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J. Am. Chem. Soc., Just Accepted Manuscript • Publication Date (Web): 16 May 2017

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# Sugar-Breathing Glycopolymersomes for Regulating Glucose Level

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KEYWORDS: glycopolymersome, polymer vesicle, self-assembly, diabetes mellitus, blood glucose level

**ABSTRACT:** Diabetes mellitus is a chronic, life-threatening illness that affects people of every age and ethnicity. It is a long-suffering pain for those who are affected and must regulate their blood glucose level by frequent subcutaneous injection of insulin every day. Herein, we propose a non-insulin and antidiabetic-drug-free strategy for regulating blood glucose level by a nano-sized "sugar sponge" which is a lectin-bound glycopolymersome capable of regulating glucose due to the dynamic recognition between the lectin and different carbohydrates. The glycopolymersome is self-assembled from poly(ethylene oxide)-*block*-poly[(7-(2-methacryloyloxyethoxy)-4-methylcoumarin)-*stat*-2-(diethylamino)ethyl methacrylate-*stat*-( $\alpha$ -D-glucopyranosyl)ethyl methacrylate] [PEO-*b*-P(CMA-*stat*-DEA-*stat*-GEMA)]. The lectin bound in the glycopolymersome has different affinity for the glucose in the blood and the glucosyl group in the glycopolymersome. Therefore, this sugar sponge functions as a glucose storage by dynamic sugar replacement: The lectin in the sugar sponge will bind and store the glucose concentration is too low. *In vitro*, this sugar-breathing behavior is characterized by a remarkable size change of sugar sponge due to the swelling/shrinkage at high/low glucose level, which can be used for blood sugar monitoring. *In vivo*, this sugar sponge showed excellent antidiabetic effect to type I diabetic mice within two days upon one dose, which is much longer than traditional long-acting insulin. Overall, this sugar sponge opens new avenues for regulating the blood glucose level without the involvement of insulin or other antidiabetic drugs.

#### **INTRODUCTION**

Diabetes mellitus is a chronic disease characterized by disorder of glucose regulation. It is predicted that by 2030 the total number of diabetes will reach 366 million.<sup>1</sup> Insulin is a peptide hormone produced by  $\beta$  cells of the pancreatic islets. It regulates the blood glucose level by promoting the absorption of glucose and its conversion into glycogen.<sup>2</sup> The treatment of type I diabetes mellitus usually involves frequent subcutaneous injection of insulin every day, which is inconvenient and accompanied with pain, local tissue necrosis, infection, nerve damage, and difficulty in achieving postprandial blood glucose control, etc.<sup>3</sup> For example, rapid-acting insulin needs to be injected before meals, while even for long-acting insulin, daily injection before sleep is necessary to imitate the basal insulin secretion. To solve these problems, non-invasive methods were developed and glucose-responsive boricacid-based polymers and hydrogels were used for controlled release of insulin.4-12 However, such systems for controlling the blood sugar still involve insulin, which may result in side effects such as hypoglycemia, insulin resistance, weight gain, allergy and hypokalemia, etc.<sup>13,14</sup>

Lectins are proteins capable of specific binding to carbohydrates. Concanavalin A (Con A) is a lectin composed

of four essential submits. In solution, Con A consists largely of dimers below pH 6 while tetramers above pH 7. Both negatively and positively charged Con A show high affinity for glucose, mannose and their derivatives while the O $\delta_1$  and O $\delta_2$  of asparagine accept hydrogen bonds from 6-OH and 4-OH of sugar, whereas N $\delta_2$  of asparagine donates such a bond to 4-OH of sugar.<sup>15,16</sup> Also, sugars play fundamental and crucial biological roles in nature. The cell-specific targeting based on carbohydrate-protein interaction, i.e., sugars and lectins, is important for cellcell and cell-matrix communication, which in turn contributes to cell recognition, microbial pathogenesis and immunological recognition, etc.<sup>15,17,18</sup> Like nucleic acids and peptides, sugars have recently gained much attention because of their significance and abundance.<sup>19,20</sup> Furthermore, sugar-containing copolymers are becoming increasingly important due to their promising potential biomedical applications.<sup>21-23</sup> Usually, the weak protein-sugar interactions can be significantly strengthened by the multivalent effect of clustered sugars (cluster glycoside effect).<sup>24,25</sup> Recently, much effort has been paid to develop sugar-containing polymers for cell sensing,<sup>25-28</sup> therapeutics,<sup>29-32</sup> and synthetic biology, etc.<sup>33-36</sup>

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Polymer vesicles, also referred to as polymersomes, have attracted much attention because of their unique structure and diverse potential applications in biopharmaceutical field, especially for stimuli-responsive vesicles.<sup>3,37,38</sup> External stimuli, such as pH,<sup>39</sup> temperature,<sup>40</sup> light,<sup>41</sup> redox,<sup>42</sup> ultrasound,<sup>43</sup> and enzyme,<sup>44</sup> have been applied to trigger the responsiveness of "smart vesicles" for various applications in drug delivery,<sup>45,46</sup> gene delivery,<sup>47,48</sup> antibacterial,<sup>49,50</sup> contrast enhancement,<sup>16,51</sup> etc. Recently, more and more concerns have been drawn to glycopolymersomes.<sup>15,19,20,52-55</sup> For example, Schlaad et al. put forward a glycopolymersome by using "click" reaction between vinyl-containing polymer and thiol-substituted glucose.<sup>19</sup> Alexander and co-workers reported a glycopolymersome with "sweet talking" ability with bacteria.15 Lecommandoux et al. fabricated a polymersome by employing polysaccharide-block-polypeptide copolymer to mimic synthetic viral capsids.<sup>33</sup> Jiang's group prepared a

glucose-coated polymersome using dynamic covalent bond between sugar and lectins.<sup>56</sup>

Herein, a non-insulin and antidiabetic-drug-free strategy is proposed to regulate the blood glucose level of diabetes based on a lectin-bound glycopolymersome. This sugar sponge can store extra glucose when the concentration is higher than normal level and release it when the concentration is too low, demonstrating a sugarbreathing behavior (Scheme 1). This sugar sponge is also functionally similar to an artificial glycogen as it can store/release glucose at high/low glucose concentrations. Furthermore, the *in vivo* test showed excellent antidiabetic effect towards type I diabetic mice within two days upon one dose of the sugar sponge.<sup>13</sup>

## Scheme 1. Preparation of Sugar Sponge and its Blood Glucose Regulation Behavior via Sugar-Breathing <sup>a</sup>



<sup>*a*</sup> Con A can be enriched on the glycopolymersome due to the electrostatic interaction with PDEA, bound with the glucosyl to form a Con A-bound glycopolymersome (sugar sponge), then immobilized in the membrane of the glycopolymersome by the photo-cross-linking of CMA under UV irradiation to form cross-linked sugar sponge. The sugar sponges could bind/release glucose at high/low glucose concentrations. The sugar sponges are capable of long circulation for regulating blood glucose level.

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## Scheme 2. Synthesis of Monomers and PEO-*b*-P(CMA-*stat*-DEA-*stat*-GEMA) Copolymer



PEO<sub>43</sub>-b-P(CMA<sub>8</sub>-stat-DEA<sub>80</sub>-stat-GEMA<sub>12</sub>) Glycopolymersome

To prepare sugar sponges, a glycopolymer, [PEO-b-P(CMA-stat-DEA-stat-GEMA)], was synthesized via atom transfer radical polymerization (ATRP) and selfassembled into glycopolymersome. The hydrophobic CMA and DEA moieties form the membrane, whereas the biocompatible and hydrophilic PEO chains form the coronas. The GEMA moieties with a glucosyl residue are embed within the membrane because of the constraints of the hydrophobic polymer chain. Con A will be bound in the membrane of the glycopolymersome when it is added into the polymersome solution due to the electrostatic effect with DEA, thus assists the recognition interactions between Con A and glucosyl, leading to the formation of the un-cross-linked sugar sponge. The photocross-linking of CMA under UV irradiation in the membrane of the glycopolymersome can stabilize its structure and prevent its disassociation upon dilution or environmental change. Also, this process can immobilize Con A in the membrane of the glycopolymersome to afford the cross-linked sugar sponges with longer circulation time in vivo.

## **RESULTS AND DISCUSSION**

Synthesis of Glycopolymer PEO-b-P(CMA-stat-**DEA-stat-GEMA**). The glycopolymer PEO-*b*-P(CMAstat-DEA-stat-GEMA) was synthesized by ATRP, followed by the deprotection of the precursor PEO-b-P(CMA-stat-DEA-stat-AcGEMA) copolymer in the presence of sodium methylate.<sup>57</sup> The synthetic details and the corresponding <sup>1</sup>H NMR spectra of PEO-Br, CMA and AcGEMA monomers, glycopolymer precursor PEO-b-P(CMA-stat-DEAstat-AcGEMA) and glycopolymer are provided in Figures SI-S6 in the Supporting Information. The degrees of polymerization (DPs) of CMA, DEA and AcGEMA are 8, 80 and 12, as calculated from Figure S5 and Table S1 in the Supporting Information. As shown in Figure S6A, the disappearance of peaks in the high field revealed the successful removal of acetyl group (comparing with Figure S5 in the Supporting Information), while the intensity of other peaks remained the same, suggesting successful synthesis of the glycopolymer. The gel permeation chromatography (GPC) trace of this glycopolymer is shown in Figure S7, showing a narrow molecular weight distribution ( $M_n$  = 9700 Da and  $M_w/M_n$  = 1.17).

of PEO-b-P(CMA-stat-DEA-stat-Self-assembly GEMA) into Glycopolymersomes. The glycopolymersome was prepared by self-assembly of PEO43-b-P(CMA<sub>8</sub>-stat-DEA<sub>80</sub>-stat-GEMA<sub>12</sub>) glycopolymer in the mixture of THF/H<sub>2</sub>O (1:3, v/v) at an initial concentration  $(C_{ini})$  of 2.0 mg/mL in THF. THF was then removed by dialysis. <sup>1</sup>H NMR study of the glycopolymersome in D<sub>2</sub>O (Figure S6B) reveals the structure of glycopolymersome. The signal from the hydrophilic PEO chains suggests that they form the coronas of the glycopolymersome. The attenuated signal from the whole P(CMA<sub>8</sub>-stat-DEA<sub>80</sub>-stat-GEMA<sub>12</sub>) block indicates that it forms the membrane of the glycopolymersome along with a fraction of hydrophilic PGEMA moieties embedded in the membrane. Full discussions on the NMR analysis of the glycopolymersomes are provided in the Supporting Information (Figure S6). The structure of the glycopolymersome is schematically presented in Scheme 2.



Figure 1. Electron microscope studies of un-cross-linked glycopolymersomes: (A) and (B) TEM images of glycopolymersomes stained by neutral phosphotungstic acid; (C) TEM images of Con A-bound glycopolymersome (un-cross-linked sugar sponge); (D) and (E) SEM images of un-cross-linked glycopolymersomes; (F) Simulation of a totally collapsed glycopolymersome in the SEM study, suggesting a membrane thickness of 24 nm. The short red lines in E and F indicate two layers of membrane (2d).

Furthermore, TEM and SEM were applied to reveal the morphology of glycopolymersomes. As shown in Figure 1A and B, the TEM images confirmed a classical collapsed membrane structure of glycopolymersomes. As mentioned before, the hydration of the embedded glucose moieties decreased the density of the membrane, resulting in the softening of the glycopolymersomes. Therefore, the glycopolymersomes were liable to collapse from different directions. This phenomenon was further verified by the SEM studies in Figure 1 where some bowl-shaped dry glycopolymersomes with a fully collapsed membrane structure (from top to bottom) and partially collapsed glycopolymersomes were visible. The average diameter of the glycopolymersomes is *ca.* 390 nm (Figure 1A), which is consistent with the dynamic light scattering (DLS) analysis where the hydrodynamic diameter  $(D_{\rm h})$  is 362 nm and

the polydispersity (PD) is 0.091 (Figure S8 in the Supporting Information), considering the collapse at dry state and the hydration in aqueous solution. The membrane thickness is *ca.* 24 nm (Figure 1F), which is consistent with the theoretical value (25.1 nm) as calculated in Scheme S1 in the Supporting Information.

The  $\zeta$ -potential of glycopolymersome is + 46.6 mV (Figure S9 in the Supporting Information), indicating that the hydration of GEMA in the glycopolymersome membrane facilitates the solubility of the DEA block in water. The positively charged glycopolymersome can interact with negatively charged Con A and then facilitate the recognition and binding between Con A and GEMA. Also, both hydrophilic PEO coronas and strong positive charges can maintain the excellent stability of glycopolymersomes in water, which is important for their long circulation *in vivo*.

Immobilization of the Vesicular Structure of Glycopolymersomes by Photo-Cross-Linking of Coumarin Moieties. To immobilize the vesicular structure of glycopolymersomes, the coumarin group was introduced to the glycopolymer, which can undergo photodimerization upon UV irradiation ( $\lambda \approx 365$  nm) to afford inter-chain covalent bonds (Figure 2). TEM images in Figure 3 revealed that the photo-cross-linking procedure didn't change the structure of the glycopolymersomes. DLS studies indicated that the photo-cross-linking procedure didn't change the size of glycopolymersomes (Figure S8). Besides, the UV-vis spectroscopy was employed to monitor the photo-cross-linking procedure. Figure 2 showed that the degree of cross-linking increased with the exposure time under UV irradiation, suggesting that the degree of cross-linking can be precisely controlled by adjusting the exposure time.



Figure 2. UV-vis spectra of photo-cross-linked glycopolymersomes with different degrees of cross-linking.

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Figure 3. TEM images of cross-linked glycopolymersomes without Con A binding.

Preparation of Sugar Sponges and Study on the Carbohydrate-Lectin Interactions. The stability of the un-cross-linked glycopolymersomes in HEPES buffer was evaluated first as this is the solution for sugar sponge preparation. As shown in Figure 4A, the diameter of the un-cross-linked glycopolymersomes barely changed at different concentrations of HEPES buffers, indicating the ionic strength did not influence the stability of glycopolymersomes. Then, the Con A-bound glycopolymersomes (un-cross-linked sugar sponge) were prepared by the recognition between Con A and glucosyl of glycopolymer based on the carbohydrate-lectin interaction. The uncross-linked sugar sponge with a concentration of FITC-Con A of 75 µg/mL was exposed under UV irradiation for 5 seconds to give a degree of cross-linking of 50% (Figure 2). The inter-chain covalent bonds ensured the stability of the vesicular structure and immobilized Con A on the membrane but did not entirely fix the structure of the glycopolymersome to provide excellent swelling/shrinkage properties. Thus the photo-cross-linked Con A-bound glycopolymersome could be regarded as a cross-linked sugar sponge.

Lectins are proteins capable of specific binding to carbohydrates. Con A is a lectin with high affinity for glucose and mannose.<sup>15</sup> Herein, the un-cross-linked FITC-Con Abound glycopolymersome (un-cross-linked sugar sponge; here FITC is fluorescein isothiocyanate) was utilized to demonstrate the specificity of carbohydrate-lectin interactions. DLS was applied to monitor the recognition between FITC-Con A and glycopolymersomes. Figure 4B revealed that the size of glycopolymersomes increased with the concentration of FITC-Con A due to the formation of Con A-bound glycopolymersome by the recognition of the glucosyl embedded in the membrane and the FITC-Con A in the solution. For example, the diameters of this sugar sponge are 375, 390, 425, 465, 504, 522 and 537 nm when the concentration of FITC-Con A increases from 50 to 200 µg/mL (Figure 4B and Figure S10A). TEM was applied to reveal the morphology of the uncross-linked sugar sponge (Figure 1C). Comparing with the un-cross-linked glycopolymersomes (without Con A) in Figure 1A, the membrane thickness of the un-crosslinked sugar sponge (glycopolymersomes with Con A) is

bigger due to the binding of Con A in the membrane of the glycopolymersomes.

It is noteworthy that the Con A binds the glucosyl groups only within the individual glycopolymersomes instead of inter-glycopolymersomes. Otherwise, the diameter of the glycopolymersomes would be expected to greatly increase. Therefore, there is little chance for Con A to bridge several glycopolymersomes together since the glucosyls were embedded into the membrane. Furthermore, Sepharose column was used to purify the un-crosslinked sugar sponge to remove free Con A (read details of that in the Supporting Information). After flowing through Sepharose column, the size of the un-crosslinked sugar sponge almost unchanged (Figure S11 in the Supporting Information), further confirming that the Con A was immobilized within the glycopolymersome.

In order to determine the loading efficiency of FITC-Con A on the glycopolymersomes, the calibration curve of FITC-Con A in HEPES buffer was plotted by fluorescence spectrometry (Figure S12). The fluorescence intensity of the un-cross-linked sugar sponge was 5797 at 520 nm after Sepharose column (Figure S13), corresponding to a Con A loading efficiency of 34%. The electrostatic interactions between DEA moieties and FITC-Con A contributed the high loading efficiency, which can be confirmed by Zeta potential studies before and after Con A binding (the  $\zeta$  value decreased from + 46.6 mV to + 10.8 mV after Con A binding, see Figure S9).



Figure 4. DLS studies of glycopolymersomes before and after cross-linking: (A) Stability of *un-cross-linked* glycopolymersomes against HEPES buffers; (B) Carbohydrate-lectin interactions between *un-cross-linked* glycopolymersomes and Con A as revealed by the variation of hydrodynamic diameters; (C) and (D) confirm that no glucose-breathing behavior for *un-cross-linked* (C) and *cross-linked* (D) glycopolymersomes without Con A binding.

**Sugar Breathing Study of Sugar Sponges Based on Carbohydrate-Lectin Interactions.** In order to verify that the sugar breathing behavior is only related to the carbohydrate-lectin interactions, control experiments were performed first using the un-cross-linked and crosslinked glycopolymersomes without the binding of Con A. As shown in Figure 4C and D, the diameters of the glycopolymersomes stay almost constant at different concentrations of glucose, which means both the un-cross-linked and cross-linked glycopolymersomes (without Con A embedded in the membrane) in glucose do not have the sugar-breathing function. By contrast, both the un-crosslinked and cross-linked glycopolymersomes with FITC-Con A embedded in the membrane possess a similar "breathing" behavior at different concentrations of glucose because of the carbohydrate-lectin interactions (Figure 5A and C). Thus, both the un-cross-linked and cross-linked Con A-bound glycopolymersomes (un-cross-linked and cross-linked sugar sponges) have sugar breathing functions, but the cross-linked sugar sponge is supposed to have a longer circulation time due to its better stability.



Figure 5. DLS and TEM studies of sugar sponges without and with cross-linking: Glucose breathing behavior of the *uncross-linked* sugar sponge (A) and the *cross-linked* sugar sponge (C) as revealed by the variation of hydrodynamic diameters; (B) Reversibility study of *cross-linked* sugar sponges determined by the variations in the hydrodynamic diameters when the glucose concentrations were switched between 0.5 mg/mL and 1.25 mg/mL; (D) Corresponding schematic illustration of breathing behavior; (E-F) TEM images of one cross-linked sugar sponge ( $C_{con A} = 75 \mu g/mL$ ) in the absence (E) and in the presence (F) of glucose ( $C_{glucose} = 0.75 mg/mL$ ), showing a remarkable sugar-induced swelling behavior of sugar sponge.

The Con A binds the glucosyl in the sugar sponge. However, in the presence of glucose, the Con A will bind the glucose instead of the glucosyl due to the Con A's higher affinity for glucose than for glucosyl. As shown in Figure 5C and Figure StoB in the Supporting Information, the size of the cross-linked sugar sponge increases from 390 to 701 nm as the concentration of glucose increases from o to 1.5 mg/mL. TEM images of the cross-linked sugar sponge before (Figure 5E and Figure St4) and after glucose binding (Figure 5F and Figure St5) also confirmed the swelling of the cross-linked sugar sponge after binding glucose (See Figures St4-St5 in the Supporting Information for more TEM images).

Considering the significant degree of swelling of sugar sponges with glucose (Figure 5 A and C), those sugar sponges can be applied in glucose detection. Since mannose can be produced from glucose *in vivo* and this may be a confounding issue in glucose detection, it was necessary to evaluate the breathing behavior of the sugar sponges against mannose.<sup>2,58</sup> As shown in Figure S16 in the Supporting Information, both un-cross-linked and

cross-linked sugar sponges have mannose-breathing functions, as confirmed by the swelling of sugar sponges with mannose. However, those sugar sponges can be still used for monitoring blood sugar levels as only small fraction of mannose exists *in vivo* (the ratio of mannose to glucose is *ca.* 1:100) and mannose undergoes fast clearance from blood ( $t_{1/2} = 30$  min).<sup>59</sup> Furthermore, extra mannose in the blood can be stored in the sugar sponge and released when needed since mannose cannot be metabolized directly.

The blood glucose level in human body is tightly regulated at a fluctuant range of  $3.9 \sim 6.1 \text{ mM}$  ( $0.7 \sim 1.1 \text{ mg/mL}$ ). TEM images show that the diameter of the cross-linked sugar sponge is around 600 nm when the concentration of glucose is 0.75 mg/mL (Figure 5F and Figure S15). These TEM images also confirm decreased membrane density of the cross-linked sugar sponge, indicating its tunable permeability. As the concentration of the glucose increases to a level higher than the normal blood glucose level (1.25 mg/mL), the cross-linked sugar sponge will absorb extra glucose. Figure 5D illustrated

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59 60 this binding process: the glucose will diffuse into the membrane and bind Con A, substituting the glucosyl on the polymer chain because of competitive effect, resulting in swelling of the cross-linked sugar sponge. Consequently, the diameter of the cross-linked sugar sponge increases to around 650 nm (Figure 5B). On the contrary, the glucose will be released from the cross-linked sugar sponge to the solution when the concentration of sugar decreases. This responsive glucose release was studied *in vitro*. As shown in Figure S17 in the Supporting Information, the glucose release content is lower compared with free glucose at the same concentration.



Figure 6. Antidiabetic test of HEPES buffer, glycopolymersome, Con A and sugar sponge to KM mice.

Cytotoxicity of Glycopolymersome. The cytotoxicity of glycopolymersome against normal liver Lo2 cells was studied (Figure S18 in the Supporting Information) as the sugar sponges contains toxic coumarin. However, the glycopolymer only contains 9.8 wt.% of coumarin moieties. The toxic coumarin monomer is polymerized and it eventually becomes one part of the glycopolymersomes for long circulation in blood. It is noteworthy that the hydrophobic coumarin moieties are embedded in the membrane of glycopolymersomes, preventing the contact with cells. Furthermore, no coumarins would be released from the glycopolymersome. Therefore, in principle, the glycopolymersomes should not be toxic. In reality, the relevant cell viability against normal liver Lo2 cells is around 80 % when the concentration of glycopolymersome is 1000 µg/mL (Figure S18), suggesting low cytotoxicity of glycopolymersomes against human cells.

*In Vivo* Antidiabetics Test. Although both type I and type II models can be used to evaluate the antidiabetic effect of the sugar sponge as it can regulate the blood glucose level independent of the type of diabetes mellitus, it is necessary to compare the antidiabetic effect of our sugar sponge with insulin in proper model. Type I diabetes occurs in about 5-15 percent of all the diabetics.<sup>3,60</sup> It is insulin-dependent and relies on the frequent subcutaneous injection of insulin to regulate blood glucose level. People with type I diabetes are seriously ill from sudden symptoms of high blood sugar. The high blood sugar phenotype is stable in this model. Compared with type II diabetes lasts lifelong and makes more

serious damage to related organs. Therefore, type I diabetes model was developed for the *in vivo* test of the sugar sponge.

In order to build the type I diabetic mice model, 31 male KM mice were injected intraperitoneally with streptozocin (120 mg/kg) in citric buffer after fasting for 12 h. A week later, the weight of the mice changed from  $40.8 \pm 2.5$  g to  $38.8 \pm 3.1$  g, while the blood glucose level increased from  $6.8 \pm 1.4$  mmol/L to  $22.3 \pm 3.5$  mmol/L. The mice with blood glucose level of 18 - 26 mmol/L were regarded as diabetic mice,<sup>61</sup> giving a modeling success rate of 74%.

The *in vivo* studies of the antidiabetic efficacy of sugar sponge were performed using these diabetic KM mice. We monitored the blood glucose level of the diabetic mice at 2, 6, 12, 24, 36, 48 and 75 h after injection of different solutions. In 2 h, the buffer in the solutions injected into the caudal vein is almost metabolized, so the dilution effect of the solution injected barely influences the blood glucose level.<sup>62</sup> The second measurement point is 6 h (after fasting 2 h) because the blood glucose level after fasting for 2 h reflects the true influence of eating.<sup>63</sup> Since the focus of this study is the long-term antidiabetic effect, we did not monitor the glucose level frequently, instead we set the measurement interval for 12 h or more.

The diabetic mice were treated with the following solutions: (1) HEPES buffer; (2) glycopolymersome; (3) Con A; and (4) sugar sponge. The normal mice treated with (5) HEPES buffer were regarded as the control. In the first 2 hours, the blood glucose levels in groups 1, 2 and 5 slightly descended because of the injection of solutions (Figure 6). However, as time went by (for 6 h), the blood glucose level of mice rose to the initial level before injection, indicating that the injection of glycopolymersome and HEPES buffer only slightly affected the blood glucose level in a very short time. By contrast, the blood glucose level of mice dramatically decreased in 2 hours in groups 3 and 4. But for group 3, the duration of efficacy is very short due to its rapid proteolysis by degradation, as confirmed by the quick recovery of the blood glucose level after 6 h. Moreover, the mice died after 36 h due to the high cytotoxicity of Con A. Notably, the blood glucose level of diabetic mice dropped to normal range after the injection of sugar sponge, and maintained at about 11 mM at least 36 h, and increased gradually in 3 days. Comparing to free Con A, the sugar sponge showed long-term efficacy of regulating blood glucose level of diabetic mice and greatly reduced the cytotoxicity of Con A.

#### CONCLUSION

In conclusion, a novel glycopolymer, PEO-*b*-P(CMA-*stat*-DEA-*stat*-GEMA), was synthesized and self-assembled into glycopolymersomes. The PDEA segment can enhance the carbohydrate-lectin interactions, resulting in a high loading efficiency of Con A (34%) on the glycopolymersomes. Those Con A-bound glycopolymersomes are sugar sponges and functionally similar to artificial glycogen. The photo-cross-linking of coumarin moieties fixes

the structure of the glycopolymersome and immobilizes the Con A within the membrane of glycopolymersome to afford the cross-linked sugar sponge. Those sugar sponges possess excellent reversible glucose responsiveness, showing a glucose regulating behavior: At high glucose concentration, the sugar sponges "breathe in" and bind glucose into the membrane, resulting in the swelling of the sugar sponges. By contrast, the sugar sponges "breathe out" glucose and shrink at low glucose concentrations. This obvious size change induced by sugar replacement can be used for monitoring glucose levels. Moreover, the in vivo test confirmed excellent antidiabetic effect to type I diabetic mice within two days upon one dose of sugar sponge. Overall, our study demonstrates a new insight for regulating blood glucose level without using insulin or other hypoglycemic drugs, which opens new avenues for the treatment of diabetes mellitus.

### ASSOCIATED CONTENT

**Supporting Information**. The experimental section and characterization of CMA monomer, GEMA monomer and glycopolymer; DLS and Zeta potential studies, TEM images, determination of loading efficiency of FITC-Con A, *etc.* This material is available free of charge via the Internet at http://pubs.acs.org.

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#### Author Contributions

<sup>1</sup>Y.X. and H.S. contributed equally.

#### Notes

The authors declare no financial competing interest.

#### ACKNOWLEDGMENT

J.D. is supported by NSFC (21374080, 21674081 and 21611130175), Shanghai International Scientific Collaboration Fund (15230724500), Shanghai 1000 Talents Plan (SH01068), and the Fundamental Research Fund for the Central Universities (1500219107). We appreciate Prof. Yao Sun in The Affiliated Stomatology Hospital of Tongji University, Prof. Shisheng He, Dongdong Wang and Shaobo Xue in Shanghai Tenth People's Hospital for their support in the animal test.

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