized microspheres, which also contained BLM 1.0 mg and gave an outside magnetic field focus on the tumor. Group 4 received an infusion of the drug-immobilized microspheres, which also contained BLM 1.0 mg but no outside magnetic field. Group 5 was the control group without any treatment.

After treatment, the tumor area was measured daily, by the same observer, with a calliper ruler (measurement scale, 0.02 mm) for several days, using the formula of $S=a\times b$ (a=width on the horizontal axis and b=length on the vertical axis). We considered change of areas as percentages of tumor areas (100%) found at day 0 (day of treatment).

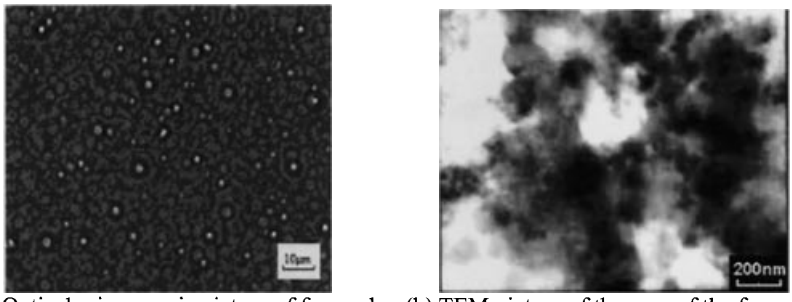
Periodically, local vein blood was taken out to determine the drug concentration. The drug concentration in the blood was determined according to the method of Shenoy [12].

Results and Discussion

Morphology

The ferrogel was observed by an optical microscope (OM) and a transmission electron microscope TEM, respectively. Fig. 2 shows the morphology of the ferrogel (a) and the ferromagnetic particles in it (b). It seems that the ferrogel dispersed homogeneously and

was global, with an average particle size of about 5 μm. It is also be seen that one hydrogel cell contained many ferromagnetic nano-particles.



(a) Optical microscopic picture of ferrogel (b) TEM picture of the core of the ferrogel

Fig.2. The morphology of ferrogel pictured by optical microscopy and TEM.

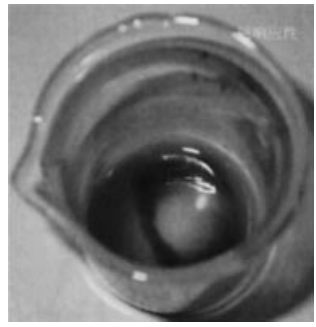


Fig. 3. The magnetic responsibility of ferrogel.

Fig. 3 shows the magnetic responsibility of the PVP ferrogel under an outside magnetic field. It is clear that the ferrogel deposited around the magnetic field, which indicates a good magnetic responsibility.

Compositions

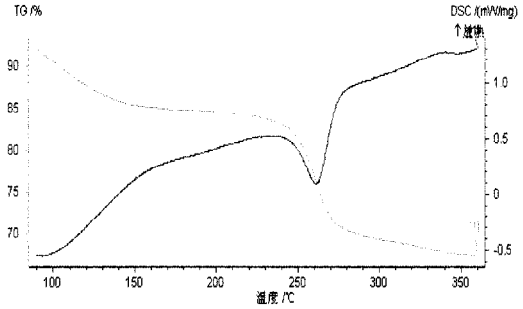


Fig. 4. Tg/DSC result of the ferrogel.

Fig. 4 shows the Tg and DSC results of the ferrogel (ferromagnetic-PVP hydrogel microspheres). The weight of the sample decreased between 230~270°. Meanwhile, the Tg/DSC indicated a heat adsorption peak near 255°. The results illustrated that the PVP decomposed near the temperature of 255°, which resulted in weight loss of the sample. On the other hand, the heat adsorption peak of DSC also indicated that the decomposition temperature of the PVP was about 255°. From the difference in Tg, we can estimate that the ferrogel contained about 30% PVP and 70% ferromagnetic nanoparticles.

Drug Release Property

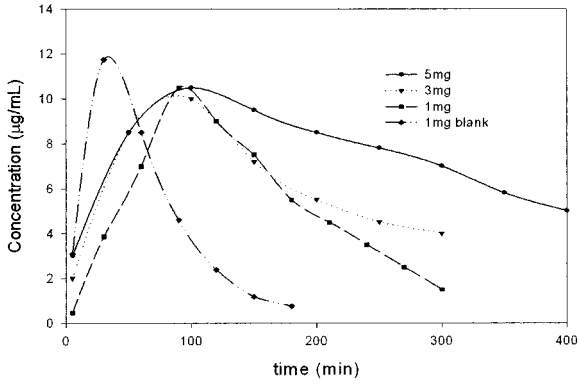
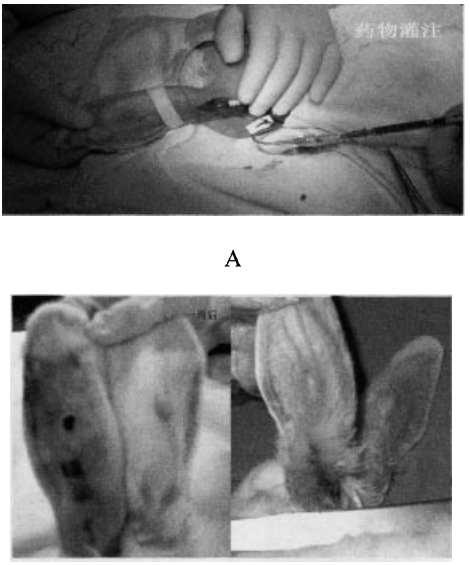


Fig. 5. Instantaneous release curve of BLM containing ferrogel at 37°C.

The drug release property of the BLM immobilized ferrogel was estimated by the determination of instantaneous drug concentration corresponding to the time, as shown in Fig. 5. The maximum concentration of the BLM appeared about 1 h after the injection, when it was in the un-immobilized state. However, it appeared 3 h later when BLM was immobilized in the ferrogel. The drug concentration decreased sharply after the maximum concentration in the un-immobilized state. The immobilized samples on the other hand, appeared there was a slower decrease of the drug concentration after the maximum value. The concentration of the drug remained relatively high for more than 8 h. This result also indicated the slower release function of the BLM contained ferrogel.



A

B C

Fig. 6. Pictures of animal examination. (A. drug injection at central artery; B. tumor morphology after a week with ferrogel; C. tumor morphology after a week without ferrogel).

A comparison of different treatments on New Zealand White rabbits is shown in Fig. 6. A is the picture of an injected intra-central artery. B and C show the tumor morphology with/without ferrogel. It was clear to observe that the surface of the tumor on the rabbit, which was injected with BLM with ferrogel, had been thanatosis and scabbed after a week. But that of the other, which was injected the same quantity of BLM without ferrogel, showed little difference.

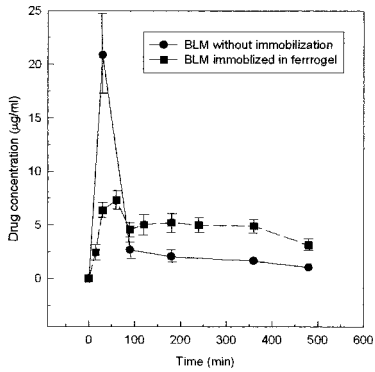


Fig. 7. Comparison of BLM concentration in blood after different treatment.

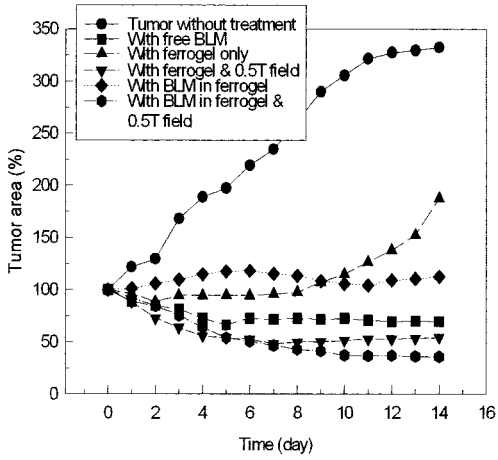


Fig.8. Area comparison of the tumor after different treatments.

Meanwhile, the blood drug concentration was determined after injection of 50 mg magnetic hydrogel microspheres immobilized 1 mg BLM. A comparison of blood drug concentration between the injection of free BLM and immobilized BLM is shown in Fig. 7.

It shows that the maximum concentration of the BLM appeared about 1 h after the injection, similar to the experiment in vitro. It remained at a relatively high concentration for more than 8h. This result also proved the slower release function of the ferrogel in vivo. Compared with the drug concentration of free BLM, we can see that the time of the maximum drug concentration and half-time was delayed, and the maximum drug concentration was obviously reduced.

From Fig. 8 we can see that after injection of 50 mg magnetic hydrogel microspheres which immobilized 1 mg BLM with an external magnetic field, this approach led to complete tumor remission. Without BLM, there was no obvious reduction of tumor area. Further, there was no obvious reduction without the magnetic fields. Under magnetic field, ferrogel containing BLM was fixed and slowly released BLM at the area of the tumor, which led to the increase of the drug concentration at that area and the drug concentration lasted for a certain long time. So, the anti-cancer effect of BLM was enhanced, and the side effect of the drug could be diminished due to the lower drug concentration in non-tumor area. Furthermore, ferrogel itself could participate in the process of cruor, and speed up the forming of thrombus in artery. Thus, the tumor was broken by the effect of ferrogel in restraining the growth of the tumor.

So this medicament offers an opportunity to treat malignant tumors locoregionally without systemic toxicity. Furthermore, it may be possible to use these magnetic particles as a "carrier system" for a variety of anticancer agents, such as radionuclides, cancer-specific antibodies, and genes.

Conclusions

1. Ferrogel with a particulate size of about 5 μm was obtained by irradiating an emulsion of ferromagnetic particles/PVP aqueous solution mixture in n-heptane.

2. The ferrogel obtained appeared to have a good magnetic response and provide slow release of drug.

3. An obvious advantageous effect of the BLM immobilized ferrogel was observed on the healing of explanted tumor.

Acknowledgments

The authors thank the Shanghai Science and Technology Committee for financial support for research. The authors also acknowledge the support of K. C. Wong educational foundation, Hong Kong.

[1] C. Rita, E. Elisabetta and N. Claudio, et al., *European Journal of Pharmaceutics and Biopharmaceutics* **1999**, 47, 153.
[2] S. Cammas, K. Suzuki and T. Okano, *J. Control. Relea.* **1997**, 48, 157.
[3] S. A. Gómez-Lopera, R. C. Plaza and A. V. Delgado, *J. Colloid Interface Sci.* **2001**, 240, 40.
[4] Gy. Török, V. T. Lebedev and L. Cser, et al., *Physica B* **2000**, 276-278, 396.
[5] S. Sershen and J. West, *Adv. Drug DeliveryRev.* **2002**, 54, 1225.
[6] V. Teixerica, I. Morfin and F. Ehrburger-Dolle, et al., *Composites Sci. Tech.* **2003**, 63, 1105.
[7] M. Zrínyi, L. Barsi and A. Büki, D. *J. Chem. Phys.* **1996**, 104 , 8750.
[8] Müler, R.H., Mssβen, S., Weyhers, H., *Int. J. Pharm.* **1996**, 138, 85.
[9] Iannone, A., Magin, R.L., Walczak, T., *Magn. Reson. Med.* **1991**, 22, 435.
[10] G. F. Hua, Y. F. Liu, Y. Ding, J. Li, J. Chen, J. Huang and G. W. Ding, *Proceeding of 03 National Workshop on Macromolecules in China*, Preprints, Hangzhou, 2003, 260.
[11] RJJ van Es, JF Nijson, AD van het Sohip, *Int. J. Oral Maxillofac. Surg.*, **2001**, 30, 407.
[12] D.B. Shenoy, R.J. D'Souza, N. Udupa, *J.Microencapsulation,* **2002**,19, 523.