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Glucose-responsive shape-memory cryogels

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ABSTRACT

Boronic ester bonds can be reversibly formed between phenyl-boronic acid (PBA) and triol moieties. Here, we aim at a glucose-induced shape-memory effect by implementing such bonds as temporary netpoints, which are cleavable by glucose and by minimizing the volume change upon stimulation by a porous cryogel structure. The polymer system consisted of a semi-interpenetrating network (semi-IPN) architecture, in which the triol moieties were part of the permanent network and the PBA moieties were located in the linear polymer diffused into the semi-IPN.

In an alkaline medium (pH = 10), the swelling ratio was approximately 35, independent of C_{glu} varied between 0 and 300 mg·dL^{-1}. In bending experiments, shape fixity $R_f \approx$ 80% and shape recovery $R_r \approx$ 100% from five programming/recovery cycles could be determined. R_r was a function of C_{glu} in the range from 0 to 300 mg·dL^{-1}, which accords with the fluctuation range of C_{glu} in human blood. In this way, the shape-memory hydrogels could play a role in future diabetes treatment options.

Keywords: shape memory, polymer, porosity

I. INTRODUCTION

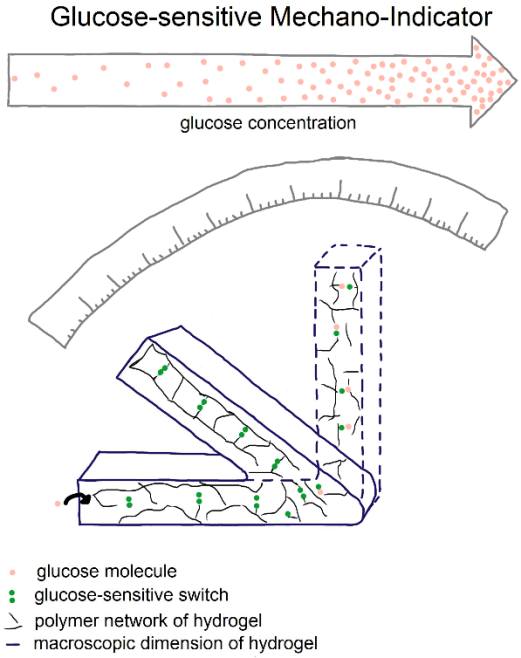
Glucose is a representative in the group of most important biomolecules as it is the primary source of energy and a metabolic intermediate for living cells.[1] For a healthy

human, blood glucose should maintain at a concentration level ranging from 70 to 100 $mg \cdot dL^{-1}$, otherwise diseases like hypoglycemia or diabetes would be caused.[2] Accordingly, there is strong interest in monitoring the glucose level. Recently, glucose-responsive polymer systems, which are capable to show physical or chemical changes in response to glucose, have been designed.[3, 4] The systems are for example expected to automatically detect the blood glucose level and further control the release of insulin in response to the glucose level, which potentially provides a painless treatment for diabetes compared to insulin injection. Three different categories of glucose-responsive systems can be differentiated: glucose oxidase (GOD), lectin, and phenylboronic acid (PBA) based systems.[3, 5] Applications of these systems are focused on either diagnosis of glucose concentration or controlled release of insulin.[6] As monitoring of the blood sugar level requires access to the blood system, which is typically realized by painful injection, other less painful methods have been developed to determine the glucose level. Among them, microneedle systems, in which a microarray of needles, typically fabricated from silicone is used to pass the transdermal barrier.[7] Microneedle-based *ex vitro* as well as *in vivo* sensors for glucose monitoring are under investigation. *Ex vitro* microneedle-based sensors could be realized by filling the microneedle cavities with carbon fibers, which contain immobilized GOD.[8] Upon the presence of glucose, H_2O_2 is generated, whose presence can be amperiometrically monitored.[9] *In vivo* sensors could be designed, which were capable to monitor the glucose level based on this technique, by accessing the interstitial fluid in the epidermis.[10] Although by this design the colorimetrical determination of glucose in interstitial fluid could be advanced[11], it remains an indirect method.

Among glucose-responsive systems, the PBA-based system shows some advantages as it is, when compared to the other two protein-based systems, a direct method and is supposed to be more stable and should not cause undesirable immune response.[3, 12-14]

PBA and its derivatives change from the uncharged to the charged state once the environmental pH value increases.[15-17] In alkaline media, the charged boronic acid groups undergo a dynamic condensation reaction with 1,2- or 1,3-diols whereby five- or six-membered cyclic esters are formed. In contrast, in acidic media, the cyclic esters are cleaved again, whereby uncharged boronic acid and the polyol are released. On the other hand, the formation of PBA/polyol complexes in alkaline media is an equilibrium reaction, whereby the two different polyols compete in their reaction with the PBA

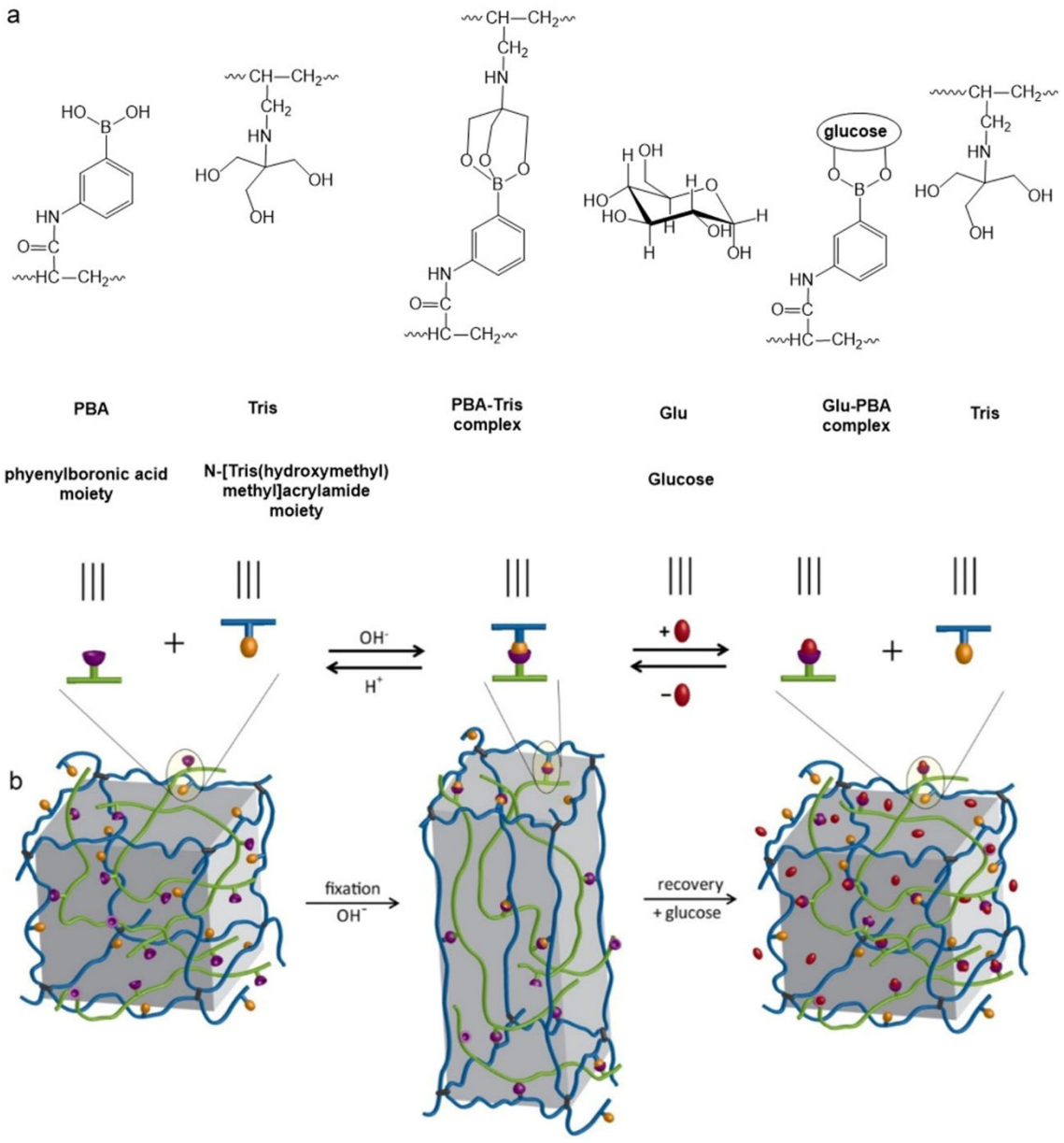
groups according to their association constants (K_{eq}).[18, 19] Recently, the implementation of PBA groups into hydrogels has been reported to achieve sensitivity toward glucose.[20, 21] PBA-based monomers, such as acrylamidophenylboronic acid (AAPBA), were copolymerized with acrylic acid (AA)[16], acrylamide (AAm)[19], *N*-vinyl-2-pyrrolidone (NVP)[22], *N*-isopropylacrylamide (NIPAAm)[12, 15, 23], or *N*,*N*-dimethylacrylamide (DMAA)[24, 25], or other monomers to gain PBA-functionalized water-soluble copolymers. PBA-based hydrogels were obtained by reacting these copolymers with macromolecular polyols like poly(vinyl alcohol) (PVA) in an alkaline aqueous medium. In a condensation reaction between PBA and polyol groups, covalently cross-linked polymer networks are formed. When glucose is added, the glucose molecules competitively react with the PBA groups and in this way, the polyol groups of PVA are substituted. As a result, the hydrogels swell in response to the glucose concentration (C_{glu}) as the average molecular chain lengths between two netpoints increases. Utilizing this shape-change capability (SCC) of the glucose-sensitive hydrogels, various glucose-concentration detection devices and insulin delivery systems have been developed.[3, 26] However, this volume change is omnidirectional and does not allow complex movements.



Scheme 1: Concept of a glucose-sensitive hydrogel providing a mechanical read-out.

We speculated that a glucose-sensitive hydrogel could be designed, which is capable of a shape-memory effect (SME) in response to glucose and provides in contrast to SCC complex and controllable movement upon stimulation (**Scheme 1**). The shape-

memory hydrogels (SMHs) should be able to be deformed into any arbitrary shape and then be capable to fix this temporary shape by immersion in an alkaline glucose-free medium. If the environmental glucose concentration (C_{glu}) would be sufficiently high, the hydrogel should recover its original (permanent) shape. As the highest glucose concentration we selected 300 mg dL^{-1}, which is significantly higher than the glucose concentration of a healthy person but still in the physiological relevant regime.



Scheme 2. a) Reversible formation of covalent bonds between the AAPBA copolymer and the AATris copolymer in response to pH or glucose. b) Schematic representation of the glucose-induced SME.

Our concept to realize such glucose-sensitive shape-memory hydrogels is a semi-interpenetrating polymer network (semi-IPN), in which the covalent netpoints from a polymer network from poly(AAm-*co*-AATris-*co*-BIS) determines the permanent shape whereas a secondary network by immobilization of poly(AAm-*co*-AAPBA) into the primary network forms the semi-IPN and provides glucose-sensitivity (**Scheme 2**). In both components, the AAm provides swellability to the system. The boronic ester bonds, formed between the PBA groups and the triol groups from AATris of the primary network, act as the reversible cross-links and are capable to fix the temporary shape. Upon the presence of glucose, the glucose molecules competitively exchange with the PBA-triol bonds to form PBA-glucose bonds: the temporary cross-links are cleaved and the shape-recovery process is initiated. However, the recovery process will hold at an intermediate state when the glucose concentration C_{glu} is that low that still PBA-triol bonds are present. In this way, a glucose-concentration indicator can be realized. The reversible formation of covalent bonds between the AAPBA copolymer and the AATris copolymer (or glucose) as well as the glucose-induced SME are schematically shown in **Scheme 2**(a) and **Scheme 2**(b) respectively.

In the following, we describe the synthesis of the precursor materials and of the semi-IPN, characterize the swelling capability as well as the change of rheology in terms of the pH and glucose concentration, and demonstrate the applicability of this concept in a simple and intuitional experiment to demonstrate the usage as a glucose-concentration indicator.

II. EXPERIMENTAL DETAILS

A. Materials

Acrylamide (AAm, ≥98%, Alfa Aesar, Karlsruhe, Germany), acrylic acid (AA, ≥99%, Fluka, Buchs, Switzerland), *N*-[Tris(hydroxymethyl) methyl]acrylamide (AAtris, ≥98%, Alfa Aesar, Karlsruhe, Germany), *N,N′*-methylenebisacrylamide (BIS, ≥99%, Merck, Darmstadt, Germany), m-aminophenylboronic acid monohydrate (AAPBA, 97%, Alfa Aesar, Karlsruhe, Germany), 1-ethyl-3-(3-methylaminopropyl)carbodiimide (EDC, 98%, Alfa Aesar, Karlsruhe, Germany), D-(+)-glucose (anhydrous, Alfa Aesar, Karlsruhe, Germany), *N,N,N′,N′*-tetramethylethylenediamine (TEMED, ≥98%, Merck, Hohenbrunn, Germany), and ammonium persulfate (APS, ≥98%, Merck, Darmstadt, Germany) were used as received. Buffer solution with a pH value of 10 was made by

combining Na_2HPO_4 and NaOH, the ionic strength of the buffer was adjusted to approximately 0.15 mol L^{-1} by addition of NaCl. *m*-Acrylamidophenylboronic acid (AAPBA) was synthesized and characterized according to the procedure described in reference [24].

B. Methods:

780 mg Acrylamide (AAm) and 120 mg *m*-acrylamidophenylboronic acid (AAPBA) were dissolved in 8 mL water to form a monomer solution in which the molar ratio of AAm:AAPBA was approximately 95:5. After 800 µL of a 4 wt% APS aqueous solution and 80 µL TEMED were added, the copolymerization was performed at 20 °C for 4 h to yield a solution of poly(AAm-*co*-AAPBA). The copolymer was purified by precipitation in ethanol for two times. Subsequently, 120 mg poly(AAm-*co*-AAPBA), 130 mg AAm, 16 mg *N*-[Tris(hydroxymethyl) methyl]acrylamide (AATris), and 0.5 mg methylenebisacrylamide (BIS) as cross-linker were dissolved in 1.5 mL deionized water in a glass vessel with an inner diameter of 25 mm (to obtain discs to measure the mechanical strength) or in a polypropylene tube with an inner diameter of 2 mm (to prepare stripes to characterize the shape-memory properties). Microporous hydrogels were synthesized by polymerization of the pre-reaction media at -18 °C for 24 h after adding 25 µL 4wt% APS aqueous solution and 5 µL TEMED. The nonporous hydrogels were synthesized at room temperature with the same feed compositions like the microporous hydrogels except that 100 µL 4wt% APS was added. Afterwards, the obtained hydrogel samples were immersed in excessive deionized water at ambient temperature for 72 h and then immersed in buffer solutions with C_{glu} between 0 and 300 mg·dL^{-1} for at least 12 h. After wiping the excessive water off with a wet filter paper (the given C_{glu} and buffer of pH = 10) the gel samples were measured gravimetrically. The swelling ratio (SR) is calculated as W_s/W_d, where W_s is the weight of the water in the swollen sample at a given condition and W_d is the weight of the dry sample.

^{1}H-NMR spectra were recorded on a 500 MHz Avance spectrometer (Bruker, Karlsruhe, Germany) in deuterated water. pHs were determined with a pH meter (SevenEasy S20, Mettler Toledo, Schwerzenbach, Switzerland). Dynamic viscoelastic properties of the SMC were performed with a rheometer (HAAKE MARS Rheometer, Thermo Electron, Karlsruhe, Germany) using parallel plates (Platte PP20 Ti) of 20 mm diameter. Before the measurements, the samples were immersed in buffer solutions with pH = 10 and the given C_{glu} for at least 12 h. The amplitude sweep was carried out with a frequency (f) of 1 Hz to determine the linear viscoelasticity region. Then, the

frequency sweep was performed in a frequency range between 0.1 and 10 Hz at a constant shear strain (γ) of 0.001. At this strain, according to the amplitude sweep, the samples were in the linear viscoelastic region. All rheology measurements were carried out at 25 ± 0.1 °C controlled by a Peltier plate. Scanning electron microscopy (SEM) was performed using a Zeiss Gemini, Supra 40VP (Zeiss, Jena, Germany) at an accelerating voltage of 3 kV, equipped with an Everhard Thornley Detector. Samples were sputtered with 4 nm Au/Pd. Cross sections were cut with a blade.

The shape-memory capability of the SMC was characterized in bending tests as follows: A straight strip (ø2 mm × 20 mm) of the cryogel was folded after immersion in pH = 2 buffer solution for 30 min and then fixed by immersion at ambient pH = 10. After a time period of 30 min, the external force was removed, and then, the fixed angle θ_f of the samples was recorded (**Figure 1**). The strain fixity ratio (R_f) is calculated as θ_f/180°. For the glucose-induced recovery test, the specimen was transferred into a buffer solution with the given C_{glu} and pH = 10. After 30 min, the angle θ_f was recorded. The strain recovery ratio (R_r) is calculated as (180° - θ_f)/180°. Measurements were repeated following these three steps: washing with acid solution (regeneration), alkaline fixation (programming), and glucose-induced recovery.

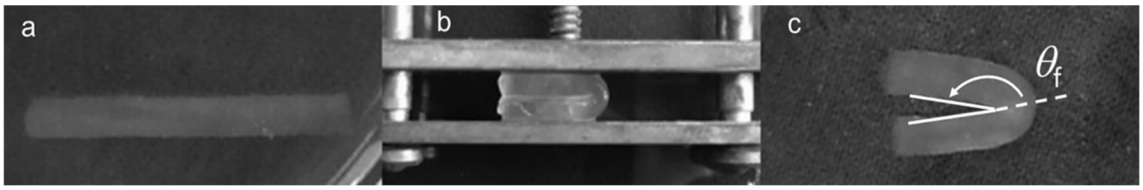


Figure 1 Bending test procedure to determine the shape fixity ratio R_f of the SMC: a) the cryogel sample was immersed in an acid solution of pH = 2 for 30 min; then b) the straight sample was folded and immersed in buffer with pH = 10 whereby the external stress was maintained; and c) after the external stress was removed, the fixed angle of the sample's temporary shape was recorded.

III. RESULTS AND DISCUSSION

A. SYNTHESIS

The glucose-sensitive SMHs were designed as a semi-interpenetrating polymer networks (semi-IPN), in which the permanent shape of the poly(AAm-*co*-AATris-*co*-BIS) network (primary network) was set by the covalent netpoints from the non-glucose-

sensitive cross-linker BIS. Immobilization of poly(AAm-*co*-AAPBA) into the primary network formed the semi-IPN.

A molar ratio between the functional groups has been calculated from the peak areas of the relative chemical groups in the ^{1}H-NMR spectra (**Figure 2**) according to equations (**1**) and (**2**). The molar ratio of the phenylboronic acid (PBA) groups relative to the repeating units in poly(AAm-*co*-AAPBA) and for the semi-IPN by the molar ratio of the PBA groups relative to the Tris(hydroxymethyl) methyl (Tris) groups:

$$\text{PBA mol\%} = \frac{1}{4} \times I_{ArH} : I_{COCH} = 5.05\% \qquad \textbf{(1)}$$

$$\text{PBA:Tris} = \frac{1}{4} \times I_{ArH} : \frac{1}{6} \times I_{C(CH_2)_3} = 92.3\% \qquad \textbf{(2)}$$

Conclusively, the molar ratio of the functional groups in the SMC is almost the same when compared to the feed composition as the polymerizable acrylamido (CH_2=CH-CO-) group is common for both monomers.

Two kinds of glucose-sensitive SMHs were prepared: films with a microporous structure and nonporous films. SMHs with microporous structure were synthesized through a cryo-polymerization method.[27] These microporous hydrogels, so-called cryogels, were denoted as SMC (shape-memory cryogels).

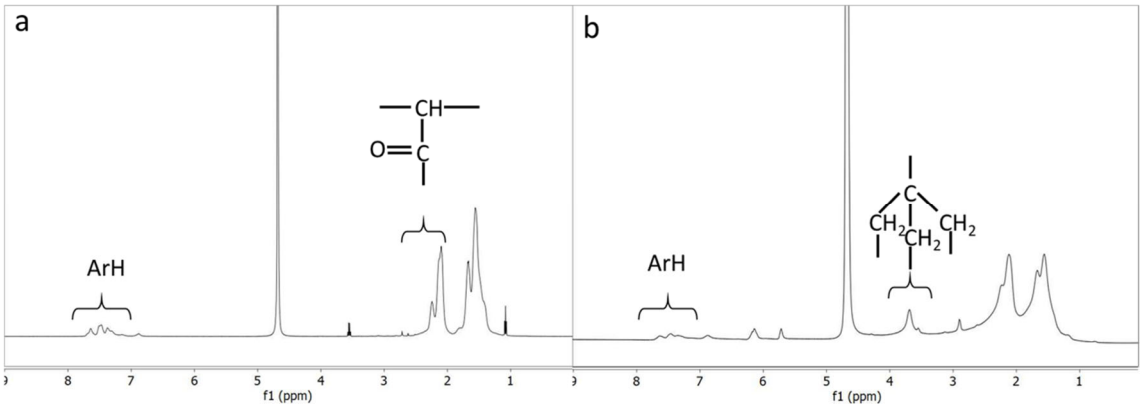


Figure 2 ^{1}H-NMR spectrum (500 MHz, D_2O) of a) the linear polymer poly(AAm-*co*-AAPBA) and b) the SMC semi-IPN.

B. SWELLING STUDIES

Swelling ratios were determined to measure the volume change of hydrogels. **Figure 5a** shows the swelling ratios of the SMC as a function of C_{glu}. The porous SMC provides

high volume stability when C_{glu} is varied. In an alkaline medium (pH = 10), the SR maintained at approximately 35 regardless of the variation of C_{glu} from 0 to 300 mg dL^{-1} so that swelling is neglectable. In contrast, the volume of the nonporous SMH increased remarkably with C_{glu} due to the cleavage of the temporary crosslinking netpoints (see **Figure 3** for the swelling ratio of the nonporous SMH reference sample). As this shape change by swelling interferes with the shape change caused by the shape-memory effect of the microporous hydrogel films were considered for the subsequent experiments only.

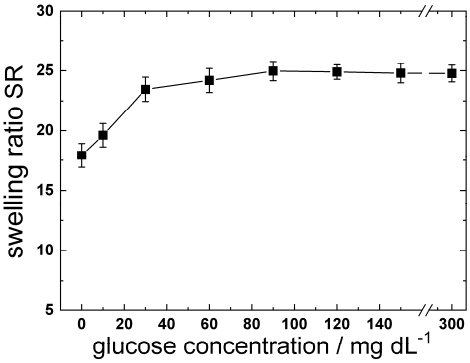


Figure 3 Swelling ratio of the nonporous SMH in solutions with various C_{glu}.

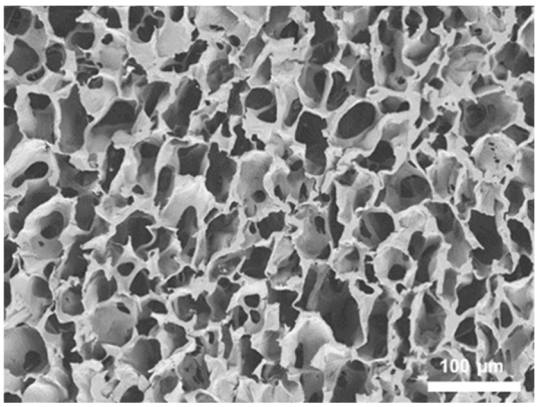


Figure 4 Morphology of the freeze-dried SMC observed by scanning electron microscopy (SEM).

In the porous cryogel matrix, the swelling of pore walls is directed into the pore volume and by this the sample maintains the outer dimension (see **Figure 4** for the morphology of the SMC in the dry state). The water content within the cryogel is approximately 97% calculated from the swelling ratio, implying that the cryogel is highly hydrophilic. In addition, the SMC is of high mechanical stability[27] and shows promising benefits in biotechnological applications such as immobilization of biomolecules and cells.[28]

C. RHEOLOGY

Mechanical performance of the swollen cryogel in solutions with different C_{glu} values was characterized by dynamic viscoelastic studies. The obtained storage shear modulus G' in the C_{glu} range from 0 to 300 mg·dL^{-1} were used to demonstrate elastic mechanical strength of the cryogel as shown in **Table 1**. It should be noted that the moduli are in the range of biological tissue. G' decreases remarkably from approximately 3800 Pa to 2200 Pa when C_{glu} increases from 0 to 90 mg·dL^{-1}. The decrease of G' can be attributed to the disassociation of the temporary cross-links as the glucose molecules exchange with the PBA-triol bonds by forming PBA-glucose bonds, which cannot act as netpoints. In the C_{glu} range from 90 to 300 mg·dL^{-1}, the G' shows a slight decrease from approximately 2200 Pa to 2000 Pa. In this C_{glu} range, glucose molecules have cleaved most of the reacted triol groups and most of the temporary cross-links are disassociated.

Table 1 Storage shear modulus G' and swelling ratio S, R_r and angle recovered θ_r of the SMC at pH = 10 at various glucose concentrations C_{glu}.

C_{glu} **[mg dL^{-1}]**	***G'*** **[Pa]**	***S*** **[%]**	R_r **[%]**	θ_r **[°]**
0	3840 ±80	37 ± 2	18 ± 3	148 ± 5
10	3650 ±150	36 ± 2	n.d.	n.d.
30	3200 ± 60	36 ± 2	28 ± 5	130 ± 9
60	2850 ± 60	39 ± 2	50 ± 5	90 ± 9
90	2260 ± 80	38 ± 2	64 ± 4	65 ±7
120	2260 ± 70	35 ± 2	78 ± 3	40 ± 5
150	2140 ± 70	37 ± 2	97 ± 3	5 ± 5
300	2010 ± 90	38 ± 2	99 ± 1	2 ± 2

D. SHAPE-MEMORY PROPERTIES

The recovery process of a SMC sample from the fixed shape (folded strip) to the permanent shape (straight strip) is shown in **Figure 6**. In an alkaline medium with pH = 10 in the absence of glucose, the folded strip could retain a bending angle of approximately 140° (Figure 6a). Upon the addition of glucose solution, the bending angle of the SMC demonstrator decreased with C_{glu}. The equilibrium bending angle correlated to C_{glu} of the environment. When the C_{glu} was 30 mg·dL^{-1} (Figure 6b), 60 mg·dL^{-1} (Figure 6c), and 120 mg·dL^{-1} (Figure 6d), the angle recovered to approximately 130°, 90°, and 40° (Supporting Video S1). In this way, the bending angle of the recovery shape could be used to indicate the environmental C_{glu}. When C_{glu} was as high as 300 mg·dL^{-1}, the strip fully recovered to its permanent shape (Figure 6e). For a certain SMC sample, the recovery force increases with the number of covalent netpoints, whereas the fixation capability increases with the amount of the reversible cross-links. The higher C_{glu} was, the lower was the fixation capability (Supporting Video S2). In this way, a potential application for SMC could be a mechanical glucose-concentration indicator. Compared to other glucose-concentration detectors such as optical sensors[29] and electrochemical sensors[30], this mechanical sensor can easily be synthesized, can be repetitively used, provides a visible read out, and does not reverse if the glucose concentration lowers again.

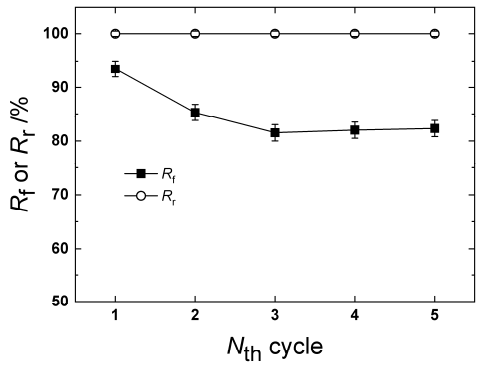


Figure 5 R_f and R_r of the SMC within five glucose-induced programming/recovery cycles (the recovery processes were performed in solutions with C_{glu} of 300 mg·dL^{-1}

The shape-memory properties of the SMC were characterized by quantification of the shape fixity ratio (R_f) and the shape recovery ratio (R_r). R_f quantifies the ability of the material to fix a certain mechanical deformation in the programming process, whereas R_r quantifies the ability of the material to memorize its permanent shape.[31] The R_f and

the R_r of the SMC have been calculated from bending tests to consider a potential volume change of the hydrogel by swelling.[32] **Figure 5** presents the R_f and the R_r within five glucose-induced programming/recovery cycles. After programmed for the first time, the SMC under alkaline condition is capable to fix very well with a high R_f of approximately 93%. In the repeated measurements, R_f decreases considerably. This observation might probably be caused by an insufficient regeneration procedure, in which the glucose molecules are not fully washed out from the cryogel. In this way, a fraction of the PBA groups would still be coordinated with glucose, losing the ability of acting as a temporary cross-linker. However, R_f was still higher than 80% after five cycles. No matter how many cycles were performed, the pre-deformed SMC can be fully recovered to its permanent shape with an R_r of approximately 100% when the environmental C_{glu} is sufficiently high (300 mg·dL^{-1}), implying that the SMC can be reversibly used. In case that C_{glu} is not high enough, the degree of recovery of the SMC changed with the C_{glu} as shown in **Figure 6** and discussed in the former sections. Here, the trend is quantified using R_r, which is also compiled in Table 1. The R_r exhibits an almost linear relationship with C_{glu} in the range from 0 to 150 mg·dL^{-1}, which is also the range of C_{glu} in human blood. When the C_{glu} is higher than 150 mg·dL^{-1}, the SMC can be fully recovered.

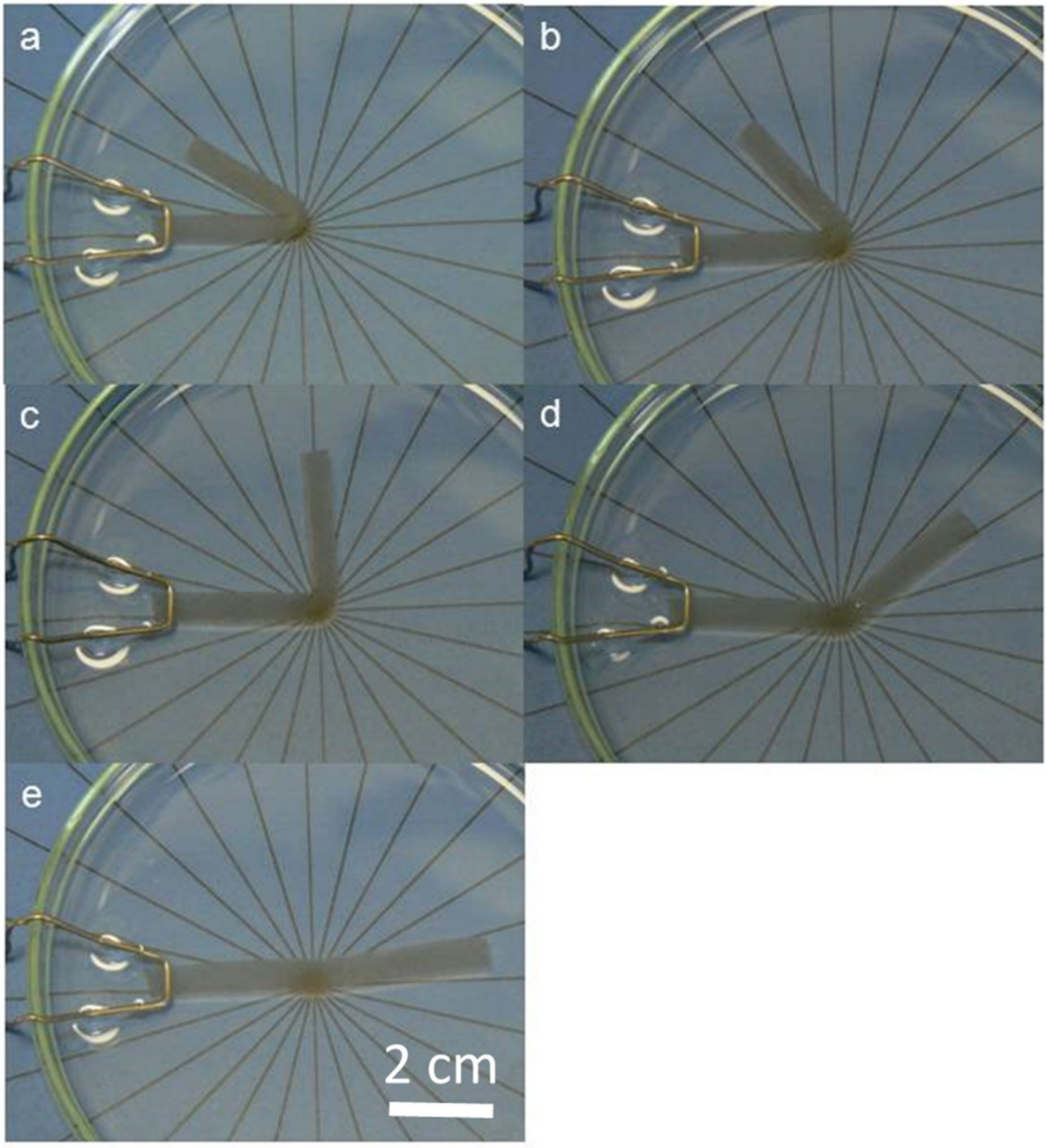


Figure 6 Photo series of shape recovery of the SMC from the folded temporary shape after retaining in solutions with various C_{glu} for 30 min: a) 0 mg·dL^{-1}; b) 30 mg·dL^{-1}; c) 60 mg·dL^{-1}; d) 120 mg·dL^{-1}; and e) 300 mg·dL^{-1}.

IV. CONCLUSIONS

A glucose-induced shape-memory effect was implemented in a microporous cryogel with a semi-IPN architecture. The design of the cryogel's pore morphology ensured

that the volume change with C_{glu} variations did not interfere with the SMC. The elastic modulus of the SMC decreased with C_{glu} due to the diminution of the temporary cross-links. The SMC could be deformed and fixed in a temporary shape under alkaline conditions when no glucose was present. When the SMC was exposed to glucose solution of concentration C_{glu}, recovery toward its permanent shape occurred. The degree of the recovery represented by R_r could be controlled by C_{glu} in the range from 0 to 300 mg·dL^{-1}. In this way, the C_{glu} could be measured using the SMC. Furthermore, the SMC can be repetitively used with $R_f \approx 80\%$ and $R_r \approx 100\%$ in the fifth programming/recovery cycle. As the C_{glu} corresponds well with the fluctuation range of glucose in human blood, the glucose-induced SMC would be a promising material for novel glucose sensors. Furthermore, as the PBA/glucose hydrogels provide elastic properties similar to those of tissue, artificial muscles could be thought of.[33]

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SUPPLEMENTARY MATERIALS

Video S1: Stepwise recovery process of the SMC obtained by various C_{glu} in a buffer with pH = 10.

Video S2: Continuous recovery process of the SMC in a buffer solution of pH = 10 and C_{glu} = 300 mg·dL^{-1}.

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