Supporting Information

Highly stable and degradable multifunctional microgel for self-regulated insulin delivery under physiological condition

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Fabrication and degradation of microgels entrapped with FITC

The same synthetic procedure, as described for the microgel preparation (**Part 2.2**) was applied, however this time FITC dye (5 mg) was dissolved in water (1 mL) and added dropwise in 10 min after the reaction was initiated. The product was purified from free FITC by intensive dialysis in distilled water (5 d, 5~6 times solvent exchange per day). The final product was lipophylized.

0.01 mg/mL (5 ml) lipophylized product were dialysis (cut-off 50000) in pH 7.4 PBS (0 mM DTT) and pH 7.4 PBS (10 mM DTT) under 37°C. 1 mL solution outside the dialysis bag was taken under certain time interval, at the same time, the same amount of fresh solution was added. Fluorescence was collected under 492 nm (E_x) and 518 nm (E_m).

Insulin loading and release

Insulin was loaded to microgels by the complexation method with the maxium loding ratio (Table S2). A stock solution of insulin was prepared by dissolving 15 mg insulin into 400 μL of 0.1 M HCl, and then the solution was diluted with 5 mL phosphate buffer solution of pH 7.4 and normalized with 400 μL of 0.1 M NaOH.[1] Solution of insulin was added dropwise to the vial containing 5 mL of 3 mg/mL microgels. The resultant solution was diluted to 15 mL by PBS of pH 7.4. After stirring overnight in ice-bath, the dispersion was centrifuged at 12000 rpm for 15 min. To remove free drug, the precipitate was redispersed in 7 mL PBS of pH 7.4 and further purified by multiple centrifugation and washing. Collecting supernatant and calculated the drug loading ratio (Dr) as follows:

$$\mathrm{Dr}\ (\mathrm{g/g}) = \frac{W_d - W_s}{W_m} \qquad (1)$$

where Wd is the weight of total insulin, Ws is the weight of insulin in supernatant and Wm is the weight of microlgels. Finally, the precipitate was redispersed in 15 mL PBS of pH 7.4 and divided equally into three samples.

In vitro release test of insulin from microgel was evaluated by the dialysis method. Three dialysis bags (cut-off 50000) filled with 5 mL of purified insulin-microgel dispersion containing 10 mM DTT, 20 mM glucose and no additive, respectively, were immersed in 200 mL PBS of pH 7.4 containing the same additive. Pure insulin in 5 mL PBS of pH 7.4 was taken as study of retardant effect of dialysis membrane. The released insulin outside of the dialysis bag was sampled at defined time intervals and assayed by spectrophotometric method at 595 nm. 1 mL of each sample was stained for 5 min in 5 mL of Coomassie Brilliant Blue solution. Cumulative release was expressed as the total percentage of drug released through the dialysis bag over time and calculated as follows:

$$\mathrm{Cumulative\ Released\ Drugs}\ (\%) = \frac{M_t}{M_\infty} \times 100 \qquad (2)$$

where M_t is the amount of drug released from the microgel at time t and M_∞ is the amount of drug loaded into the microgel. The release experiment was also carried out to show the evolution of released kinetics in response to different outside stimuli.

In vitro cytotoxicity measurement

Cell viability was assayed with respect to Hela cells by the MTT assay. The cells were cultured in DMEM with 10% of fetal bovine serum (FBS) and a humidified atmosphere (5% CO_2, 37°C). Hela cells were seeded in a 96-well plate at an initial density of 7000 cells/well and further incubated with 100 μL fresh medium. Micogel dispersions were sterilized by autoclave and then added to achieve varying final concentrations. After incubation for 24 h at 37°C， MTT reagent (10 μL in PBS, 5 mg/mL) was added to each well for further 4 h incubation at 37°C. Then, the culture medium was removed and replaced with 100 μL DMSO to dissolve the formed formazan crystals. Untreated cells which were taken as control had 100% viability (%) that correlates with amount of liable cells compared with cell control. Each experiment was done in triplicate. The data were shown as the mean value plus a standard deviation (± SD).

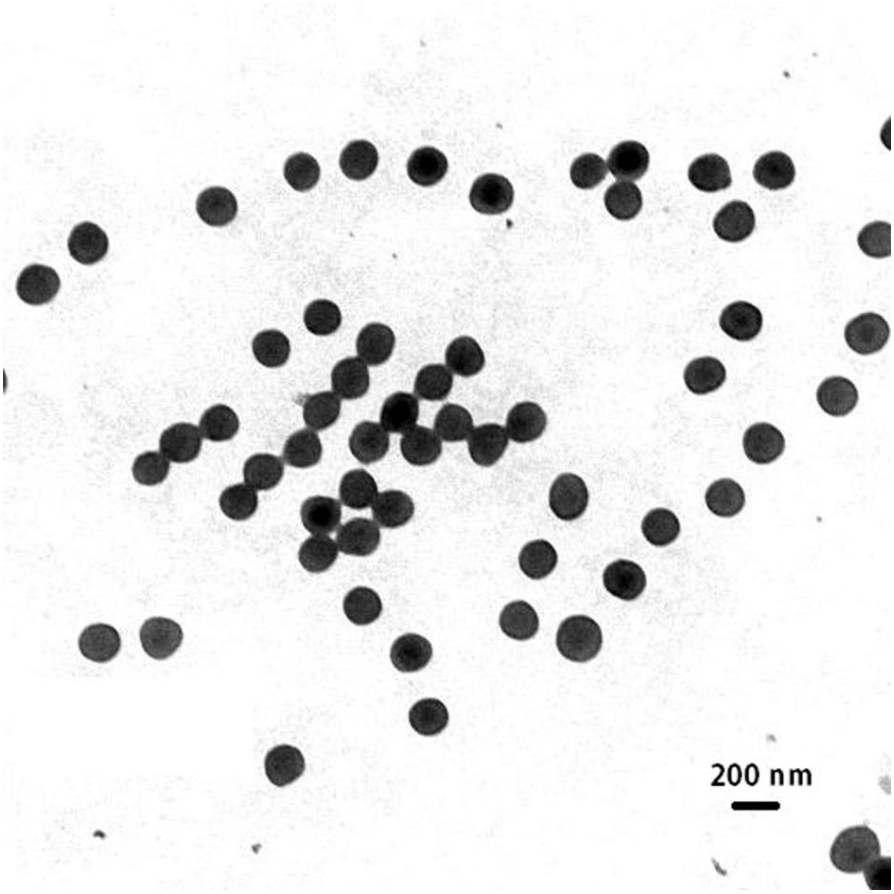

Figure S1 TEM image of microgels achieved at pH 12.

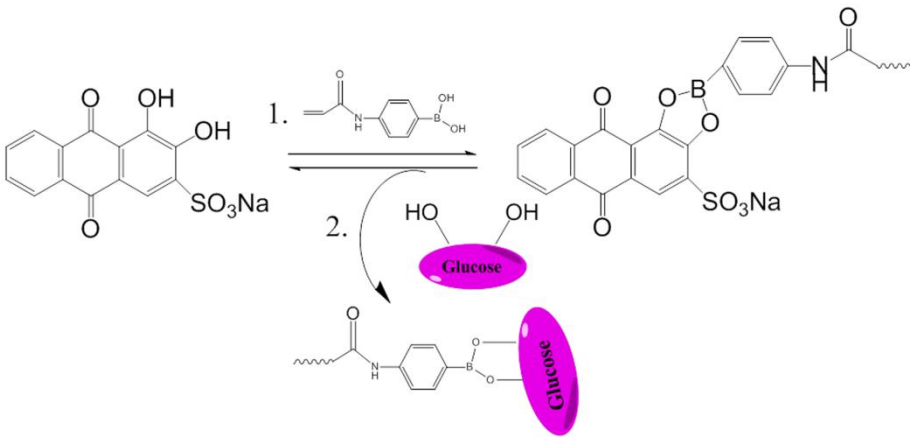

Figure S2 Schematic scheme illustrates the incorporation of PBA functional group competing between ARS and glucose.

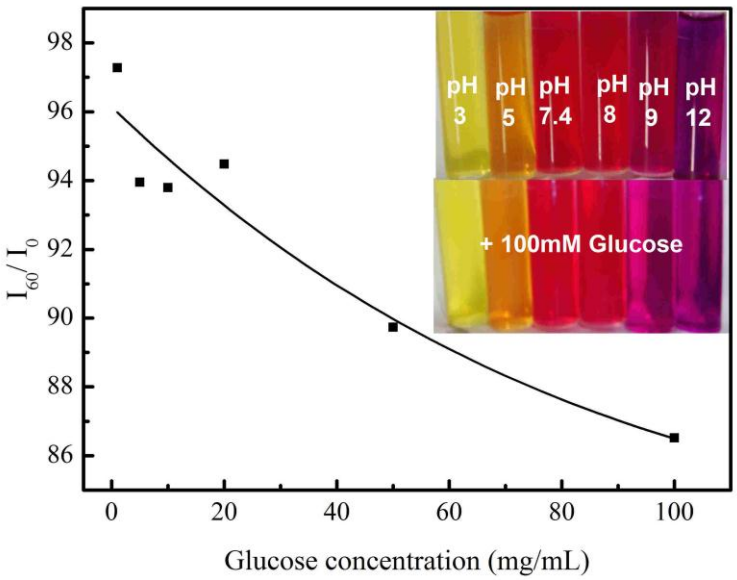

Figure S3 Fluorescence ratios of microgels response to glucose. I_{60} is the fluorescence detected 60 min with the adding of glucose. I_0 is the fluorescence achieved from initial microgels dispersion with addition of ARS but without any glucose

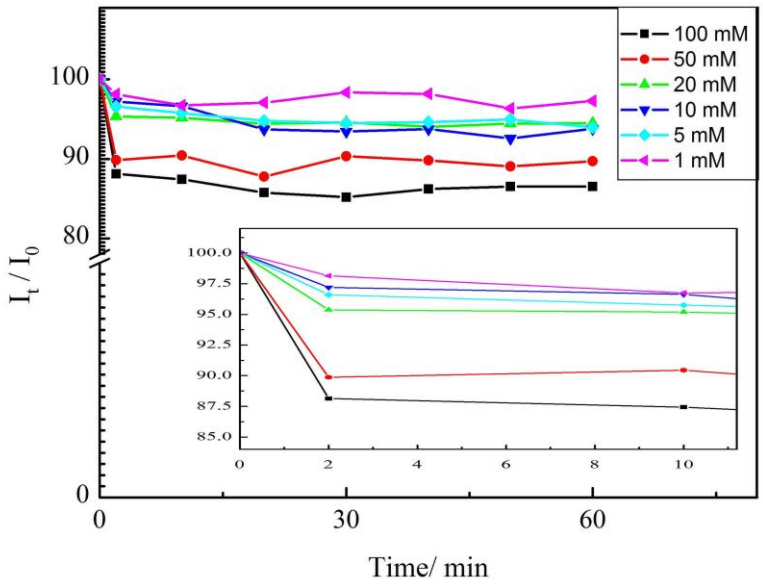

Figure S4 Fluorescence ratios of microgels vary due to a competing between ARS and glucose as time runs. I_t represents fluorescence at a fixed time and I_0 is the initial fluorescence of microgels in the presence of ARS but without any glucose.

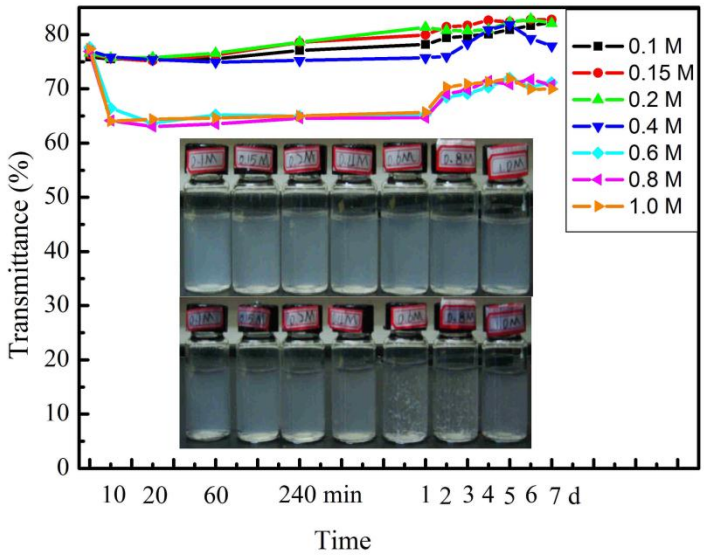

Figure S5 Stability of microgels in distilled water depends on salt concentration as a function of time at 37°C. Inset pictures illustrate the changes of microgel dispersion after one day incubation under physiological temperature.

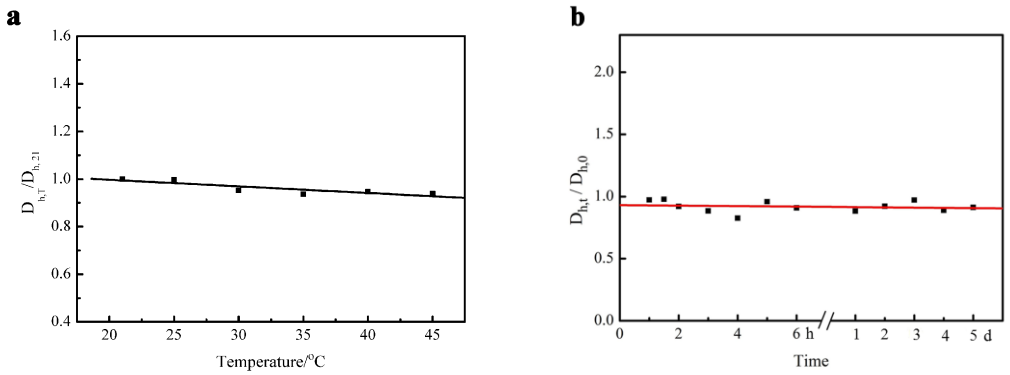

Figure S6 Stability of microgels test by DLS under certain physiological condition (pH 7.4, 37°C, 0.15 M NaCl). (a) Stability depends on temperature; (b) Stability changes as a function of time at 37°C. Both of two images show there is no obvious change on size, indicating stable of microgels under physiological condition.

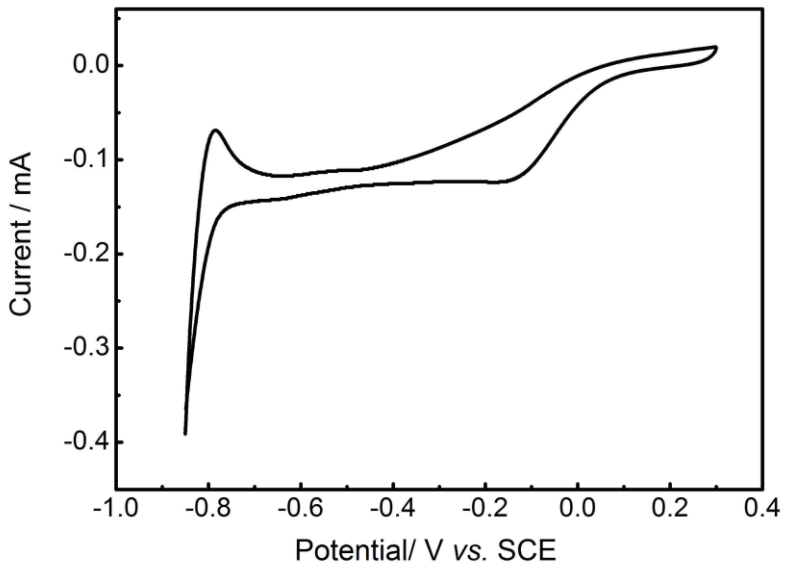

Figure S7 CV curve of microgels shows redox property in the presence of disulfide bonds.

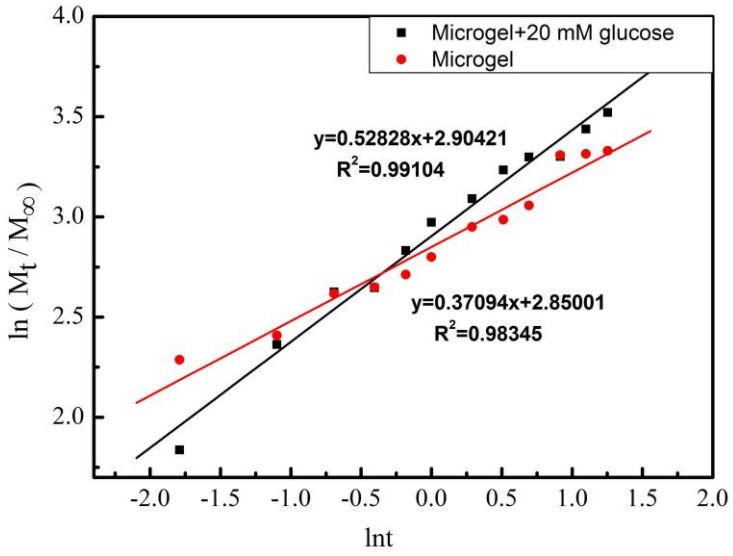

Figure S8 Data of microgels and microgels with 20 mM glucose best fit model (Fickian diffusion model).

Fickian diffusion model is defined as follow.

$$M_t/M_\infty = kt^n$$

Where M_t is the amount of drug released from the microgel at time t and M_∞ is the amount of drug loaded into the microgel. K is a constant and t is the elapsed time. n is the release exponent which indicate the drug release mechanism. When $n \leq 0.5$ correspond to Fick diffusion and higher values of n, between 0.5 and 1.0, or n=1.0, for mass transfer follow a non-Fickian model. Here, n of micrgels and micrgels with 20 mM glucose are both below 0.5.[2] That is, their release mechanism is in agreement with Fickian diffusion model.

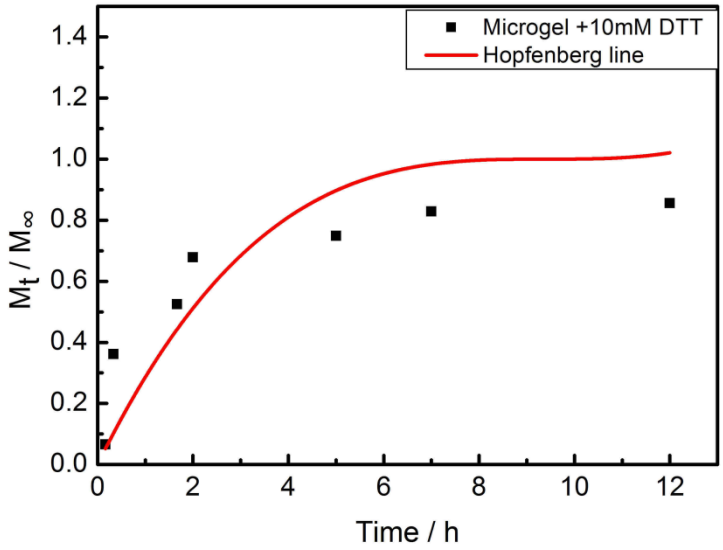

Figure S9 Data of microgels and microgels with 10 mM DTT best fit model is Hopfenberg experience model.

Hopfenberg experience model is defined as fllow.

$$\frac{M_t}{M_\infty} = 1 - (1 - \frac{k_0 t}{C_0 r})^n$$

Where M_t is the amount of drug released in time t, M_∞ is the total amount of drug loaded, Mt/M is the fraction of drug released, K_0 is the erosion rate constant, C_0 is the initial concentration of drug in matrix and r is the initial radius for a sphere or cylinder or the half-thickness for a slab. 1, 2, and 3 value of n represents slab, cylinder and sphere, respectively.[1] Here n is taken as 3 because the microgel is sphere. From the fitting curve, it can be found that microgels with addition of DTT have similar trend to the model of Hopfenberg experience model.

Table S1 Microgels swelling ratio change as glucose concentration vary.

Clucose (mM)	0	1	5	10	20	50	100
D_h (nm)	315	332	350	367	383	393	420
Sr [a]	1	1.05	1.11	1.17	1.22	1.25	1.33

a swelling ratio (Sr) = $\frac{D_{h,\,m}}{D_{h,\,o}}$

$D_{h,m}$ represents hydrodynamic diameter in certain glucose concentration; $D_{h,0}$ represents micrgels diameter without glucose

Table S2 The maximum loading capacity of the microgels.

Insulin (mg)	Microgel (mg)	Ratio	Loading Ratio (%)
5	5	1:1	38.67
5	2.5	2:1	36.87
5	10	1:2	36.00
5	3.3	1.5:1	36.36
5	7.5	1:1.5	36.54

Table S3 The molecule weight decrease as degradation of microgels.

	- DTT	+ DTT (2h)	+ DTT (1d)
M_w (g/mol)	69820	55320	7700

References

1. K. Sonaje, Y. H. Lin, J. H. Juang, S. P. Wey, C. T. Chen and H. W. Sung, Biomaterials, 2009, 30, 2329-2339.

2. Costa, P.; Sousa Lobo, J. M. *European Journal of Pharmaceutical Sciences* **2001,** 13, (2), 123-133.