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RESEARCH PAPER

A Floating Hydrogel System Capable of Generating CO_2 Bubbles to Diminish Urinary Obstruction After Intravesical Instillation

Tingsheng Lin • Yifan Zhang • Jinhui Wu • Xiaozhi Zhao • Huibo Lian • Wei Wang • Hongqian Guo • Yiqiao Hu

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ABSTRACT

Aim Intravesical instillation is commonly used to decrease the tumor recurrence after transurethral resection. However, most drug solutions would be eliminated from bladder after the first voiding of urine, so its clinical efficacy is limited. To overcome this obstacle, we developed a floating hydrogel system for controlled delivery of antitumor drugs.

Methods The floating hydrogel was made of Adriamycin, thermo-sensitive polymer (Poloxamer 407) and NH_4HCO_3, which was liquid at low temperature while forming hydrogel at high temperature. Meanwhile, at high temperature, NH_4HCO_3 decomposed to produce CO_2 bubbles, which helped hydrogel float in bladder without urinary obstruction.

Results The mixture containing 45% P407 and 6% NH_4HCO_3 was selected as the optimal formulation. At 37°C, the mixture formed hydrogel and produced many bubbles which could be observed by B ultrasound. The vitro study showed that the antitumor drug Doxorubicin was released in a controlled manner. After the mixture was instilled into rabbit bladder, it formed hydrogel and floated in the bladder. The bladder stimuli was reduced and antitumor drugs could be released continuously in the bladder.

Conclusion Our results suggested that the floating hydrogel was a feasible intravesical drug delivery system and may have application prospects in intravesical therapy for bladder cancer.

KEY WORDS bladder irritation symptoms · floating hydrogel · intravesical instillation · thermo-sensitive hydrogel · urinary obstruction

INTRODUCTION

Bladder cancer is one of the most common human cancers and thus has a profound impact on health care (1). In newly diagnosed cases of urothelial carcinoma of the bladder, approximately 75% present as non-muscle-invasive bladder cancer (NMIBC), and 25% present as muscle-invasive bladder cancer (MIBC) (2). Transurethral resection of the bladder tumor (TURBT) in combination with intravesical chemotherapy is considered the standard therapy for NMIBC. For transurethral resection alone, NMIBC recurs at a rate of 50–80% and has a 14% chance of progression to MIBC, so intravesical chemotherapy after TURBT is crucial for the treatment of NMIBC. For the NMIBC patients receiving postoperative intravesical chemotherapy, the time before first recurrence is significantly prolonged and the recurrence rate per year is reduced by nearly 50% (3).

Intravesical drug administration is commonly performed by instilling chemical drugs to bladder using a urethral catheter. It provides high drug concentration in bladder and

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T. Lin • Y. Zhang • J. Wu (✉) • Y. Hu (✉)
State Key Laboratory of Pharmaceutical Biotechnology
Nanjing University, 22 Hankou Road, Nanjing 210093, China
e-mail: wuj@nju.edu.cn
e-mail: huyiqiao@nju.edu.cn

T. Lin • X. Zhao • H. Lian • W. Wang • H. Guo
The Department of Urology, Nanjing Drum Tower Hospital
The Affiliated Hospital of Nanjing University Medical School
Nanjing 210093, China

J. Wu • Y. Hu
Jiangsu Key Laboratory for Nano Technology, Nanjing 210093, China

H. Guo (✉)
321 Zhongshan Road, Nanjing 210008, Jiangsu, China
e-mail: dr.guohongqian@gmail.com

simultaneously reduces systemic exposure. However, the chemical drug is usually kept inside bladder less than 2 h and is eliminated due to the bladder stimuli. The efficacy of intravesical instillation is determined by the drug residence time in bladder, therefore, a controlled delivery system for intravesical instillation is necessary (4,5).

In order to realize the control release and to prolong the resident time of drug in the bladder, nanoparticles carriers and mucoadhesive carriers were developed for intravesical drug delivery. Nanocarriers can form a variety of structures and can be formulated from materials including lipids, synthetic and biopolymers, proteins, metals, inorganic and organometallic compounds. These carriers could provide prolonged residence of drug in the bladder. Lu *et al.* used gelatin nanoparticles to deliver the hydrophobic drug paclitaxel (PTX) to bladder cancer. Results showed paclitaxel gelatin nanoparticles had favorable bladder tissue/tumor targeting and retention properties with pharmacologically active concentrations retained in tumors for at least 1 week (6). A study by Burjak used microspheres made of Eudragit polymer coated with different mucoadhesive polymers, such as chitosan, carboxy methylcellulose (CMC) and polycarbophil (PC). The use of microspheres increases the surface area of contact between the drug and the tissue. In addition, this adhesion to the mucous membrane slowed down the release of drug from the matrix, prolonging its residence time in the bladder (7).

Though the control release of drugs is realized, nanoparticles still disperse in urine homogenously, so most of them are washed out during the first voiding of urine. The repeated infusions of drug solutions justify the search for other carriers. A recent study was to use thermo-sensitive hydrogels as drug depots on the bladder wall (8–10). Due to the adhesion, hydrogels could attach to the bladder wall even after the urine voiding. Thus, the drug residence time in bladder was significantly extended. For example, Tyagi developed hydrogels with thermosensitive polymer (PEG-PLGA-PEG) as a vehicle for extending drug exposure in the bladder beyond the voiding of urine post instillation. Results showed that the hydrogel greatly prolonged the resident time of drug in bladder (8). Ke *et al.* presented a D/DMP-F system, wherein the deguelin-loaded DMP nanoparticles (D/DMP) were incorporated into a thermo-sensitive Pluronic F127 hydrogel. The system could significantly extend the hydrophobic drug residence time and increase the drug concentration within the bladder (9).

Nevertheless, due to the high viscosity of hydrogel, one serious problem is urinary obstruction. Gels adhering to the bladder may block the urinary tract, such as internal urethral orifice and ureteric orifice with narrow opening. As hydrogels attach to the bladder wall, another problem may be serious bladder irritation symptoms, just like blood clots in bladder (11). Therefore, despite that the hydrogel has many advantages in intravesical drug delivery, its application is limited due to these two shortcomings.

To overcome the critical defects arising from the hydrogel, a floating hydrogel delivery system was designed in our study. The hydrogel could float in urine by producing micro-bubbles, so it would not cause urinary obstruction and serious bladder irritation symptoms. In our study, poloxamer 407 was chosen as the matrix for preparing the drug loaded floating hydrogel. It is polyoxyethylene-polyoxypropylene-polyoxyethylene(PEOn-PPOn-PEOn) tri-block. The thermosensitive, biodegradable and non-toxicity characteristics of this tri-block copolymer have widely been reported (12–14). P407 solution exists in a sol state at room temperature or below but converts to a gel state at elevated temperature such as 37°C. P407 solution would form gel after injected into bladder. To make hydrogels float in urine, Ammonium bicarbonate (NH_4HCO_3) was incorporated into hydrogels as micro-bubbles producers. Ammonium bicarbonate is a raising agent commonly used in the food industry to generate gas bubbles in baked goods (15). It is relatively stable below 27°C but easy to decompose above about 37°C into ammonia, carbon dioxide, and water, in addition, the rate of decomposition increases with temperature (16). Thus, NH_4HCO_3 in hydrogel decomposed at body temperature, and generated bubbles inside which made hydrogel float in urine. Our study showed that this floating hydrogel may be an optimal carrier for Dox (Doxorubicin) to prolong the drug residence time in bladder.

MATERIALS AND METHODS

Materials

Poloxamer 407 (P407), Ammonium bicarbonate (NH_4HCO_3) and Doxorubicin (98%, chemical grade) were purchased from Meilun Biology Technology Company, Ltd. (Dalian, Liaoning, P.R. China). Twelve New Zealand white male rabbits, weighting on average 2000 g (range, 1800–3200 g) were obtained from the Experimental Animal Center, University of Yangzhou, China. All animal protocols were approved by Institutional Animal Care and Use Committee (IACUC) of Nanjing University.

Preparation and Characterization of Hydrogel

The non-floating hydrogel is the mixture of P407 and distilled water. To determine the optimal P407 concentration of the hydrogel, four solutions of 30, 35, 40, and 45% (w/v) P407 were prepared. The gelation temperature of the floating hydrogels was observed. The gelation temperature was the lowest temperature at which P407 solution coverts to gel. The tube inversion was used to determine the gelation temperature (17).

Preparation and Characterizations of Floating Hydrogel

To prepare the floating hydrogel, the powder of NH_4HCO_3 was added in the 45% P407 solution mentioned above. To evaluate the floating, the time to float of hydrogels with different concentrations of NH_4HCO_3 was recorded. To furthers optimize the floating hydrogel, the erosion time of floating hydrogel with 6% NH_4HCO_3 of different P407 concentrations and volumes were recorded respectively. The time to float was time needed for gel to float stably in water. The erosion time was time needed for gel to dissolved completely in water. The apparent viscosity was determined by using a NDJ-1 viscometer (Shanghai Balance Instrument Factory, Shanghai, China). Twenty milliliter of hydrogel solution were put in a 25 ml beaker and placed in water bath. Then the solution was heated at a speed of 1°C/min and the viscosity was recorded. The storage stability of the hydrogel of 45% P407 and 6% NH_4HCO_3 was also recorded. A digital timer (Thermo Fisher Scientific) was used for recording.

Incorporation of Dox in Floating Hydrogel

A calculated amount of P407 (45%) and NH_4HCO_3 (6%) were successively dissolved in Dox solution at 4°C to form a Dox-loaded floating hydrogel solution. The concentration of Dox was 0.05% (w/v).

Morphological Studies

Scanning electron microscopy was performed on hydrogels (the samples were plunged in liquid nitrogen and freeze-dried to maintain the porous structure without any collapse) to obtain information on the pore structure of hydrogels. The samples were mounted on the base plate and coated with gold. The morphology was investigated using a Hitachi (Tokyo, Japan) S-570 Scanning Electron Microscope.

Verification of Bubbles Produced in Hydrogel

As the temperature increased, NH_4HCO_3 would decompose to generate CO_2, NH_3 and H_2O. Bubbles produced in hydrogel with 45% P407 were detected by B ultrasound (B/K, Denmark). The hydrogels with and without NH_4HCO_3 at different temperatures were also detected by B ultrasound respectively.

Release Study *In Vitro*

The release study was performed in the dissolution tester (ZRS-8G, TDTF, China). The PBS (pH=7, 400 ml) in the beaker of dissolution tester was heated to 37°C. Four groups of Dox-loaded floating hydrogels (5 ml) with 6% NH_4HCO_3 and different concentrations of P407 were injected into solution, respectively. At predetermined time points, 4 ml solution was collected and substituted with the same volume of fresh solution. The amount of Dox was determined by UV spectrophotometry at 480 nm.

Verification of Hydrogel Floating *In Vivo*

Rabbit was anesthetized with pentobarbital (30 mg/kg, intravenous injection), Dox loaded floating hydrogel was intravesically instilled into bladder using a catheter. The floating hydrogel in bladder was detected by B ultrasound.

Release Study *In Vivo*

The rabbits were randomly selected. They were maintained in a controlled atmosphere of 12 h dark/light cycle, 22±2°C temperature and 50–70% humidity, with free access to pellet feed and fresh tap water. The animals were supplied with dry food pellets commercially available.

In fact, the gel solution was stored at 0°C condition before injected to bladder and the viscosity of 45% P407 was about 400 mPa.s at this temperature. The viscosity was low. Therefore, it is easily to inject the gel solution from the catheter to bladder. Rabbits were intravesically instilled with 5 ml normal saline, free Dox, Dox-loaded floating hydrogel and Dox-loaded non-floating hydrogel solution respectively. The Dox-loaded non-floating hydrogel solution did not contain NH_4HCO_3. All animals were fastened on a desk. Intravesical administration was accomplished using a catheter (9F) inserted into the bladder through the urethra. Urine samples were collected. The concentration of Dox was determined by a fluorescence microplate reader. The frozen section was immediately prepared after isolating rabbit bladder tissues. The fluorescence in the bladder section was observed using a Zeiss M2Bio fluorescence microscope.

RESULTS AND DISCUSSION

We successfully developed a floating hydrogel system for intravesical drug delivery. The floating hydrogel was composed of 45% P407 and 6% NH_4HCO_3. There are mainly three advantages:

(1) Both the gelation of P407 and the decomposition of NH_4HCO_3 were thermo-sensitive. 45% P407 changed from sol state to gel state immediately after injected into bladder. So this system could change to gel state immediately after intravesical instillation.
(2) The suspension of our hydrogel avoided of urinary obstruction and severe irritation. 6% NH_4HCO_3 contained in hydrogel decomposed into CO_2, NH_3 and H_2O at

body temperature, thus the micro-bubbles were generated in hydrogel and made hydrogel float in urine.

(3) The ingredients of our floating hydrogel were biologically safe. The drug delivery system was merely made of P407 and NH_4HCO_3. P407 is a biocompatible polymer widely used in medical and pharmaceutical purposes. It is suitable for drug delivery because of its low toxicity and weak immunogenic properties (18). In terms of biocompatibility studies *in vivo*, there were no damages observed in muscle cells after injection of 20% P407, and no signs of tissue injury, necrosis as well as severe inflammation in the microscopy examination (19). In this study, NH_4HCO_3 was used since it could decompose at normal body temperature (37°C). We have used the NaHCO3 for preparation of floating hydrogels in our previous study (20). However, we need to acidify the urine to provide enough acid environment (pH <5.6) for hydrogel floating in urine. Therefore, NH_4HCO_3 may be better than $NaHCO_3$ for preparation of floating hydrogels.

Preparation and Characterization of Floating Hydrogel

There are two parameters to evaluate the hydrogel formation and endurance time *in vivo*, the gelation temperature and erosion time, as defined in the method part. The gelation process must be accomplished in aqueous environment (urine) immediately after the gel solution injected into bladder in case of gel solution diluted by urine. The gelation process accelerates in the body temperature (37°C) along with the decrease of gelation temperature. Erosion time of hydrogel was also important for intravesical administration, because the release of drug ended once hydrogels totally dissolved in water. So the hydrogel was optimized to harbor a minimal gelation temperature and maximal erosion time.

At first, the optimal concentration of P407 was screened by increasing the P407 proportion from 30 to 45% (w/w) to record the gelation temperature and erosion time of non-floating hydrogels and floating hydrogels respectively. In addition, the floating hydrogels were designed to contain 6% NH_4HCO_3 tentatively for simplifying this optimizing process. As a result, the gelation temperature of non-floating hydrogels (without 6% NH_4HCO_3) decreased from 19.6 to 12.2°C, and the gelation temperature of floating hydrogels (with 6% NH_4HCO_3) decreased from 17.8 to 10.2°C (Fig. 1a). As the poloxamer concentration increased, the gelation temperature decreased. The gelation mechanism of P407 is based on micelles packing and entanglements (21). The P407 consists of a large population of micelles in aqueous phase. In addition, the number and volume of micelles were determined by the concentration of poloxamer. Therefore, as the poloxamer concentration increased, more micelles existed in aqueous solution causing gel formation in a lower temperature (22). The erosion time of floating hydrogels increased from 2 to 3.5 h with the concentration of P407 increased from 30 to 45% (w/v) (Fig. 1c). So we chose the 45% P407 as the hydrogel matrix for the following study.

To avoid urinary obstruction, the suspension performance of our floating hydrogel was optimized. The parameter we used to evaluate the floating was the time to float as defined in the method part. Gels containing 2% NH_4HCO_3 failed to float, besides, time to float of other gels containing 4, 6, and 8% NH_4HCO_3 were about 7, 2, and 2 min respectively (Fig. 1b). Time to float of gel containing 6 and 8% NH_4HCO_3 was similar. Therefore, 6% NH_4HCO_3 was selected as a pharmaceutical additive for floating hydrogel in our study. The process was recorded visually (Fig. 3).

The volume of hydrogels used in our experiments above was 5 ml, referring to the volume of rabbit bladder, but the empty human bladder was much bigger (about 50 ml). To evaluate the effect of gel volume, we recorded the erosion time of hydrogels with different volumes from 5 to 20 ml. The erosion time of floating hydrogel was prolonged as the hydrogel volume increased (Fig. 1d), increasing from 3.5 to 13.5 h as the hydrogel volume increased from 5 to 20 ml.

The viscosity of gels was increased as the concentration of P407 increased, and the sol-gel transition temperature of gels was decreased as the concentration of P407 increased (Fig. 1e). The viscosity of gels was increased as the temperature increased. When the temperature up to the sol-gel transition temperature, the viscosity of gels increase significantly and reached a maximum. The viscosity of floating hydrogel was slightly higher than non-floating hydrogel when the temperature under the sol-gel transition temperature (Fig. 1f).

The storage stability of the hydrogel was also tested. The hydrogel could be stored up to 6 months without significant change of the gelation. The drug entrapment efficiency was 100% due to the gelation of hydrogel.

The gel morphologies were investigated by SEM. Results were showed in Fig. 2. The morphologies of non-floating hydrogel (45% P407) and floating hydrogel (45% P407 with 6% NH_4HCO_3) were all porous. Micro-bubbles produced by NH_4HCO_3 in floating hydrogel could be seen in gel networks.

Verification of Bubbles and Floating of Hydrogels *In Vitro*

We developed the floating hydrogel based on hypothesis that NH_4HCO_3 can decompose to ammonia, carbon dioxide and produce bubbles in the surface of the hydrogel to float it, and the bubbles amount rises with temperature. To support the assumption directly, we recorded the floating hyrogel images

(Fig. 3) and captured images of bubbles produced by floating hydrogels with B ultrasound (Fig. 4).

The hydrogels changed from solution state to gel state with temperature increasing from 0 to 37°C (Fig. 4a and c). No bubble was produced in hydrogel without NH_4HCO_3 at 0, 25, and 37°C as we expected (Fig. 4b). For the hydrogel containing NH_4HCO_3, no bubbles observed in the hydrogel at 0°C and only a small amount of bubbles produced in hydrogel at 25°C, but a large amount of micro-bubbles were observed at 37°C (Fig. 4d). The data manifested that the

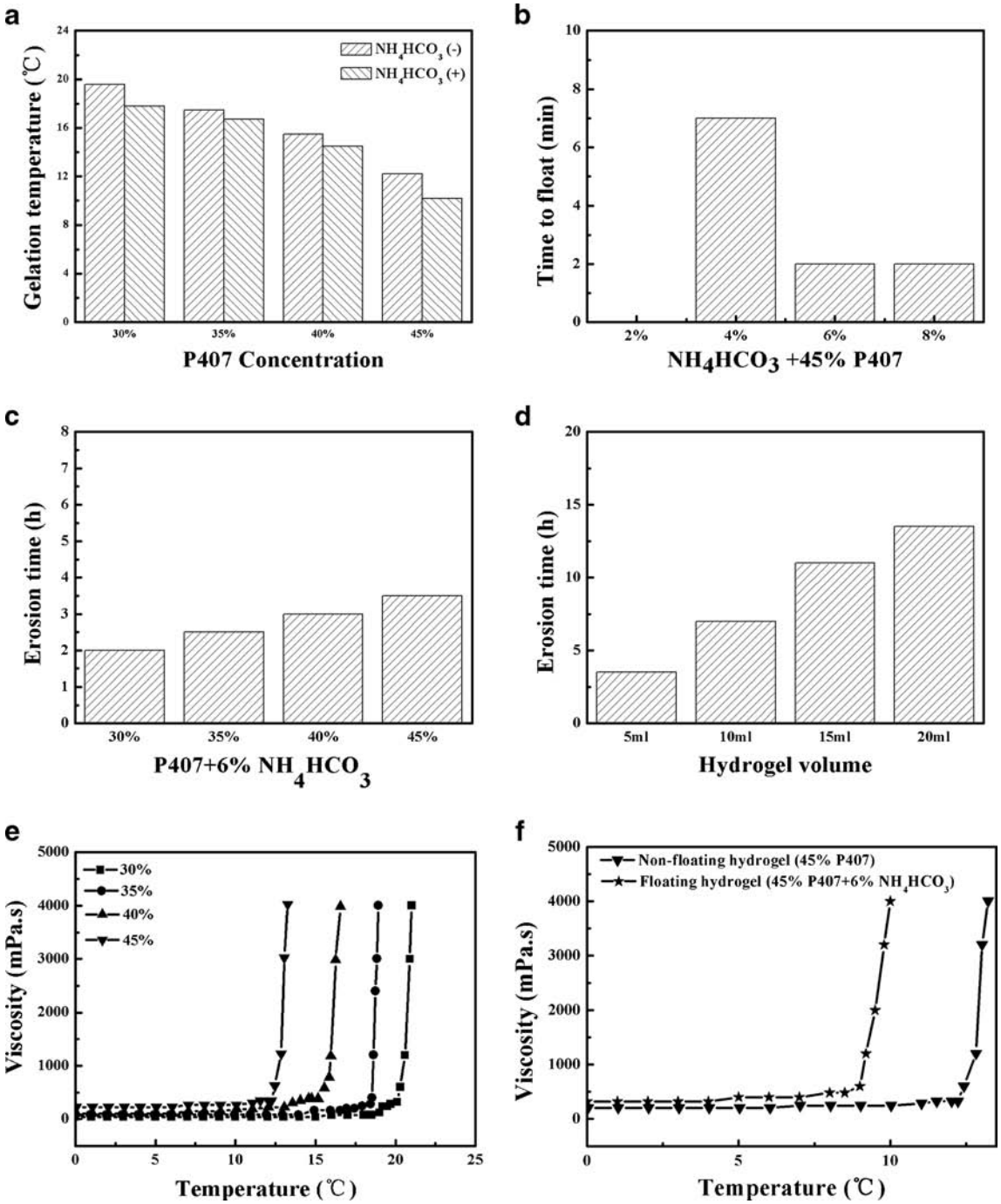

Fig. 1 Optimization of hydrogel to get a minimal gelation temperature, minimal time to float and maximal erosion time ($n = 2$). (**a**) Gelation temperature of non-floating and floating hydrogel (with 6% NH_4HCO_3) with different concentration of P407, (**b**) time to float of floating hydrogel with 45% P407 and different concentration of NH_4HCO_3, (**c**) erosion time of floating hydrogel with 6% NH_4HCO_3 and different concentration of P407, (**d**) erosion time of floating hydrogel with 6% NH_4HCO_3, 45% P407 and different hydrogel volume. (**e**) The viscosity of non-floating hydrogel with different concentration of P407. (**f**) The viscosity of non-floating and floating hydrogel with 45% P407.

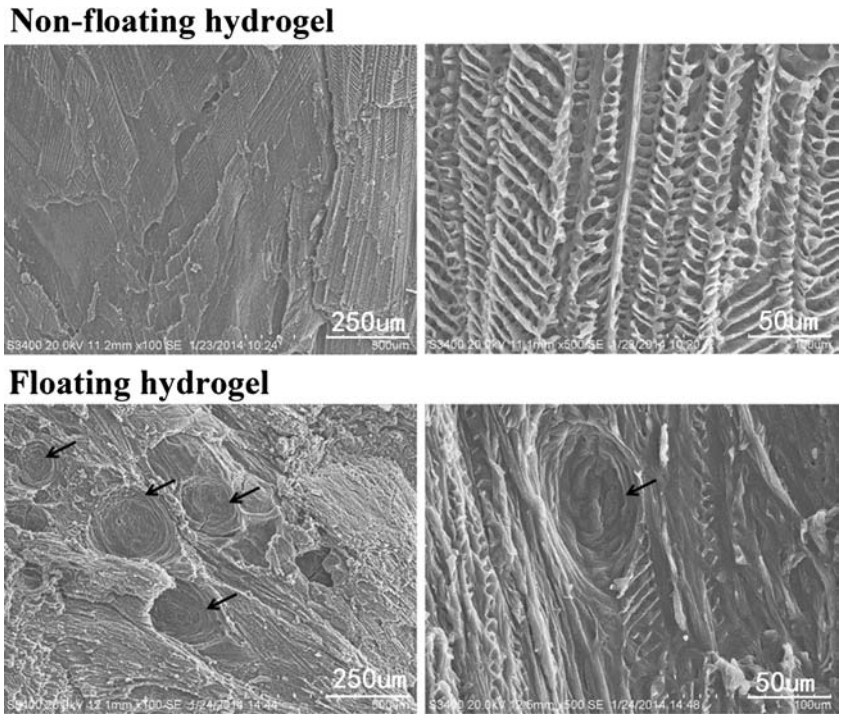

Fig. 2 Scanning electron microscopy images of non-floating hydrogel and floating hydrogel. Microbubbles were pointed by *black arrows*.

bubbles did exist to make the hydrogels float, and the body temperature is ideal for the functioning of floating hydrogels.

Release Study *In Vitro*

The release of drug ended with the complete dissolution of hydrogels, therefore, the erosion time of hydrogels is a key factor of drug release (23). Release behavior of Dox-loaded floating hydrogels with 6% NH_4HCO_3 containing different concentrations of P407 *in vitro* was investigated (Fig. 5). The curves depicted the cumulative amount of Dox released as a function of time. The control (free Dox) dispersed immediately to form homogenous solution in water, and the cumulative release reached 93.6% in 0.5 h after injection. While after injecting gels with different concentrations of P407 into water, floating hydrogels could float on the surface of water. After 2, 2.5, 3, and 3.5 h, almost the total amount of Dox was released from gels with 30, 35, 40, and 45% P407, respectively. As the concentration of P407 increased, the erosion time of floating hydrogels was prolonged. Therefore, the floating hydrogel containing 45% P407 had the best sustained release.

As the P407 consists of a large population of micelles in aqueous phase, the incorporated drug may be released by diffusion through gel matrix. Drug release correlates well with P407 dissolution and the drug release is controlled by erosion of the gel (23). We found that the Higuchi model ($Qt = KH\sqrt{t}$) (24) could predict the release from gel perfectly in which the release constant (K_H) is 52.32 and the correlation coefficient (R) is 0.9982, respectively. It indicated that drug release from gels was a controlled-release process and square root time dependent.

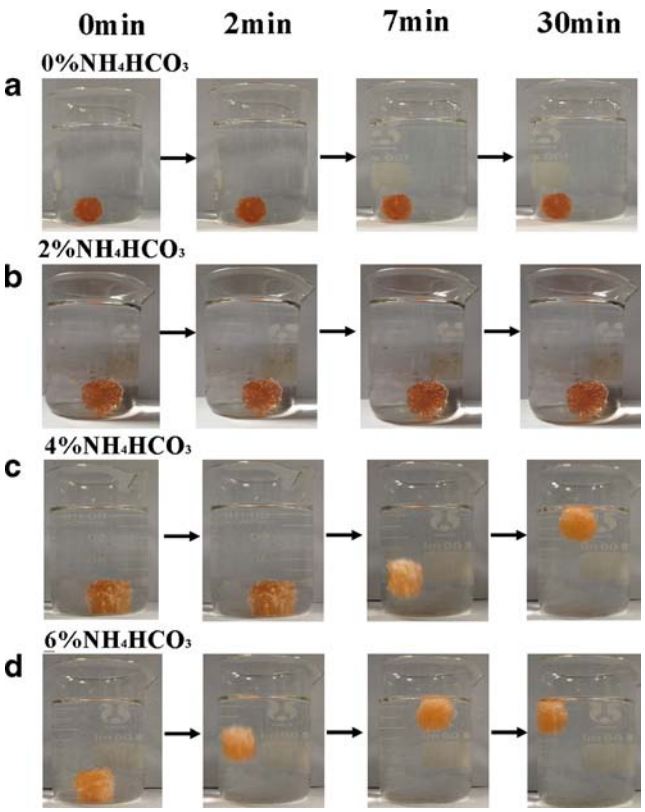

Fig. 3 Images of floating hydrogel with different concentration of NH_4HCO_3 from 0 to 6% (**a**) to (**d**).

Verification of Hydrogel Floating *In Vivo*

We evaluated the floating of hydrogels in rabbit bladder. Thermo-sensitive gels were injected into rabbit bladder using catheter. Hydrogels floating *in vivo* were confirmed by B ultrasound (Fig. 6). The white dashed curves in the pictures represented the rabbit bladder wall, and the white dashed circles represented gels in rabbit bladder. The floating of floating hydrogel was shown (Fig. 6a). The floating hydrogel was solution state at low temperature *in vitro*, but changed to gel immediately after intravesically injected to bladder, and the hydrogel did not float in the beginning (Fig. 6a-2). About 2 min later, the hydrogel stably floated in urine (Fig. 6a-3). Figure 6b showed the floating of hydrogel without NH_4HCO_3. The hydrogel could not float in the rabbit bladder (Fig. 6b-2 and b-3). Figure 6c showed B ultrasound images of rabbit bladder after intravesical instillation of free Dox. More information about the floating process could be seen in the video (Suppl.).

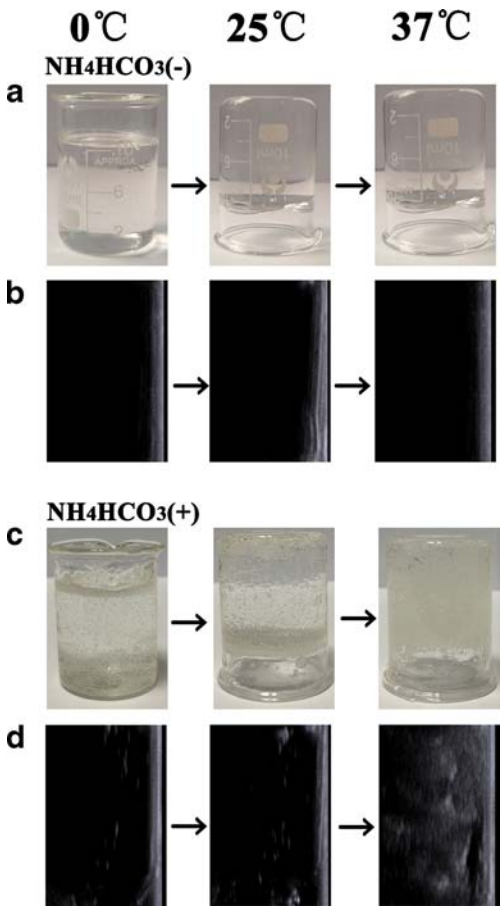

Fig. 4 Gelation and bubbles of non-floating and floating hydrogel. (**a**) Gelation of non-floating hydrogel, (**b**) bubbles produced by non-floating hydrogel recorded by B ultrasound, (**c**) gelation of floating hydrogel, (**d**) Bubbles produced by floating hydrogel recorded by B ultrasound.

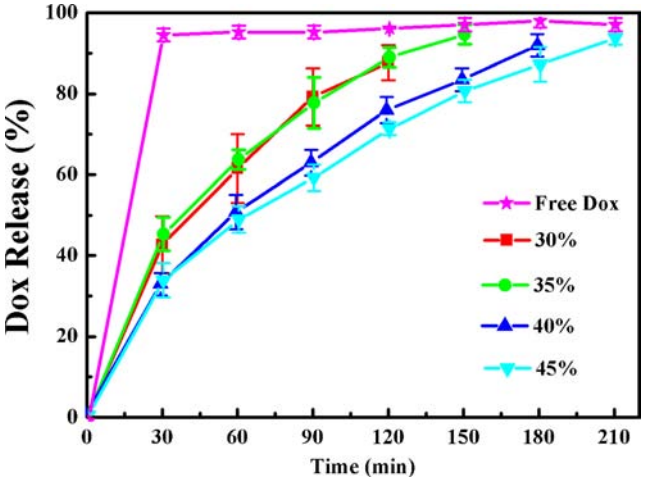

Fig. 5 Cumulative release of free-Dox and Dox-loaded floating hydrogels with different concentrations of P407 (30~45%).

Release Study *In Vivo*

We measured the Dox release rate of non-floating and floating hydrogels, compared with free Dox *in vivo* (Fig. 7a). After intravesical instillation of free Dox, the drug concentration in bladder reached the peak immediately, but dropped sharply after the first voiding of urine. Because of the irritation problem, most non-floating gels were expelled from bladder during the first voiding of urine 10 min after injection, with

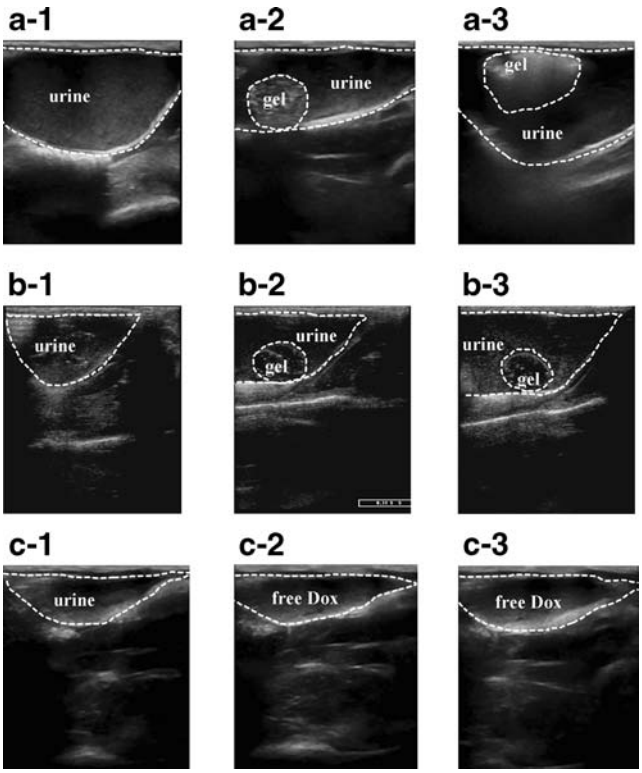

Fig. 6 B ultrasound images of floating hydrogel, non-floating hydrogel and free-Dox in rabbit bladder. (**a**) Floating hydrogel, (**b**) non-floating hydrogel, (**c**) free Dox.

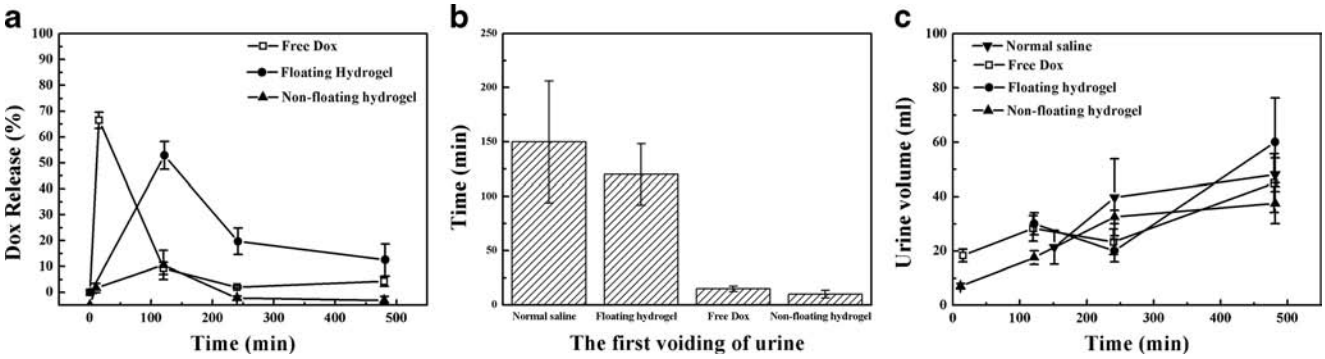

Fig. 7 (**a**) Dox release of floating hydrogel, non-floating hydrogeland free Dox, (**b**) time of first urination of normal saline, floating hydrogel, free Dox and non-floating hydrogel group, (**c**) urine volume of normal saline, floating hydrogel, free Dox and non-floating hydrogel group (Mean ± SD, $n = 3$).

only a few remained. In contrast, the intravesical delivery of floating hydrogel showed sustained release of Dox from gel. The amount of Dox released from floating hydrogel reached 52.75% before the first voiding. After voiding, floating hydrogel remained releasing, reached 19.61% before the second voiding, and 12.47% of Dox was released before the third voiding. Compared to free Dox and non-floating hydrogel, floating hydrogel could significantly extend the residence time of Dox in bladder and hence increase the bioavailability.

To assess the degree of bladder irritation that floating hydrogels caused, the time of first urination was recorded (Fig. 7b). The first time of urination was about 15, 10, 120, and 150 min after intravesical administration of free Dox, non-floating hydrogel, floating hydrogel and normal saline, respectively. This indicated that the free Dox and non-floating hydrogels had strong irritation to the rabbit bladder wall and caused rabbits to void their bladder. In contrast, the floating hydrogels could float in urine, thus the irritation to bladder wall was greatly reduced, indicated by the extended time of first urination of 120 min, almost close to the control (normal saline) of 150 min.

Hypothetically, the urine volume will be reduced if there is urinary obstruction, so we measured the urine volume of rabbit after intravesical administration of the four groups as mentioned above. In terms of the viscosity of non-floating gels, the urine volume should be less than the control. However, no significant difference among the floating, non-floating hydrogels and control group (Saline) was observed, because most non-floating gels were expelled from bladder during the first voiding of urine 10 min after injection (Fig. 7c).

We further investigated the residual amount of Dox in normal saline, free Dox, Dox-loaded non-floating hydrogel and Dox-loaded floating hydrogel groups in bladder (Fig. 8). Our purpose to design floating hydrogel is to increase the Dox concentration in bladder tissue, and the residual amount of Dox in the bladder tissue was proportional to the fluorescent intensity. The result showed that the fluorescent intensity of floating hydrogel group was much higher than free Dox and non-floating hydrogel, and no fluorescence of Dox could be

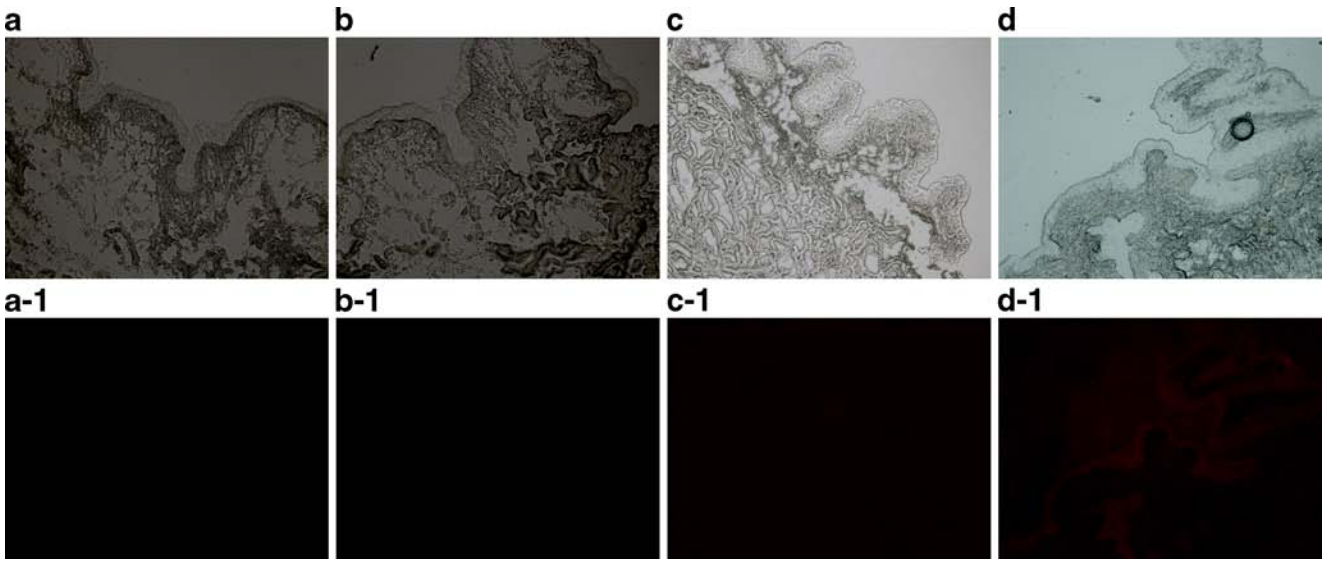

Fig. 8 Phase and fluorescence picture of frozen sections of bladder tissue to evaluate residual of Dox in the bladder. (**a**) Normal saline, (**b**) free Dox, (**c**) non-floating hydrogel, (**d**) floating hydrogel.

observed in the normal saline group (Fig. 8). Therefore, the efficiency of intravesical instillation could be raised by our floating hydrogel.

CONCLUSION

This paper presents a floating hydrogel intravesical drug delivery system with application prospects by incorporating gas-forming materials to P407 gel. This system not only inherits the advantages of hydrogels as biodegradability and thermosensitivity, but also remedys the defects of hydrogels as urinary obstruction and irritation symptoms due to the suspension. As a result, this floating system prolongs the drug exposure time to bladder tissue and therefore raises the efficiency. In further studies, we will use the animal bladder cancer model to investigate its efficacy.

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Tingsheng Lin and Yifan Zhang contributed equally to the article.

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