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Design of an "Active Defense" System as Drug Carriers for Cancer Therapy

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A novel intelligent "active defense" system that can specially respond to cancerous tissues for drug release was designed and prepared. The "active defense" system consists of a biodegradable dextran microgel core cross-linked by a Schiff's base and a surrounding layer formed by Layer-by-Layer (LbL) assembly. The loading and release of macromolecular model drug, dex-FITC, as well as antineoplastic drug, DOX, was investigated. The in vitro cell inhibition and drug release behavior of the drug delivery system were studied and the results showed that the entrapped drug could be explosively released from the microcapsules and thereafter taken up by cancer cells upon the trigger of the acidic environment around tumor tissues.

1. Introduction

Drug delivery approaches have a great influence on the efficacy of drug treatments.[1] Since traditional antineoplastic drugs can not discern between cancerous and healthy cells, various drug delivery systems (DDSs) have been proposed to realize selectively drug release at cancerous tissues. Among these DDSs, most of them have been focused on introducing targeting groups to drug carriers to realize the targeted delivery.[2–10] Although such devices are able to reach the targeted sites, the applications of these systems are limited by the unexpected sustained release of drugs in the process of blood circulation before arriving at the target sites. Due to the above deficiencies, it is of great importance to design intelligent drug carriers that can specifically respond to physiopathological signals and allow explosive release of the loaded drugs while entrapping the drugs efficiently during the process of blood circulation.

The development of satisfactory drug delivery systems which could explosively release the drug in response to environmental stimuli still remains a great challenge. For example, nano-explosions of nanoparticles for the sudden release of substances by a thermal treatment were reported by Landfester et al.[11] However, the method was not suitable for biological applications since it required a high temperature (above 100 °C). Self-exploding capsules that consist of a degradable core surrounded by a semi-permeable shell have been reported.[12–14] For these capsules, when the microgel core degraded, the swelling pressure caused leakage of the shell membrane to release its payload.[15] These systems, however, were limited by the base-driven requirement, which was difficult to be satisfied in biological systems. In our previous study, we also prepared microcapsules that could release the drug rapidly under physiological conditions upon addition of DTT.[16] Obviously, this need of external trigger is the limitation of this system.

There are many examples existing in nature that could explosively release of particular materials upon stimuli. A typical example is the well-known flower, balsamine, which can project ripe seeds to environment at the slightest touch.[17] Another example is the bombardier beetle which has the ability to eject a hot noxious chemical spray when threatened, initiated by a reaction between hydroquinone and hydrogen peroxide catalyzed by enzymes.[17] Inspired from these "active defense" behaviors in nature, in the current study, we designed and fabricated explosive microcapsules with the "active defense" ability, i.e. the "active defense" system could effectively entrap the drug in the blood and normal tissues whereas allow the explosive drug release under physiopathological stimuli once reaching the cancerous tissues.

It is well known that the pH around cancerous tissues is more acidic (pH 5-6) as compared with the blood and normal tissues,[18,19] which allows the adjusted drug release from the delivery devices that could respond to acidic environment.[20,21] Based on this characters of tumor tissues, we fabricated an "active defense" system consisting of degradable dextran containing C=N bonds as a microgel core and a LbL[22–25] membrane as a outer layer. The introduction of a Schiff's base to the system lies in the fact that the Schiff's base will hydrolyze in acidic environments. When the "active defense" system reaches the tumor tissue, the crosslinkages containing C=N bonds will cleave upon the hydrolyzation of the Schiff's base, leading to the increased swelling pressure of the core. When the swelling pressure exceeds that the membrane can resist, the surrounding LbL membrane will rupture, followed by an explosive release of the entrapped drug. In this study, we prepared larger microcapsules with a mean diameter of 20 μm because the large microcapsules require less pressure to explode compared with smaller ones.[26] In most literatures in this fields, only the

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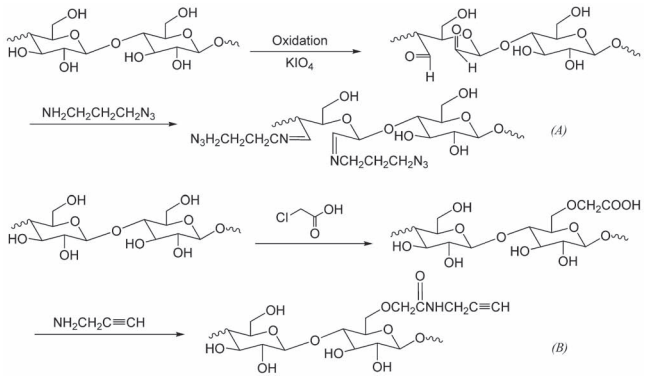


Scheme 1. Synthesis routes of (A) dex-C = N-N_3 and (B) dex-C≡C.

releases of macromolecular model drugs or nanoparticles were demonstrated.[12–14] Since many anti-cancer drugs are small molecules, we therefore, besides macromolecules model drug dex-FITC, also studied the explosive release of small molecules, anti-cancer drug doxorubicin (DOX) from the microcapsules. The results of drug release, cell viability and cell uptake experiments proved that our "active defense" microcapsules could specifically respond to **the** acidic environment of cancerous tissue and allow the explosive release of loaded drugs.

2. Results and Discussion

In the current study, the precursors polyaldehyde dextran (PAD) and carboxymethyl dextran (CMD) were synthesized via oxidation and substitution reactions of dextran, respectively. As shown in **Scheme 1**A, the pendant azide groups were introduced into the structure of PAD to obtain dex-C = N-N_3 via the reaction between the aldehyde groups of PAD and the amine groups of aminopropylazide. In this study, to ensure the complete reaction between the aldehyde and amine groups, aminopropylazide with a 1.2-fold molar excess was employed. From the FT-IR spectrum (Figure S1 in the Supporting Information), the signal located at ~2169 cm^{-1} indicates the presence of azide pendant groups. The ^{1}H NMR characterization of dex-C=N-N_3 was also performed (Figure S2 in the Supporting Information). It can be found that the signals corresponding to the methylene protons of aminopropylazide are located at ~1.76 and ~2.90 ppm, implying the success reaction between the aldehyde groups of PAD and the amine groups of aminopropylazide to form C=N bonds. Based on the ^{1}H NMR spectrum, the degree of substitution (DS) of azide functionality was calculated as 4%.

To prepare dex-C≡C, the pendant alkyne groups were incorporated into CMD via the coupling reaction between the amine groups of propargyl amine and the carboxyl groups of CMD in the presence of a coupling reagent EDC (Scheme 1B). From the FT-IR spectrum (Figure S3 in the Supporting Information), the typical absorbance of alkyne groups appears at ~2120 cm^{-1}. Base on the ^{1}H NMR spectrum of dex-C≡C (Figure S4 in the Supporting Information), the signal of alkyne proton (–C≡CH) located at ~2.6 ppm confirms the success of the reaction between propargyl amine and CMD. Herein, the DS of alkyne groups was calculated as ~20% as according to the ^{1}H NMR spectrum.

In this study, the cores of the microcapsules are crosslinked microgels. The microgel cores were fabricated by slowly mixing of an aqueous solution containing dex-C≡C, dex-C=N-N_3 and $CuSO_4$ and another aqueous solution containing poly(ethylene glycol) (PEG) to obtain a water-in-water emulsion due to the immiscibility of the two phases.[27] Then microgels with a three-dimensional network structure was formed through the click reaction between the pendant alkyne groups of dex-C≡C and the pendant azide groups of dex-C = N-N_3. Since the crosslinking degree of the microgels determines their degradation rate, the degree of azide functionalization of dex-C=N-N_3 was kept as low as 4% to endow our "active defense" system with the adequate response to acidic environments. The confocal laser scanning microscopy (CLSM) image of the dextran microgel core loaded with fluorescein isothiocyanate modified dextran (dextran-FITC) is displayed in **Figure 1**A. The image shows that dex-FITC is successfully encapsulated in the microgels and the average size

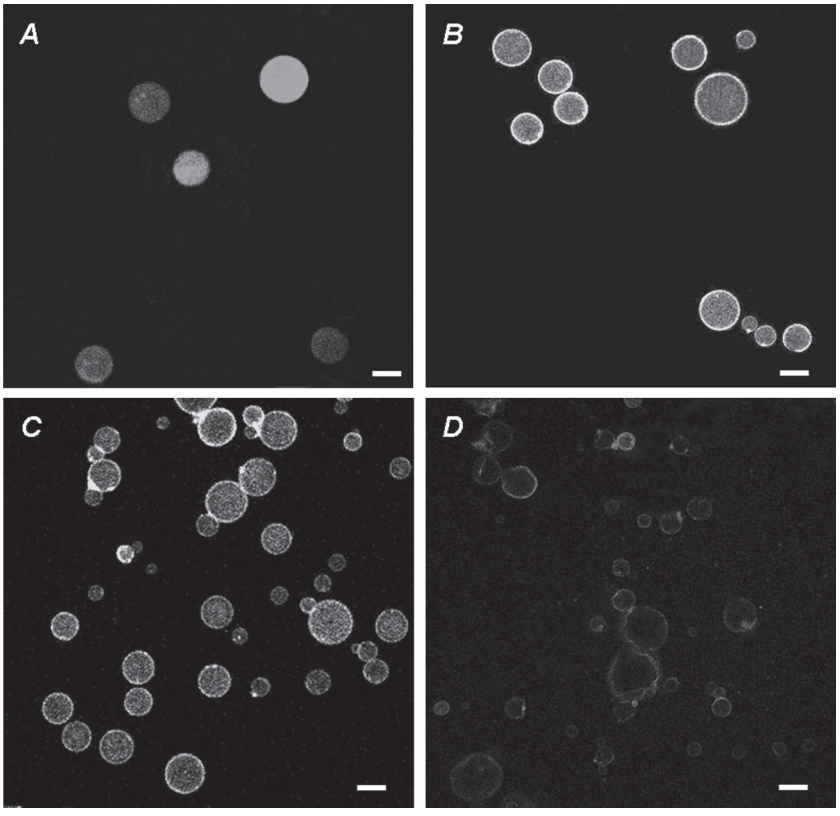


Figure 1. CLSM images of A) dextran based microgel core, B) microcapsules, and microcapsules after 24 h incubation in aqueous medium at pH 7.4 (C) and pH 5.5 (D) at 37 °C. (The scale bar is 20 μm.)

of the microgels is around 20 μm. To enable sequent LbL assembly, the microgel cores should have charged surfaces. Therefore, during the synthesis of dex-C≡C, only partial carboxyl groups of CMD (Scheme 1B) were reacted with propargyl amine to obtain the microgels with negatively charged surfaces. The residual carboxyl groups of the dextran chains enable the resultant microgels to hold a ζ-potential of –27.8 mV. Since the azide groups are connected to dextran backbone via C=N bonds, the degradation of the microgel core will occur in acidic environments.

To achieve the ability of explosive release of drugs, the dextran based microgel cores were coated with two bilayer LbL membrane. During the LbL assembly, rhodamine B labeled poly(allylamine hydrochloride) (PAH-Rb) was used as a polycation and polyaspartic acid (PASP) was used as a polyanion. The CLSM image of the LbL membrane coated microgel cores, the microcapsules, is shown in Figure 1B. It can be found that the surface of the microcapsule is a regular red ring, indicating the polyelectrolyte membrane is successfully coated on the microgel core.

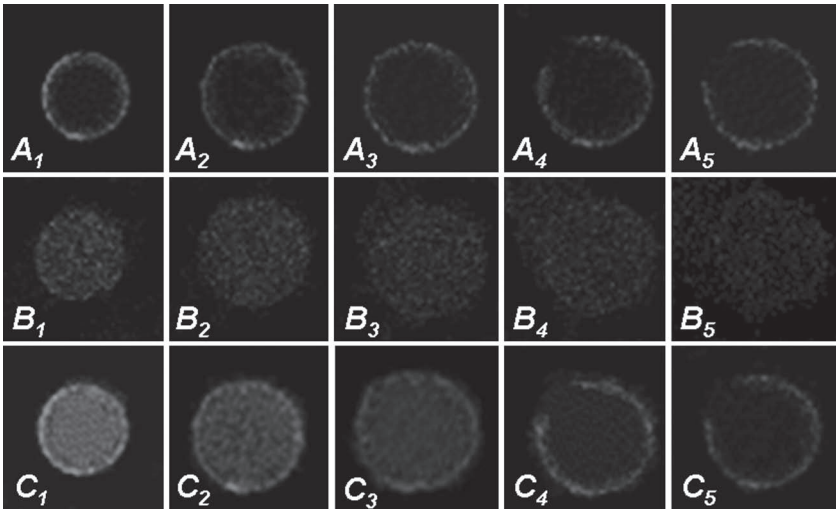


Figure 2. Snapshots of exploding process of the dex-FITC loaded microcapsules at pH 2 and 37 °C. A_1–A_5) The LbL membrane in the exploding process; B_1–B_5) The dex-FITC encapsulated in the exploding process; C_1–C_5) Overlap of A_1-A_5 and B_1-B_5. (The time interval of snapshots is 30 s)

To investigate the degradation behavior of the microcapsules, the microcapsules were incubated in the aqueous media with different pHs of 5.5 and 7.4 for 24 h and then observed under CLSM. As presented in Figure 1C, all the microcapsules remain intact structures filled with dex-FITC, demonstrating that drug can be hosted by microcapsules at pH 7.4. In contrast, only debris of broken polyelectrolyte shells could be observed after 24 h incubation at pH 5.5 (Figure 1D). The result demonstrates that the microcapsules are ruptured at pH 5.5 and dex-FITC is released, implying that the microcapsules could be used as an "active defense" system to rapidly release the drugs in acidic environments.

For the exploding microcapsules, the degradation rate of the core governs the time point of explosion and thus the release of the loaded drug.[16] Since the result described above indicates that the microcapsules degradation at pH 5.5 (37 °C) takes a few hours, we monitored the degradation of the microcapsules at pH 2, which only takes a few seconds to a few minutes, to observe the rupture of the microcapsules. In order to monitor the degradation process, 1 M HCl aqueous solution was employed to adjust the pH of the microcapsule dispersion to 2 to accelerate the degradation process at 37 °C. Subsequently, the microcapsules were scanned immediately by CLSM. **Figure 2** provides five snapshots of a microcapsule during the degradation. Figure $2A_1$–A_5 presents the membrane of the microcapsule and Figure $2B_1$–B_5 shows the encapsulated dex-FITC. In addition, the overlap of Figure $2A_1$–A_5 and Figure $2B_1$–B_5 was performed and the corresponding results are exhibited in Figure $2C_1$–C_5. **From** the images of Figure $2C_1$ and C_2, the swelling of the microcapsule could be clearly observed because the C=N bonds are cleaved to produce free polymer chains, resulting in increased osmotic pressure force. This pressure formed inside reaches the maximum that the membrane can resist and the membrane begins to rupture (Figure $2C_3$). As a consequence, rupture of the coating membrane occurs and the encapsulated dex-FITC is explosively released from the carrier (Figure $2C_4$). From the image of Figure $2C_5$, the encapsulated dex-FITC is completely released into the surrounding environment.

To evaluate the potential application of the "active defense" system for anti-tumor drug delivery, doxorubicin (DOX) was loaded in the "active defense" system. It was found that DOX can be hosted well by the microcapsule (Figure S5 in the Supporting Information). The percent loading of DOX in the microcapsule was 1.36% (mg/mg). Similarly, to monitor the degradation process of DOX-loaded microcapsule, 1 M HCl was also used to adjust the pH of the microcapsule dispersion to 2 to accelerate the degradation at 37 °C and the microcapsules were scanned immediately by CLSM. Similar explosive release was observed for the DOX-loaded microcapsule as shown in **Figure 3**. Upon changing the pH of the microcapsule dispersion to 2, the dextran microgel core degrades quickly due to the cleavage of Schiff's base. The free dexran chains formed inside can also produce an osmotic pressure and thus force the membrane to rupture followed by the explosive release of encapsulated DOX.

To investigate the pH-triggered drug release behavior of the DOX-loaded microcapsules, the drug release was carried out at different pHs. As shown in **Figure 4**, at pH 7.4, the drug release is very slow and less than 6% of drug is released within 12 h. When altering the pH to 5.5, within the initial 2.25 h, the drug release rate is slightly faster than that at pH 7.4. We believe that in the first 2.25 h, C=N bonds get cleaved to produce free polymer chains, and the resulting osmotic pressure forces the microcapsules to swell up, leading to an accelerated drug release. After 2.25 h, the drug release rate increases dramatically. It is supposed that after this time point, the inside osmotic pressure exceeds the maximum value that the membrane can resist and the membrane begins to rupture, followed by the explosive release of DOX. In other words, the exploding of the microcapsules occurs at 2.25 h.

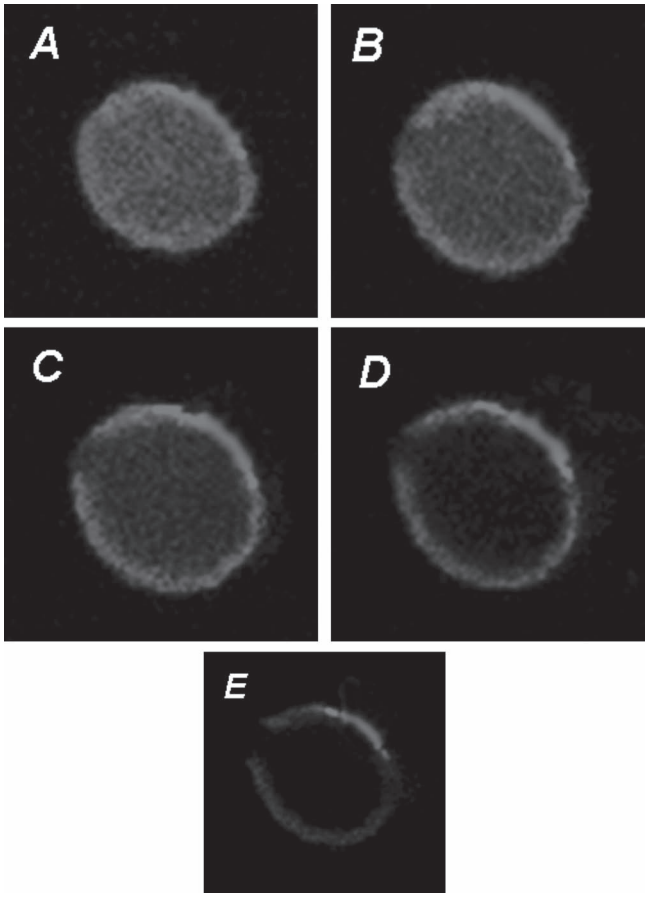


Figure 3. Snapshots of exploding process of the DOX loaded microcapsule at pH 2 and 37 °C. (The time interval of snapshots is 30 s).

In this investigation, to study the "active defense" behavior (**Scheme 2**) of the microcapsules, the dex-FITC loaded microcapsules were co-incubated with HeLa cells at different pHs, i.e., pH = 5.5 and pH = 7.4. As shown in **Figure 5**A–C, green fluorescence can not be observed for HeLa cells after 4 h incubation with the microcapsules at pH = 7.4, implying that dex-FITC can

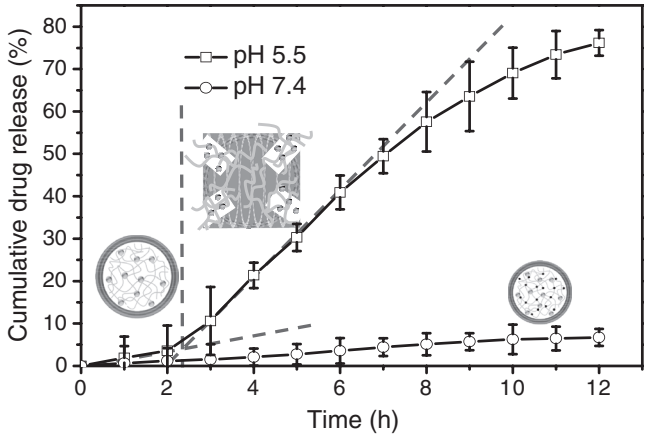
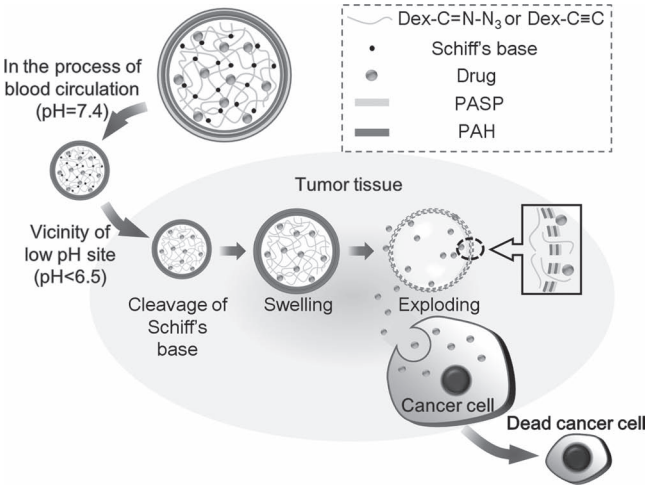


Figure 4. Drug release behaviors of microcapsules at pH 5.5 and pH 7.4.



Scheme 2. Structure of a microcapsule and tumor triggered exploding of the microcapsule. A) Structure of microcapsules, B) microcapsules host drugs at pH 7.4, C) Schiff's base cleaves at low pH site, D) increased pressure causes the microcapsule to swell, E) exploding of the microcapsule, F) uptake of drugs released from exploding microcapsules by cancerous cell, and G) apoptosis of tumor cells.

be hosted by our microcapsules in normal physiological conditions. In contrast, green fluorescence can be observed clearly in HeLa cells incubated with microcapsules at pH = 5.5 (Figure 5D–F), indicating that dex-FITC can be released and uptaken by HeLa cells under this condition. This is due to the degradation of the dextran microgel cores, resulting in the exploding of the microcapsules followed by explosive release of encapsulated dex-FITC.

The *in vitro* experiment was also carried out to prove the environment triggered release and uptake of DOX. As shown in **Figure 6**A–C, red fluorescence can be negligible in HeLa cells incubated with the DOX loaded microcapsules at pH 7.4. This is consistent with the result shown in Figure 4. According to the data of Figure 4, only 2% of loaded DOX is released within 4 h. Therefore, the red fluorescence is very low in Figure 6A. In contrast, at pH 5.5, about 21% of DOX can be released within the same time frame. So the dose of DOX uptaken by HeLa cells is much more at pH 5.5 than that at pH 7.4. And thus the fluorescent intensity of HeLa cells incubated at pH 5.5 is dramatically enhanced (Figure 6D–F). Moreover, in comparison with the cells in Figure 6B with normal elliptical elongate shape, the cells in Figure 6E are small and collapse since the cytotoxic DOX released from the microcapsules is uptaken by the cells and leads to the cell apoptosis. All these results strongly demonstrate that the release of drug from our microcapsules as well as subsequent cell apoptosis can be triggered by acidic environment of tumor tissues.

To evaluate the tumor specific therapy of our DOX loaded microcapsules, the cell viability was determined and the corresponding data are presented in **Figure 7**A. It can be found that the HeLa cell viability of the DOX loaded microcapsules at pH 7.4 is ~70% at the concentration of 50 μg/mL (containing ~0.68 μg DOX) (Figure 7A) while the cell viability decreases dramatically to around 30% at the same concentration when altering the medium pH to 5.5, which is attributed to the release of DOX

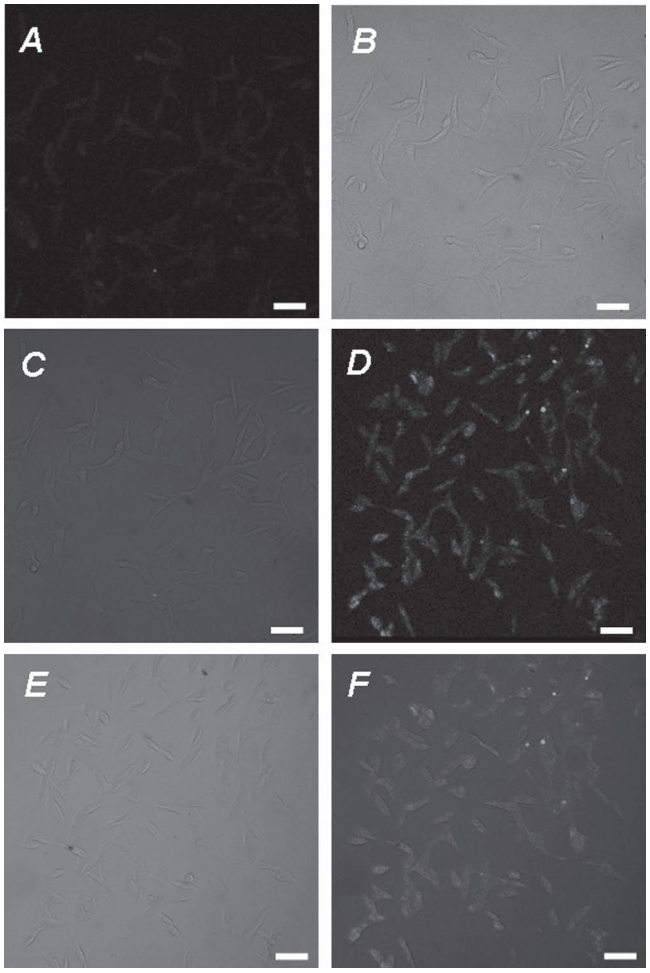


Figure 5. CLSM images of HeLa cells treated by dex-FITC loaded microcapsules at pHs of 7.4 (A–C) and pH 5.5 (D–F). A,D) confocal fluorescence images; B,E) bright field images; C, F: overlap of confocal fluorescence and bright field images. (The scale bar is 50 μm).

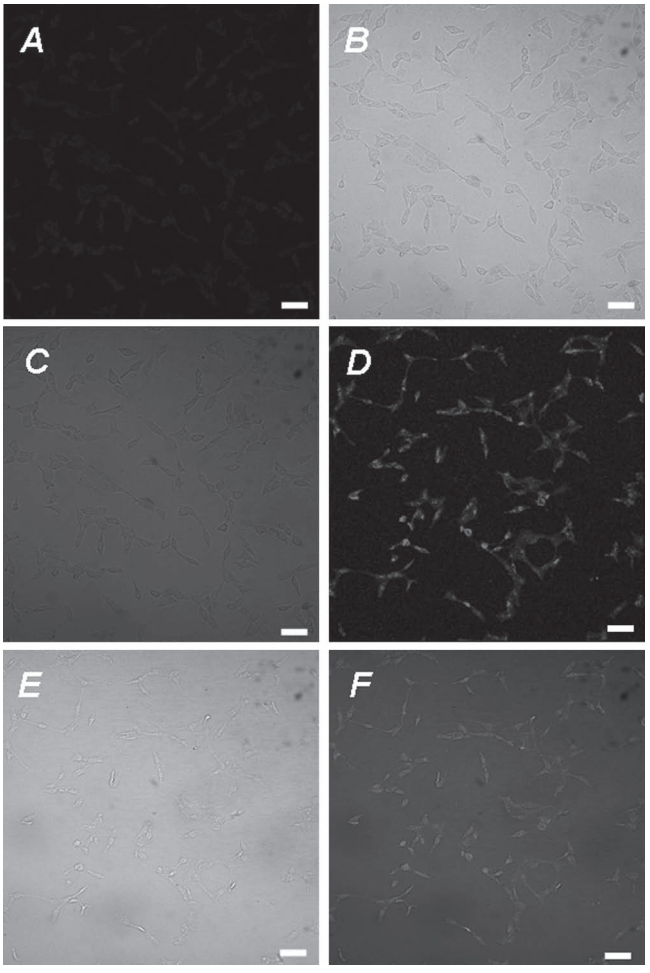


Figure 6. CLSM images of HeLa cells treated by DOX loaded microcapsules at pH 7.4 (A–C) and pH 5.5 (D–F). A,D) confocal fluorescence images; B,E) bright field images; C,F) overlap of confocal fluorescence and bright field images. (The scale bar is 50 μm).

under the acidic condition, leading to the inhibition effect on the tumor cells. Cell viability of HeLa cells incubated with free DOX at the same concentration as that loaded in the microcapsules was further studied and the corresponding data are shown in Figure 7B. According to the data of Figure 4, less than 6% of loaded DOX is released within 12 h when the medium pH is 7.4. In other words, when the concentration of the microcapsules is 50 μg/mL, the concentration of released DOX within 12 h is about 0.0408 μg/mL. As shown in Figure 7B, at the same concentration of free DOX, the cell viability remains above 95%, indicating that such DOX dose is low enough and will not affect normal cells obviously. Moreover, from Figure 7B, the viability of HeLa cells incubated with free DOX is almost the same when the medium pH is 5.5 or 7.4, indicating that cell activity would not be affected by different pH values. Thus, the desired therapy to cancerous cells with our microcapsules might be achieved due to the drug release upon the trigger of the acidic environment of tumor tissues. Here, it should be noted that the microcapsules prepared in this study still have some drawbacks if used in practice such as the relatively large size of microcapsules that can be cleared from blood and lack of passive targeting property. However, these drawbacks could be overcame by further functionalizing the microcapsules, including the change of the size of the microcapsules, incorporation of PEG to prolong the blood circulation period and incorporation of functional peptide sequences such as RGD to endow the microcapsules with targeting properties.

3. Conclusions

In summary, we have demonstrated a novel intelligent "active defense" system that can specially respond to cancerous tissues. Antineoplastic drug can be effectively loaded in this "active defense" system and can be explosively released triggered by the acidic environment of tumor tissues. Cell viability and cell-uptake experiments demonstrate that the encapsulated drug will not be uptaken by cells under normal physiological condition. Upon the trigger of acidic environment of tumor, the entrapped drug can be explosively released from the microcapsules and

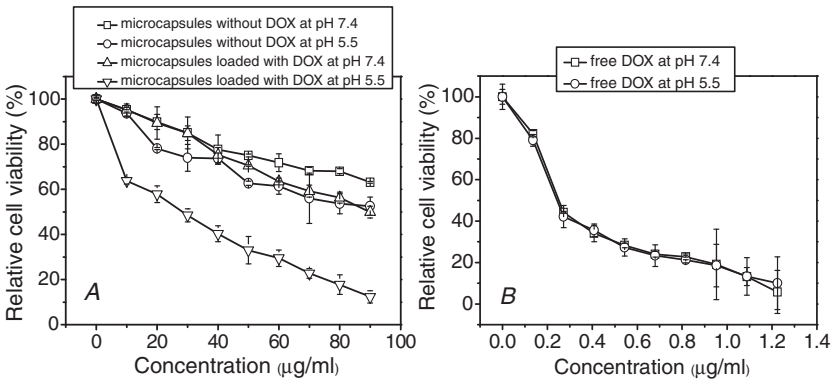


Figure 7. Viability of HeLa cells after being incubated with microcapsules: A) with or without DOX at different pHs for 24 h and B) with free DOX at different pHs for 24 h (B).

then uptaken by cancerous cells to achieve the desirable therapy effect. In this way, the microcapsules can specifically respond to physiopathological signals of tumor tissues and the side effects on normal tissues could be minimized can be avoided. This "active defense" system will thus have great potential in the caner therapy.

4. Experimental Section

Materials: Dextran (M_w ~ 60–90 k & 200 kDa), polyethylene glycol (20kDa), L-aspartic acid, phosphoric acid (H_3PO_4), 3-chloropropylamine hydrochloride, sodium azide (NaN_3), potassium iodide (KI), copper sulfate ($CuSO_4$), sodium ascrobate, chloroacetic acid, isopropanol, methanol, hydrochloric acid (HCl), 1-ethyl-3-3-dimethylaminopropyl carbodiimide (EDC), rhodamine B, sodium hydroxide (NaOH), potassium periodate (KIO_4), anhydrous sodium sulfate (Na_2SO_4), potassium bromide (KBr), and dimethylsulfoxide (DMSO) were purchased from Shanghai Reagent Chemical Co. (China) and used as received. Fluoresceinamine isomer (FITC) was obtained from Acros and used directly. Doxorubicin (DOX) was purchased from Zhejiang Hisun Pharmaceutical Co. (China). Poly(allylamine hydrochloride) (PAH) (M_w = 70 kDa) was purchased from Sigma-Aldrich. Polyaspartic acid (PASP) was synthesized according to a literature procedure.[28] Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS) and Dulbecco's phosphate buffered saline (PBS) were purchased from Invitrogen Corp. All other reagents and solvents were provided by Shanghai Reagent Chemical Co. (China) and used without further purification.

Synthesis of Aminopropylazide: 3-Chloropropylamine hydrochloride (10 g, 78 mmol), NaN_3 (15 g, 231 mmol) and KI (1.3 g, 7.8 mmol) were dissolved in de-ionized (DI) water (150 mL). After reaction at 80 °C for 18 h, the mixture was concentrated and the solution pH was adjusted to ~10 by using 4 M NaOH aqueous solution. Subsequently, the solution was extracted using diethyl ether and the organic phase was dried over anhydrous Na_2SO_4. After filtration and concentration, the product was collected by distillation. Yield: 3.9 g (50%). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 1.14 (bs, 2H, -NH_2), 1.74 (m, 2H, -$CH_2CH_2CH_2$-), 2.81 (t, 2H, -CH_2NH_2), 3.38 (t, 2H, -CH_2N_3).

Synthesis of Polyaldehyde Dextran (PAD): Polyaldehyde dextran (PAD) was synthesized via the periodate oxidation of dextran. In brief, dextran (1.00 g, 6.17 mmol of repeat unit) was dissolved in DI water (20 mL) and KIO_4 (28.4 mg, 0.123 mmol, 2% of dextran repeat unit) was subsequently added. After stirring at 25 °C for 24 h under N_2 atmosphere, the solution was dialysized (MWCO: 3500 Da) against DI water for 6 days and then lyophilized for 3 days to obtain white solid PAD (0.98 g, yield: 98%).

Synthesis of Azide Modified Dextran (dex-C = N-N_3): Dex-C = N-N_3 was synthesized via the reaction between the aldehyde groups of PAD and amide groups of aminopropylazide. Briefly, PAD (0.5 g, 3.09 mmol of repeat unit) was dissolved in DI water (15 mL) and then aminopropylazide (15.4 mg, 0.154 mmol, 5% of PAD repeat unit) was added. The mixture was stirred at 35 °C overnight under N_2 atmosphere. Subsequently, the solution was dialysized (MWCO: 3500 Da) against DI water for 6 days and then lyophilized for 3 days to obtain brown solid (0.4 g, yield: 80%). The number of azide group to the anhydroglucose unit of dextran was defined as degree of substitution (DS) and the DS of dex-C = N-N_3 was evaluated by ^{1}HNMR analysis (Figure S2 in the Supporting Information).

Synthesis of carboxymethyl dextran (CMD): CMD was synthesized according to a literature procedure.[16] Briefly, dextran (1 g, 6.17 mmol of repeat unit) was added into the mixture of isopropanol (22 mL) and 14.3 M aqueous NaOH solution (4 mL). After stirring at room temperature (22 °C) for 1 h, chloroacetic acid (3.4 g, 3.6 mmol) dissolved in isopropanol (7 mL) was added dropwise and the suspension was stirred for 90 min at 60 °C. After cooling to room temperature, isopropanol was removed and methanol was added to wash the residue. Thereafter, methanol was removed and the residue was dissolved in distilled water (20 mL). After adjusting the solution pH to 2~3 by using 1 M HCl aqueous solution and then dialyzing (MWCO: 3500 Da) against DI water, the lyophilized product was collected as white solid (1.28 g, yield: 93%). The number of carboxyl group to the anhydroglucose unit of CMD was defined as degree of substitution (DS) and the DS of CMD was calculated as 79.4% according to the corresponding ^{1}HNMR spectrum.

Synthesis of Alkyne Modified Dextran (dex-C≡C): CMD (1.28 g, 7.88 mmol) was dissolved in H_2O (20 mL). To this solution, propargyl amine (0.22 g, 3.94 mmol) was added. After adjusting the solution pH to 5 ~ 6, EDC (1.51 g, 7.88 mmol) was added and the mixture was stirred overnight at 50 °C. After dialyzing (MWCO: 3500) against DI water and freeze-drying, the product was obtained as white fluffy powder. (1.42 g, yield: 95%). The number of alkyne group to the anhydroglucose unit of dextran was defined as degree of substitution (DS) and the DS of dex-C≡C was evaluated based on the ^{1}HNMR spectrum (Figure S4 in the Supporting Information).

Fluorescent Labeling of PAH and Dextran: PAH was fluorescently labeled with a dye, rhodamine B. In brief, PAH was dissolved in distilled water and then rhodamine B (10 mol.% based on amine groups of the PAH) as well as EDC (1.2-fold molar excess compared to rhodamine B) was added. After stirring at room temperature for 24 h, rhodamine B labeled PAH (PAH-Rb) was obtained by dialyzing against water and freeze-drying.

Fluorescent Labeling of Dextran: Dextran was dissolved in DMSO and then FITC (5 mol.% of repeat unit of dextran) was added. After stirring at room temperature for 24 h, fluorescein isothiocyanate modified dextran (dextran-FITC) was collected by dialyzing against water and freeze-drying.

Fabrication of Dextran Microgel Core: dex-N_3 (60 mg), dex-C≡C (60 mg) and $CuSO_4$ (10 mg) were dissolved in DI water (360 μL) to form homogeneous solution. Subsequently, 24% (w/w in DI water) PEG solution (3.2 mL) was added. The mixture was stirred continuously until homogenous dispersion was formed. Thereafter, sodium ascorbate (200 μL, 100 mg/mL) was introduced to initiate click chemistry and the reaction was allowed to proceed for 4 h. Subsequently, DI water (20 mL) was added and the microgels were collected after centrifugation (8000 r/min × 5 min)/wash for three times. Herein, dextran-FITC was encapsulated in the microgels by the addition of dextran-FITC (6 mg) before the emulsification. DOX was loaded in the microgels by immersing the isolated microgels into DMSO containing DOX (1 mg/mL) and then shaking for 24 h, followed by washing with DI water (7 × 1 mL).

LbL Coating of the Dextran Click Microgels: The isolated microgels were transferred into 1 mL pipette and further washed with DI water (3 × 1 mL). Then the microgels were dispersed in DI water (0.5 mL) and polyelectrolyte solution (1 mL, PAH-Rb or PASP, 1 mg/mL in DI water at a pH of 7) was introduced. After shaking for 10 min, the microgels were isolated via centrifugation at 8000 r/min for 5 min. The purification of the microgels was performed by washing with DI water and then centrifuging (8000 r/min × 5 min) for two times. This procedure was repeated until two polyelectrolyte bilayers were obtained. In this study, we defined this LbL coated dextran microgel as microcapsules.

In Vitro Drug Release at Different pHs: The DOX loaded microcapsules (120 mg) were isolated with centrifugation (8,000 rpm for 5 min) and then dispersed in DI water (2 mL). The microcapsule dispersion was departed into two parts evenly and respectively put into two dialysis tubes (MWCO: 8000-12,000 Da) quickly. Subsequently, the dialysis tubes were immersed into buffer solution (0.1 M, 100 mL) with a respective pH of 7.4 and 5.5. Aliquots of 2 mL were withdrawn from the solution periodically. The volume of solution was held constant by adding fresh buffer solution (2 mL) after each sampling. The amount of DOX released from the microcapsules was measured based on a RF-530/PC spectrofluorophotometer (Shimadzu) absorbance at 556 nm (excitation wavelength used is 480 nm). The cumulative drug release was calculated as follows: Cumulative amount released (%) = $(M_t/M_\infty) \times 100$, where M_t is the amount of drug released from the microcapsules at time t and M_∞ is the amount of drug loaded in the microcapsules.

Co-Incubation of Drug-Loaded Microcapsules with Cells at Different pHs: Human cervix carcinoma (HeLa) cells were incubated in DMEM medium with 10% FBS and 1% antibiotics (penicillin-streptomycin, 10,000 U/mL) at 37 °C in a humidified atmosphere containing 5% CO_2. The cells were seeded respectively in a 6-well plate at a density of 1×10^5 cells/well, and then cells were incubated in DMEM (1 mL) containing 10% FBS for 24 h. Thereafter, dex-FITC loaded microcapsules (50 μg, containing ~2.5 μg dex-FITC) dispersed in DMEM medium (1 mL) with 10% FBS and 1% antibiotics were added and the cells were further incubated at 37 °C for another 4 h. After removing the medium and then washing with PBS (1 mL), the cells were observed by using CLSM.

In vitro Cytotoxicity of DOX-loaded Microcapsules and Free DOX: HeLa cells were seeded into a 96-well plate (1.5×10^4 cells/well) containing DMEM (200 μL). After incubation for 24 h (37 °C, 5% CO_2), the culture medium was removed, and DMEM (200 μL) containing a fixed amount of DOX-loaded microcapsules or free DOX were added in each well. The pH value of some wells was adjusted to 5.5 using 1 M HCl. After incubated at 37 °C for 24 h, MTT solution (20 μL, 5 mg/mL) was added to each well and the cells were further incubated for another 4 h. Subsequently, the MTT medium was removed and DMSO (200 μL) was added to each well. The optical density (OD) was measured at 570 nm with amicroplate reader, model 550 (BIO-RAD, USA). The cell viable rate was calculated as follows: Viable rate = $(OD_{treated}/OD_{control}) \times 100\%$.

^{1}H Nuclear Magnetic Resonance (^{1}H NMR): ^{1}H NMR spectra of aminopropylazide, PAD, dex-C = N-N_3, CMD and dex-C;C were recorded on a Mercury VX-300 spectrometer at 300 MHz (Varian, USA) by using $CDCl_3$ or D_2O as the solvent.

Fourier Transform-Infrared Spectroscopy (FT-IR): The samples were analyzed by using a FT-IR (Perkin-Elmer, USA) spectrophotometer. Before the measurements, the samples were pressed into potassium bromide (KBr) pellets.

Confocal Laser Scanning Microscopy (CLSM): The dextran microgel core and microcapsules samples in water were viewed by using CLSM (Nikon C1-si, Japan, BD Laser at 488 nm).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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[1] E. E. Bakken, K. Heruth, *Ann. N.Y. Acad. Sci.* **1991**, *618*, 422.
[2] K. Shigeta, S. Kawakami, Y. Higuchi, T. Okuda, H. Yagi, F. Yamashita, M. Hashida, *J. Controlled Release* **2007**, *118*, 262.
[3] M. Elfinger, C. Maucksch, C. Rudolph, *Biomaterials* **2007**, *28*, 3448–3455.
[4] N. Nasongkla, X. Shuai, H. Ai, B. D. Weinberg, J. Pink, D. A. Boothman, *Angew. Chem. Int. Ed.* **2004**, *43*, 6323.
[5] P. A. Bertin, J. M. Gibbs, C. K. F. Shen, C. S. Thaxton, W. A. Russin, C. A. Mirkin, *J. Am. Chem. Soc.* **2006**, *128*, 4168.
[6] V. P. Torchilin, A. N. Lukyanov, Z. G. Gao, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 6039.
[7] D. Q. Wu, B. Lu, C. Chang, C. S. Chen, T. Wang, Y. Y. Zhang, S. X. Cheng, X. J. Jiang, X. Z. Zhang, R. X. Zhuo, *Biomaterials* **2009**, *30*, 1363.
[8] Z. Y. Poon, D. S. Chang, X. Y. Zhao, P. T. Hammond, *ACS Nano* **2011**, *5*, 4284.
[9] Y. A. Shieh, S. J. Yang, M. F. Wei, M. J. Shieh, *ACS Nano* **2010**, *4*, 1433.
[10] N. Stephanopoulos, G. J. Tong, S. C. Hsiao, M. B. Francis, *ACS Nano* **2010**, *4*, 6014.
[11] M. Volz, P. Walther, U. Ziener, K. Landfester, *Macromol. Mater. Eng.* **2007**, *292*, 1237.
[12] B. G. De Geest, C. Déjugnat, G. B. Sukhorukov, K. Braeckmans, S. C. De Smedt, J. Demeester, *Adv. Mater.* **2005**, *17*, 2357.
[13] B. G. De Geest, C. Déjugnat, M. Prevot, G. B. Sukhorukov, J. Demeester, S. C. De Smedt, *Adv. Funct. Mater.* **2007**, *17*, 531.
[14] B. G. De Geest, M. J. McShane, J. Demeester, S. C. De Smedt, W. E. Hennink, *J. Am. Chem. Soc.* **2008**, *130*, 14480.
[15] B. G. De Geest, S. De Koker, J. Demeester, S. C. De Smedt, W. E. Hennink, *Polym. Chem.* **2010**, *1*, 137.
[16] J. Zhang, C. Li, Y. Wang, R. X. Zhuo, X. Z. Zhang, *Chem. Commun.* **2011**, *47*, 4457.
[17] L. Dahne, *Angew. Chem. Int. Ed.* **2009**, *48*, 4106.
[18] M. Krämer, J. F. Stumbé, H. Türk, S. Krause, A. Komp, L. Delineau, S. Prokhorova, H. Kautz, R. Haag, *Angew. Chem. Int. Ed.* **2002**, *41*, 4252.
[19] P. Vaupel, F. Kallinowski, P. Okunieff, *Cancer Res.* **1989**, *49*, 6449.
[20] N. Murthy, Y. X. Thng, S. Schuck, M. C. Xu, J. M. J. Fréchet, *J. Am. Chem. Soc.* **2002**, *124*, 12398.
[21] M. C. Parrott, J. Chris Luft, J. D. Byrne, J. H. Fain, M. E. Napier, J. M. DeSimone, *J. Am. Chem. Soc.* **2010**, *132*, 17928.
[22] G. Decher, J. D. Hong, J. Schmitt, *Thin Solid Films* **1992**, *210–211*, 831.
[23] G. Decher, *Science* **1997**, *277*, 1232.
[24] F. Caruso, R.A. Caruso, H. Möhwald, *Science* **1998**, *282*, 1111.
[25] E. Donath, G. B. Sukhorukov, F. Caruso, S. A. Davis, H. Mohwald, *Angew. Chem. Int. Ed.* **1998**, *37*, 2202.
[26] B. G. De Geest, S. De Koker, J. Demeester, S. C. De Smedt, W. E. Hennink, *J. Controlled Release* **2009**, *135*, 268.
[27] O. Franssen, W. E. Hennink, *Int. J. Pharm.* **1998**, *168*, 1.
[28] C. Li, J. Zhang, S. Yang, B. L. Li, Y. Y. Li, X. Z. Zhang, R. X. Zhuo, *Phys. Chem. Chem. Phys.* **2009**, *11*, 8835.