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Hydrogen-bonded and ionic crosslinked high strength hydrogels exhibiting Ca^{2+}-triggered shape memory and volume shrinkage for cell detachment

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Abstract

A hydrogen-bonded and calcium ion crosslinked hydrogel, termed as PVDT-PAA, was synthesized by one-step photo-polymerization of 2-vinyl-4,6-diamino-1,3,5-triazine (VDT), acrylic acid (AA), and polyethylene glycol diacrylate (PEGDA, Mn=4,000). Combined physical crosslinkings from inter-diaminotriazine and coordination of Ca^{2+} with carboxyls contributed to a significant enhancement in the mechanical properties of PVDT-PAA hydrogels. Furthermore, reversible Ca^{2+} crosslinking imparted shape memory function to the hydrogel which were able to firmly memorize multiform shapes and return to the initial state in response to Ca^{2+}. Interestingly, the PVDT-PAA hydrogels with weaker H-bonding interaction demonstrated a sharp volume change phenomenon induced by Ca^{2+}. This volume change could be utilized to trigger unharmful cell detachment from hydrogel surface supposedly due to Ca^{2+}-induced marked variation of

mechanotransduction between cells and substrate interface. This H-bonding and ion-crosslinking strategy opens a new opportunity for designing and constructing multifunctional high strength hydrogels for the biomedical applications.

Keywords: hydrogel, hydrogen-bonding, ionic crosslinking, shape memory, cell detachment.

Introduction

Shape memory (SM) hydrogels are a class of smart, soft and wet materials that are featured by an ability to memorize a deformed shape and recover to their original shape under external stimuli.[1-10] Although SM hydrogels were reported earlier,[2] the relevant work progressed at a slow pace. In recent years, SM hydrogels began to attract increasing attention thanks to the achievements in materials chemistry and ingenious utilization of diversified trigger stimuli.[11,12] We, for the first time, reported on a new type of high strength shape memory hydrogels triggered by zinc ions stimulus responsive association/dissociation of dipole–dipole pairs and reversible complexation/decomplexation.[13] Recently, ferric ions responsive shape memory hydrogel system was also fabricated.[14] The SM behavior originates from the high complexation ability of oxidized ferric ions with phosphate groups, which served as the reversible physical crosslinking that could firmly lock the temporary shape. Chen et al[15] reported phenylboronic acid modified sodium alginate/poly(vinyl alcohol)(PVA) supramolecular hydrogels with both self-healing and shape memory properties based on the dynamic interactions of PVA as well as complexation of alginate with Ca^{2+}, respectively.

In developing new shape memory hydrogels, it is of great importance to explore SM hydrogels in biomedical applications. Based on ion-regulated shape memory effects, our group has previously developed cells-encapsulated hydrogel scaffolds.[16] Further, we realized the controllable pre-seeding of human mesenchymal stem cells (hMSCs) on different growth positions of a high strength hydrogel by utilizing double-ion-triggered automatic shape changing and memory effect.[17] We demonstrated that the differentiation behavior of hMSCs was highly sensitive to their growth position on the hydrogel scaffold. Another important work is the use of shape memory polymers to provide new insight into how cells respond to their physical environment.[18] To overcome the limit of static and non-responsive cell culture substrates, Henderson et al[19] developed a temperature-sensitive shape memory polymer substrate that could be programmed to change surface pattern in response to the variation of culture temperature, thereby controlling the cell orientation.

In the present study, we aimed to design and construct an ion-crosslinking and hydrogen bonding reinforced hydrogel by copolymerizing 2-vinyl-4,6-diamino-1,3,5-triazine (VDT, hydrogen-bonding monomer), acrylic acid (AA, calcium ion responsive monomer) and a hydrophilic crosslinker, polyethylene glycol diacrylates (PEGDA, M_n = 4,000). We found that the dual crosslinking consisting of Ca^{2+} coordination and diaminotriazine-diaminotrazine (DAT-DAT) hydrogen bonding interaction could contribute to a significant increase in mechanical strength, and at an appropriate ratio of VDT/AA, the hydrogels exhibited a shape memory behavior in response to calcium ions. Another beneficial phenomenon was

that the cells were well adhered on the gel surface, and with addition of Ca^{2+}, hydrogels at a smaller mass ratio of VDT/AA contracted considerably. The sharp shrinking of hydrogel volume was accompanied with dramatic change of modulus. This Ca^{2+}-induced variation of mechanotransduction of hydrogels was hypothesized to be able to allow for cell detachment.

Experimental section

Materials

2-Vinyl-4,6-diamino-1,3,5-triazine (VDT, >95%,Tokyo Kasei Kogyo Company, Japan), acrylic acid (AA, >99%, Sigma-Aldrich), 2-hydroxy-4-(2-hydroxyethoxy)-2-methylpropiophenone(IRGACURE-2959, 98%, Sigma-Aldrich), acryloyl chloride (>95%, Tokyo Kasei Kogyo Company, Japan), triethylamine (>99%, Tokyo Kasei Kogyo Company, Japan) were of analytical grade and were used without further purification. Poly(ethylene glycol) (PEG, M_w=4,000, Sigma-Aldrich) was dried in a vacuum oven at room temperature for 24 hours before use. PEGDA was synthesized from PEG (M_w=4000) and acryloyl chloride as the reported method.[20,21] All the other solvents were of analytical grade.

Synthesis of PVDT-PAA copolymer hydrogels

An appropriate amount of VDT, AA, PEGDA and IRGACUREA 2959 photoinitiator (3 % w/w relative to total monomers fed) were dissolved in DMSO. The concentration of total monomers was set at 15 wt % and the mass ratio of PEGDA to VDT and AA was fixed at 1/2. The mixture was cast into rectangular molds (length 7

cm, width 5 cm, thickness 0.5 mm) or tubular molds (inner diameter 10 mm, length 12 mm), and the photo-polymerization was carried out for 40 minutes in a crosslink oven (XL-1000 UV Crosslinker, Spectronics Corporation, NY, USA). The obtained hydrogels were then immersed in pH 7.4 phosphate buffered saline (PBS) solution with PBS changed every12 h for 3 days to remove the unreacted reactants, impurities and DMSO. The synthesized hydrogels were named as PVDT-PAA-x-PBS (in PBS) and PVDT-PAA-x-Ca-y (in Ca^{2+} solution), where the x and y represent the mass ratio of VDT/AA and concentration of calcium ion solution.

FTIR characterization

Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) conducted on PerkinElmer spectrum 100 (USA) was used to confirm the successful the formation of PVDT-PAA hydrogels.

Determination of equilibrium water contents (EWCs)

The EWCs of hydrogels were measured at room temperature using a gravimetric method. The hydrogel samples fully swollen in PBS or 100mmol L^{-1} calcium chloride solutions were taken out, blotted with wet filter paper to remove water on the surface, and weight on a microbalance. Then the hydrogels were dried to constant weights in a vacuum oven at 50 ℃. The EWC was defined as:

$$EWC = \frac{m_{wet} - m_{dry}}{m_{wet}} \times 100\ \% \quad (1)$$

Where m_{wet} and m_{dry} represent the wet weight and dry weight of the hydrogel sample, respectively. At least, three measurements were taken for each sample

Measurement of mechanical properties

All of the mechanical properties of hydrogels were tested on the WDW-05 electromechanical tester (Time Group Inc, China) at room temperature in air environment. The hydrogels were fully swollen in PBS solution or calcium chloride solution and at least five specimens were tested for each measurement. For tensile strength, hydrogel sheets were cut into dumbbell specimens in accordance to GBT 528-20094 (width: 2 mm, gauge length: 10 mm, thickness: 0.5 mm by a customized cutter (Xuan Yu Inc, China). The crosshead speed was set at 50 mm min^{-1}. For compression tests, the fully swollen hydrogels were cut into cylinders (10 mm in diameter and 8 mm in height) and measured on the same tester with a crosshead speed of 10 mm min^{-1}. In this experiment, all the stresses measured are engineering stresses.

Shape memory performance assay

The shape memory performance of the PVDT-PAA-10-PBS hydrogels was evaluated at room temperature. Firstly, hydrogels fully swollen in PBS solution were cut into designed strips. Then the hydrogels were curled into a specific shape, and were immersed into 100 mmol L^{-1} Ca^{2+} solution for 12 hours to fix the temporary shape. Finally, the hydrogels were taken out and placed into 50 mmol L^{-1} EDTA·2Na solution at 37 ^{o}C to observe the recovery of the temporary shape.

Shape memory cycle assay

The quantitative shape memory cycle was determined by the reported method.[1] A

straight strip of PVDT-PAA-10-PBS hydrogel was bent into a U-form shape and immersed into 100 mmol L^{-1} Ca^{2+} solution for 12 hours. Then the deformed hydrogel was transferred into 50 mmol L^{-1}EDTA·2Na solution at 37 ^{o}C to extract Ca^{2+} from the gel. The shape memory efficiencies were evaluated by measuring the angles at specific time points. The test was repeated three times. The shape fixity ratio (R_f) and shape recovery ratio (R_r) were defined by the following equations:

$$R_f = \frac{\theta_t}{\theta_i} \times 100\ \% \quad (2)$$

$$R_r = \frac{(\theta_i - \theta_f)}{\theta_i} \times 100\ \% \quad (3)$$

Where θ_i is the given angle, θ_t is the temporarily fixed angle, and θ_f is the final angle. We also tested the mechanical properties of PVDT-PAA-10-Ca-100 hydrogels immersed in 50 mmol L^{-1} EDTA.2Na solution for different times.

Calcium ions induced volume change of hydrogels

The hydrogels fully swollen in the PBS solution were cut into discs (diameter 15mm, thickness 0.5 mm) and immersed in different concentrations of Ca^{2+} solutions. Within the first 30 minutes, the diameter of each disc was measured every 1.5 minutes. The volume change of hydrogels was calculated according to the equation given below:[22]

$$\frac{V}{V_0} = (\frac{D}{D_0})^3 \quad (4)$$

Where V and D are the hydrogel disc's volume and diameter at each measured point, V_0 and D_0 are the hydrogel disc's volume and diameter at initial state.

Cytotoxicity assay of PVDT-PAA-PBS hydrogels

Mouse fibroblast cells (L929, obtained from Peking Union Medical College) were seeded in a 48-well plate coated with PVDT-PAA-PBS hydrogels and incubated for

48 hours to evaluate the cytotoxicity of hydrogels with MTT assay method. The culture medium was replaced with 400μL fresh medium containing 40μL MTT (3-(4,5-dimethyl-2-thia-zoyl)-2,5-diphenyl tetrazolium bromide, 5 mg mL^{-1} in PBS), and the cells were incubated for another 4 hours. Finally the whole medium was replaced by 300 μL DMSO per well to dissolve the formed crystal and the plate was gently shaken for 15 minutes. The absorbance (Abs) of each well was measured at 490 nm on a ∑960 plate-reader (Metertech) with pure DMSO as a blank. The non-treated cells (in DMEM) were used as a control and the relative cell viability (RCV, mean% ± SD, n = 3) was expressed as below:

$$RCV = \frac{Abs_s}{Abs_c} \times 100\% \quad (5)$$

Where Abs_s and Abs_c are the absorbance of sample and control group, respectively.

Ca^{2+}-induced cell detachment assay

Hydrogel samples were sterilized by immersing in 75 % medical alcohol for 12 hours and then transferred into a 96-well culture plate, soaked with PBS solution for 12 hours to reach swelling equilibrium. The L929 cells were seeded onto the hydrogels at ~1×10^4 cells per well and cultured with RPMI 1640 supplemented with 10% fetal bovine serum (FBS; Hyclone) at 37 ^oC in 5 % CO_2 for 24 hours to achieve cell spreading.

In the cell detachment assay, the culture medium was replaced with PBS, and then pipetted out carefully. Immediately, 100 mmol L^{-1} $CaCl_2$ solution was added and left for standing 15 minutes. After that, the hydrogel surface was washed gently with 100 mmol L^{-1} $CaCl_2$ solution. In order to evaluate the cell viability after detachment, the

released cells were cultured again on a cell plate for 24 hours. The cell detachment efficiency (CDE, mean% ± SD, n = 3) was calculated as follows:

$$CDE = \frac{Abs_{dc}}{Abs_{dc} + Abs_{rc}} \times 100\% \quad (6)$$

Where Abs_{dc} and Abs_{rc} are the absorbance of detached cells and residual cells on the hydrogel sample

Results and Discussion

Characterization of PVDT-PAA hydrogels

Scheme 1 presents the molecular structure of PVDT-PAA hydrogel. As shown in the Scheme, the carboxylic acid group was in de-protonated state. The pKa of poly(acrylic acid) was 6.72 in pure water and decreased as the ionic strength increased,[23] thus, the carboxyl groups will be de-protonated in PBS solution (pH = 7.4, ionic strength = 0.001 M). Fig. 1 shows the ATR-FTIR spectra of hydrogels. In the FTIR spectra, the characteristic peaks at 2915, 1070 and 1437 cm^{-1} are separately attributed to the stretching vibrations of C-H, C-O-C and bending vibration of $–CH_2-$ in PEGDA. The feature peaks of VDT are located at 3200, 1640, 1544 cm^{-1}, corresponding to the stretching vibrations of N-H, C=N, and C-N, respectively.[24,25] The strong absorption band at 3330 $cm^{-1}$is assigned to hydroxyl stretching of AA.[17] These characteristic peaks suggest the formation of PVDT-PAA hydrogels via photo-polymerization.

The EWCs of PVDT-PAA-PBS hydrogels were measured at room temperature (Table 1). In this study, the effect of VDT/AA on the EWCs of hydrogels was

examined at a fixed initial monomer concentration and cross-linker content. The results show that with an increment in VDT content, the EWCs of hydrogels decreases from 97.6% to 88.6 % over the range of VDT/AA ratio from 1 to 10. It is understandable that introducing an increased VDT content means more intermolecular hydrogen-bondings are formed from diaminotriazine residues, which results in a denser network, thus limiting the uptake of the water.[26] DAT-DAT hydrogen bonding was well-established.[27,28] Previously, we constructed high strength hydrogels based on DAT-DAT hydrogen bonds.[26] In PVDT-based hydrogels, DAT-DAT hydrogen bonds could form hydrophobic microdomains, which were shown to decrease the equilibrium water content of hydrogels, and stabilized the hydrogels immersed in water. Fig. S1 clearly shows the marked difference in swelling state of PVDT-PEGDA hydrogel immersed in neutral PBS (pH = 7.4) and acidic PBS (pH = 3). The hydrogel disc in acidic PBS is highly swollen compared to the one in neutral PBS, indicating that the presence of DAT-DAT hydrogen bonding was broken due to the protonation of diaminotriazine (pKa=5.15).[29]

As shown in Table 1 the mechanical properties of hydrogels are positively related to the content of VDT. In particular, while the mass ratio of VDT/AA is raised to 6 or 10, the comprehensive mechanical properties of PVDT-PAA-PBS hydrogels are enhanced significantly. For PVDT-PAA-10-PBS hydrogel with 88 % EWC, its tensile strength, elongation at break, Young's modulus and compressive strength are up to 512.0 kPa, 262.1 %, 731.3 kPa and 3.4 MPa, respectively. Due to its appropriate EWC

and outperformed mechanical properties, PVDT-PAA-10-PBShydrogel will be used for the following shape memory assay.

Effect of calcium ions on the mechanical properties of hydrogels

In this study, we aimed to fabricate calcium ions-triggered high strength shape memory hydrogels. Thus, the complexation of calcium ions with carboxyl groups is the foundation to achieve shape memory effect. To investigate the influence of calcium ions on the mechanical properties of the hydrogels, we immersed PVDT-PAA-10-PBS hydrogel into three different concentrations of Ca^{2+} solutions. After reaching a fully swollen equilibrium, the tensile strengths of hydrogels were measured.

Fig. 2 exhibits the tensile stress-strain curves of PVDT-PAA-10-PBS hydrogel in different concentrations of calcium ion solutions. Obviously, Ca^{2+} soaking leads to a remarkable increase in tensile strength and elongation at break compared to PBS swollen hydrogels. In the selected 50 mmol L^{-1}, 100 mmol L^{-1}, and 500 mmol L^{-1} Ca^{2+} solutions, the tensile strengths of hydrogels increase from 0.5 MPa in PBS solution to 1.0 MPa, 1.2 MPa and 1.3 MPa respectively. Meanwhile the Young's moduli are enhanced from 0.7 MPa to 1.7 MPa, 1.7 MPa and 1.7 MPa, respectively. Also, the elongations at break are increased 1.5-2 fold along with the increments of Ca^{2+} concentration. It is worth noting that 100 mmol L^{-1} and 500 mmol L^{-1} Ca^{2+} exhibita nearly similar enhanced effect on the mechanical properties of hydrogels. Therefore,

100 mmol L^{-1} Ca^{2+} solutions will be chosen for the shape memory experiment. Taking into account a possible influence of the adsorption of calcium ions to amino groups of VDT on DAT-DAT hydrogen bonding,[25] the PVDT-PEGDA-PBS hydrogels were fabricated and immersed in PBS and 100 mmol L^{-1} Ca^{2+} solutions, respectively. Then the tensile strength was measured (Table S1). Evidently, calcium ions have a negligible effect on the mechanical properties of the PVDT-PEGDA hydrogel, implying that the Ca^{2+}- adsorption does not affect the DAT hydrogen bonding interactions at this concentration. These results indicate that the coordination of calcium ions with carboxyl groups can act as physical crosslinks, which increase the stiffness of the hydrogels combined with chemical crosslinking. Furthermore, the ionic crosslinking is reversible since the hydrogels could recover to their original mechanical properties when calcium ions were extracted by EDTA·2Na solution. The mechanical properties of other proportions of PVDT-PAA-PBS and PVDT-PAA-Ca-100 hydrogels are summarized in Table 1. Similarly, with Ca^{2+} crosslinking, the mechanical performances of all the hydrogels are enhanced. Particularly, for PVDT-PAA-1-PBS hydrogel with lower VDT content, 100 mmol L^{-1} Ca^{2+} coordination can contribute to 40-, 6-, 15-, 11-fold increase in the tensile strength, elongation at break, Young's modulus and compressive strength relative to the parent hydrogel. Therefore, the dual physical crosslinking from DAT-DAT hydrogen bonding and Ca^{2+} complexation can act synergistically to greatly increase the mechanical strength of hydrogels. We need to point out that besides physical crosslinking, the shielding effect of Ca^{2+} on COO^- may exert an influence on the mechanical properties

and water content to a certain extent. It is known that the shielding effect is dependent on the concentration of the counter ions. As demonstrated in Table S2, the Young's moduli show negligible difference when Ca^{2+} concentration increases from 50 mmol L^{-1} to 500 mmol L^{-1}, suggesting that the ionic crosslinking is the main factor contributing to the enhancement of mechanical properties of the hydrogels. It is noteworthy that the PVDT-PAA-PBS and PVDT-PAA-Ca hydrogels exhibited the same level of tensile/compressive strengths and Young's moduli at similar equilibrium water content, compared to the reported high strength hydrogels.[26, 30]

Shape memory performance

The above-mentioned reversible change in mechanical properties in response to calcium ions provides a premise for shape memory. To embody this idea, the original hydrogel sheets were curled into a cube, a pyramid-or a spring-like shape, and then immersed in 100 mmol L^{-1} Ca^{2+} solutions at 37°C for 12 hours. We found that these temporary shapes could be well fixed (Fig. 3b, 3c, 3d). Subsequently, the hydrogels were transferred into 50 mmol L^{-1} EDTA·2Na solution at 37 °C to remove the chelated calcium ions. The hydrogels were shown to lose its deformed shape and recover to its permanent shape within 165 minutes. Based on the above phenomenon and analysis, a shape memory mechanism is proposed (Fig. 3a). While the deformed hydrogels were immersed in Ca^{2+} solution, the Ca^{2+} selectively chelated with carboxyl groups to form additional physical crosslinking points, thus enhancing the elastic modulus and meanwhile firmly locking the temporary shape. After addition of a complexing agent,

the Ca^{2+}-carboxyl complexation would be dissociated, causing a decrease in crosslink density and eventually giving rise to the recovery of the temporary shape.

Next, we measured the shape fix ratio (R_f) and shape recovery ratio (R_r) of the PVDT-PAA-10-PBS hydrogels by the reported angle-control method.[1] All the R_f values of PVDT-PAA-10-PBS hydrogels reach 100% in three cycles. The R_r can also increases to 100% in 165 minutes (Fig. S2), indicating that coordination of calcium ions with carboxyl groups can well lock the temporary shape and the decomplexation of this coordination can lead to full recovery of the original state. The reproducible shape memory effects could be demonstrated after three cycles.

The stress-strain curves of PVDT-PAA-10-Ca-100 hydrogels immersed in 50 mmol L^{-1} EDTA·2Na solution for different times are shown in Fig. S3. As presented in the figure, with increasing treating time of EDTA·2Na, more of ionic crosslinkages are dissociated due to the extraction of Ca^{2+}; hence, the tensile strength declines gradually. At 150 min, almost 100 % tensile strength recovers compared to the pristine gel. This result indicates that it takes almost the same time for the full recovery of shape as the restoration of mechanical properties of hydrogels.

Effect of calcium ions on the volume of hydrogels

Apart from the shape memory behaviors, we also found a prominent volume change occurred to the hydrogels with addition of Ca^{2+}. In this case, the extent of volume change is strongly dependent on the ratio of VDT/AA. Herein, in light of Ca^{2+}-induced marked variation in the mechanical properties, PVDT-PAA-1-PBS

hydrogel was chosen to investigate the influence of calcium ions on the volume of hydrogels. In our experiment, the PBS-swollen hydrogels discs were immersed in 5 mmol L^{-1}, 50 mmol L^{-1}, 100 mmol L^{-1} and 500 mmol L^{-1} Ca^{2+} solutions. The diameter of each hydrogel disc was measured every 1.5 minutes over 30 minutes. As shown in Fig. S4a, different concentrations of Ca^{2+} induce different decreasing speed and extent of volume change of the hydrogel discs. The higher Ca^{2+} concentration, the faster decreasing speed. At 5 mmol L^{-1}, the volume of hydrogel remains almost unchanged within the test time frame due to much fewer ionic crosslinkings formed. Upon increasing Ca^{2+} concentrations up to 50 mmol L^{-1}, 100 mmol $L^{-1}$and 500 mmol L^{-1}, the gels shrink markedly within the test time. At higher contents of 100 mmol $L^{-1}$and 500 mmol L^{-1}, the PVDT-PAA-1-PBS gel's volume decreases by 80% within 15 minutes compared with its initial value. To elucidate the shrinking behavior more clearly, we also calculated the equilibrium swelling ratio (ESR) of the hydrogels in the presence or absence of Ca^{2+}. The PVDT-PAA-1-PBS and PVDT-PAA-1-Ca-100 hydrogels possess 4120 % and 911 % ESRs, respectively. Obviously, treating with calcium ions, the swelling ratio change of PVDT-PAA-1-PBS hydrogel is about 78 %, very close to the volume change.

The above Ca^{2+}-induced dramatic variation in the volume of PVDT-PAA-1-PBS hydrogel is accompanied with surface mechanotransduction, which could be utilized to accelerate cell detachment. Taking into account the decreasing speed and extent of volume change as well as possible cytotoxicity, 100 mmol L^{-1} Ca^{2+} was chosen for the

following cell detachment. At this concentration, the volume of PVDT-PAA-2-PBS also displays a reducing trend with time (Fig. S4b), however, the shrinking speed was slower than that of PVDT-PAA-1-PBS hydrogel, and the ultimate volume shrinks to 75% its initial value due to decreased coordination of Ca^{2+} with carboxyl groups in 30 minutes.

Cytotoxicity assay and calcium ions-induced cell detachment

Cytotoxicity is one of the critical factors determining whether the hydrogel can be used in the biomedical field. Prior to performing cell detachment induced by calcium ions, we evaluate the cytotoxicity in the culture containing 100 mmol L^{-1} calcium ions. As shown in Fig. 4b, after introducing 100 mmol L^{-1} calcium ions, the cell morphologies show no difference compare to the initial state of cells cultured on the cell plate (Fig. 4a). Furthermore, addition of 100 mmol L^{-1} calcium ions did no harm to the cells due to the short contact time with calcium ion solutions. Fig. 4c and Fig. 4e show the phase contrast microscopy images of L929 cells cultured on the surfaces of PVDT-PAA-1-PBS and PVDT-PAA-2-PBS hydrogels, respectively. The cells can adhere uniformly and assume spread morphology on the gel surfaces. Fig. S5 demonstrates that the PVDT-PAA-1-PBS and PVDT-PAA-2-PBS gels can maintain above 80% cell viability at the selected proportions, in comparison with non-treated cells used as a control.

In order to detach cell from the gel surface, the cells on both PVDT-PAA-1-PBS and PVDT-PAA-2-PBS were treated with 100 mmol L^{-1} Ca^{2+} for 15 minutes. During

this time, an obvious volume shrinkage of hydrogel was observed. The volume of PVDT-PAA-1-PBS and PVDT-PAA-2-PBS hydrogels are reduced to about 20 % and 35 % their original volume, respectively. The dramatic contraction of volume may cause a notable mechanotransduction between cells and substrate, which can lead to the highly efficient cell detachment (Fig. 4d, 4f). The mechanism of this unharmful cell detachment was illustrated in Scheme S1.

To quantitatively evaluate the detached cells from PVDT-PAA-PBS hydrogels, the cell detachment efficiency (CDE) on PVDT-PAA-PBS hydrogels was estimated by MTT assay. Fig. 5 reveals that more than 90 % and 80 % cells are released from PVDT-PAA-1-PBS and PVDT-PAA-2-PBS hydrogels, respectively. The lower CDE of PVDT-PAA-2-PBS is resulted from the decreased extent of its volume change. To examine the effect of Ca^{2+} on cell viability, the detached cells were then re-cultured on another plate. After 12 h, the cells could resume normal shape and growth as shown in Fig. S6, suggesting that the viability of the detached cells can be well maintained.

Conventionally, confluent cells are detached by enzymatic digestion, which affects adversely the cell functions. To address this problem, temperature and light stimuli responsive polymers have been developed as platforms for unharmful cell detachment.[31, 32] In this work, we found the calcium ions-induced volume shrinkage of hydrogels and this function was extended to manipulate cell detachment. It is well known that calcium ions are biocompatible and have been widely used to crosslink alginate hydrogels for cell encapsulation in tissue engineering.[33] We have

demonstrated that the cells could adhere on the hydrogel surface, and the confluent cells were conveniently detached by simply adding calcium ions without influencing their viability. Thus, this proof of concept work offers a facile and benign method to harvest seed cells for potential tissue engineering application.

Conclusions

In summary, we have demonstrated that dual physical crosslinkings from diaminotriazine-diaminotriazine hydrogen bonding interaction and coordination of calcium ion with carboxyl groups acted synergistically to greatly enhance the comprehensive mechanical properties of the PVDT-PAA-PBS hydrogels. Reversible complexation and decomplexation Ca^{2+} with carboxyl groups enabled the PVDT-PAA-PBS hydrogels to memorize multiform of shapes and recover to their original states. Furthermore, for PVDT-PAA-PBS hydrogels with weak H-bonding interaction, a dramatic volume shrinkage occurred under Ca^{2+} stimulus. This calcium ions-induced volume change could be translated into unharmful cell detachment from PVDT-PAA-PBS hydrogel surface. This work opens up a new way to fabricate high strength shape memory hydrogels for a variety of biomedical applications, such as non-destructive cell harvest and soft tissue substitutes.

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Captions

Scheme 1 Schematic molecular structure of PVDT-PAA hydrogel.

Fig. 1 ATR-FTIR spectra of PVDT-PAA-2 and PVDT-PAA-6 hydrogels.

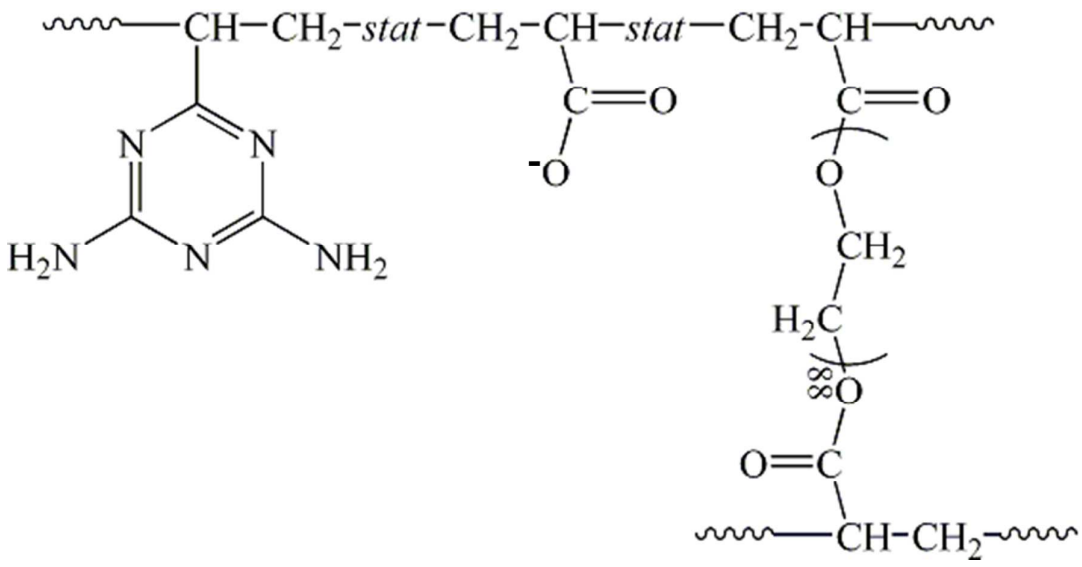
Fig. 2 Tensile stress-strain curves of PVDT-PAA-10-PBS hydrogels immersing in PBS and different concentrations of calcium ion solutions.

Fig. 3 (a) Mechanism of calcium ion-triggered reversible shape memory behavior of PVDT-PAA-PBS hydrogel. Actual observation of the shape memory behavior of PVDT-PAA-10-PBS hydrogel. The original cruciform hydrogel strip was curled into a (b) box-like shape, (c) a pyramid-like shape and (d) a spring-like shape, and then fixed in 100 mmol L^{-1} Ca^{2+} solution.

Fig. 4 (a) Phase contrast microscopy image of L929 cells cultured on the cell plate at 37^{o}C.(b) Phase contract microscopy image of Ca^{2+}-treated L929 cells cultured on the cell plate at 37 ^{o}C. Phase contract microscopy images of L929 cells cultured on (c) PVDT-PAA-1-PBS and (e) PVDT-PAA-2-PBS hydrogels. Phase contract microscopy images of L929 cells cultured on Ca^{2+}-soaked (d) PVDT-PAA-1-PBS and (f) PVDT-PAA-2-PBS hydrogels. Scale bar: 100μm.

Fig. 5 Cell adhesion rate and cell detachment efficiency measured by MTT assay on the surface of the PVDT-PAA-1-PBS and PVDT-PAA-2-PBShydrogels. Results are

presented as mean ± SD.



Scheme 1

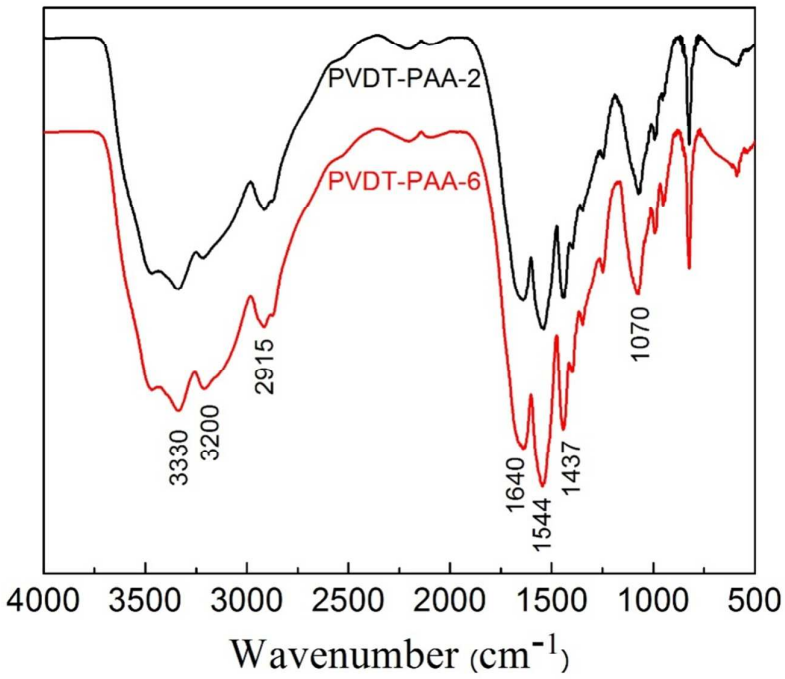


Fig. 1

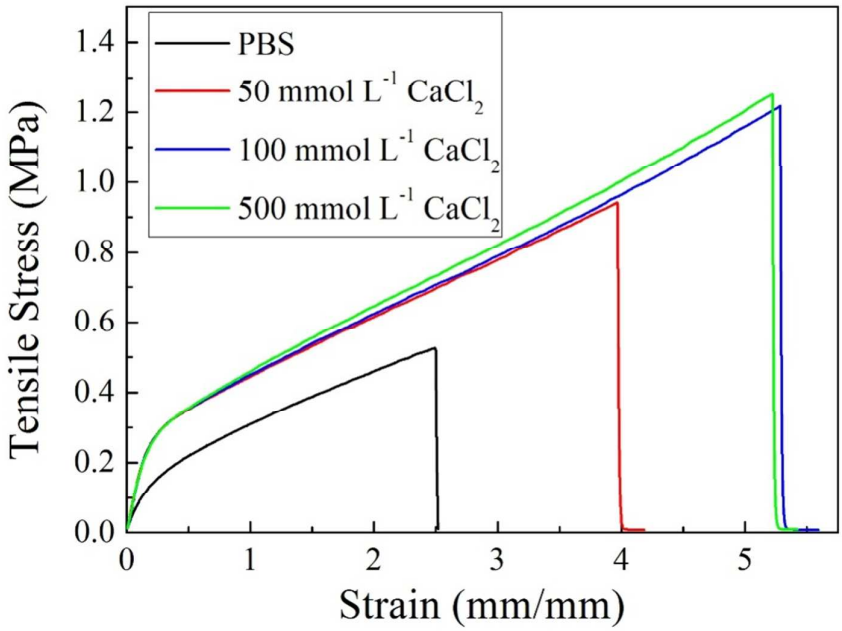


Fig. 2

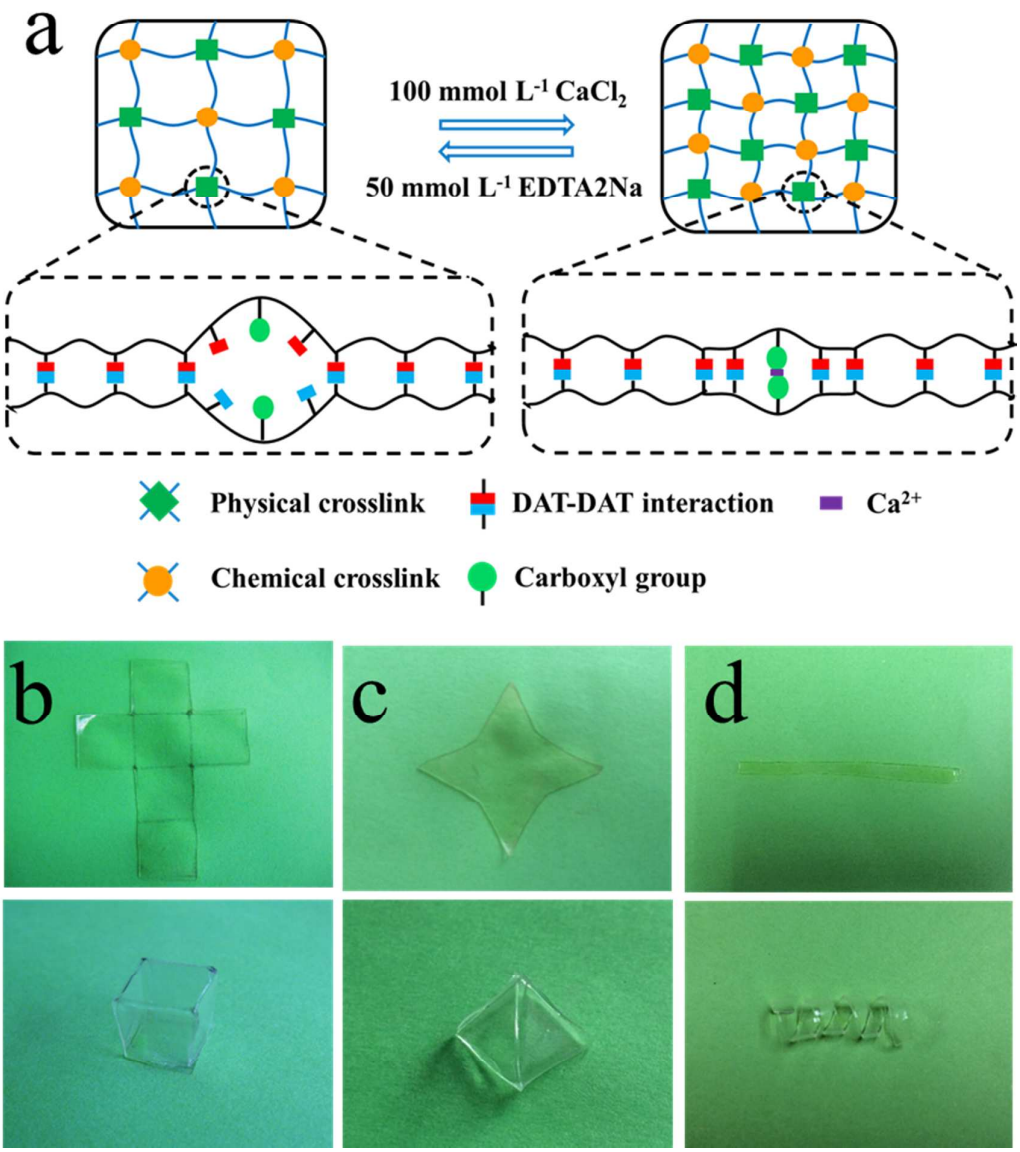


Fig. 3

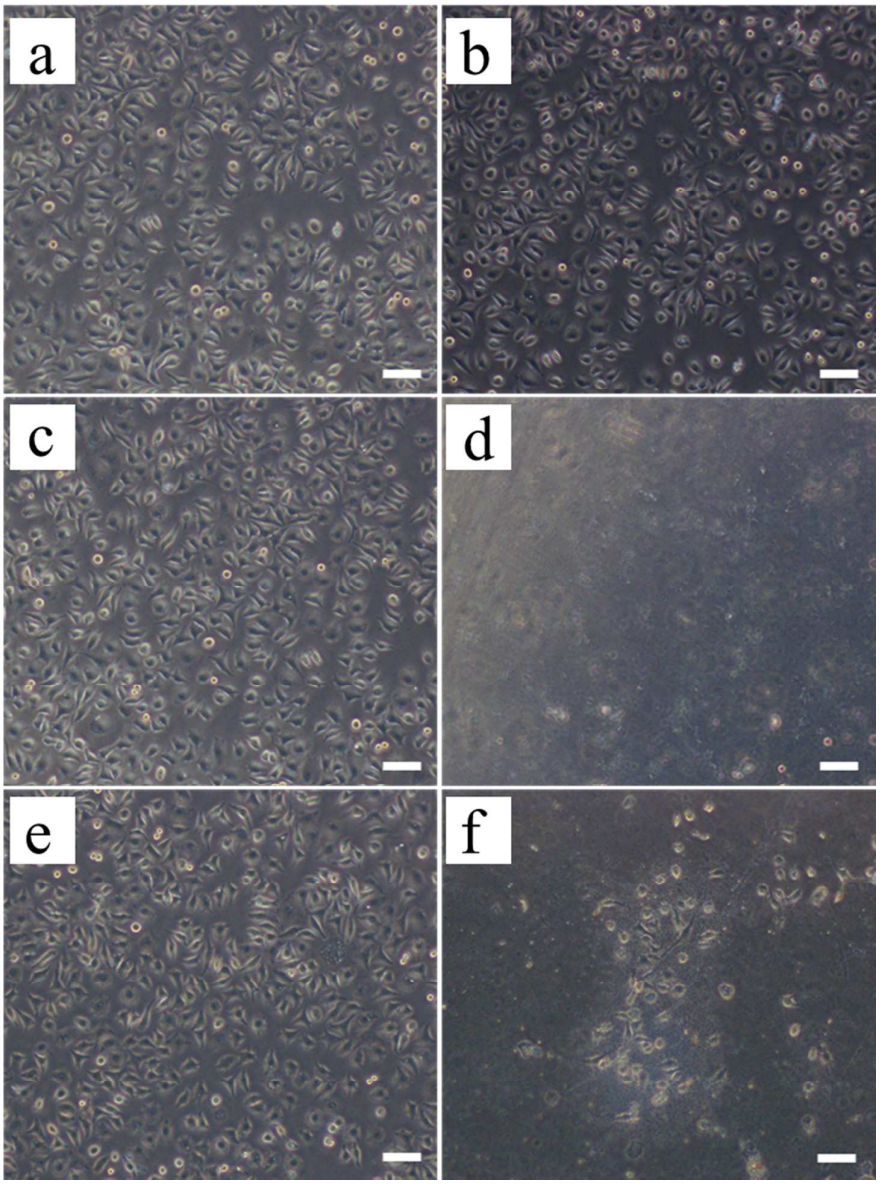


Fig. 4

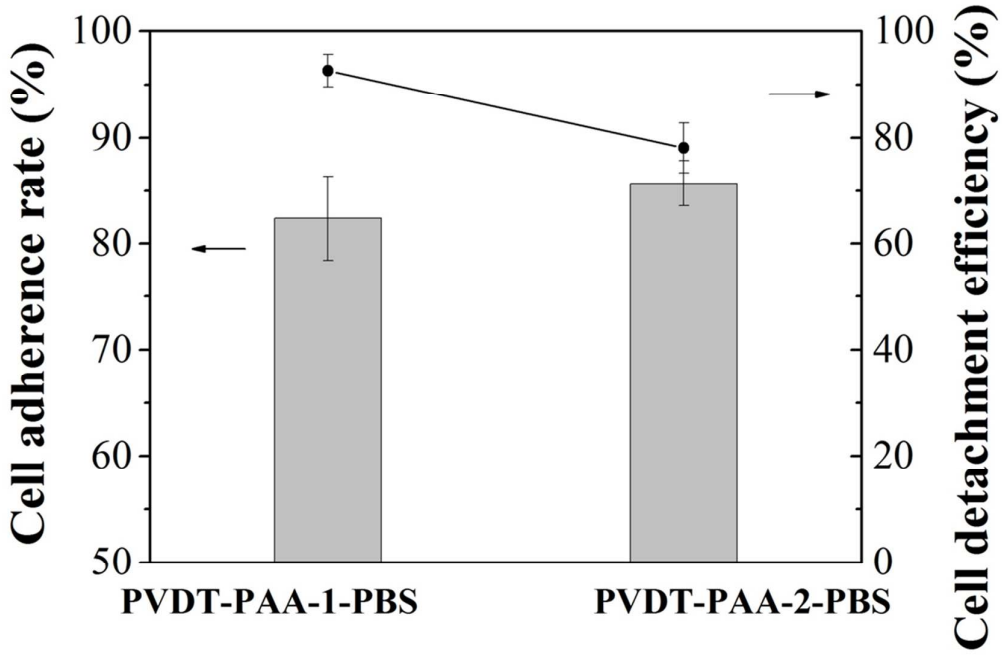


Fig. 5

Table 1. Mechanicalproperties and EWCs of PVDT-PAA-PBS and PVDT-PAA-Ca hydrogels

Sample ID	Tensile Strength (kPa)	Elongation at Break (%)	Young's Modulus (kPa)	Compressive Strength (kPa) at 90% Strain	Compressive Modulus (kPa)	EWC(%)
PVDT-PAA-1-PBS	11.9±5.6	46.4±12.4	13.3±7.1	234.0±14.0*	14.9±1.3	97.6±0.6
PVDT-PAA-2-PBS	16.5±1.7	47.5±7.7	17.5±2.1	1977.1±7.0*	31.2±2.0	96.2±0.4
PVDT-PAA-6-PBS	375.6±28.8	225.2±36.3	139.0±10.8	2703.2±367.1	207.4±29.0	91.8±0.9
PVDT-PAA-10-PBS	512.0±12.1	262.1±7.5	731.3±66.1	3405.8±256.4	384.1±71.9	88.7±0.2
PVDT-PAA-1-Ca-100	493.9±7.3	271.2±6.1	192.0±5.8	2561.0±175.2	194.9±68.9	90.1±1.5
PVDT-PAA-2-Ca-100	447.2±9.7	256.5±5.8	369.6±13.1	2893.3±282.0	368.1±12.3	90.4±0.6
PVDT-PAA-6-Ca-100	815.2±25.2	393.6±16.9	1018.4±89.4	3422.4±312.1	1631.4±61.4	88.8±0.7
PVDT-PAA-10-Ca-100	1214.1±185.4	526.5±25.5	1707.7±142.3	4768.2±296.3	4429.2±73.8	85.5±0.5

*Breaking compressive strength

Graphic Abstract

Diaminotriazine hydrogen bonding reinforced and Ca^{2+}-crosslinked high strength shape memory hydrogels are fabricated. Ca^{2+}–induced dramatic volume shrinkage is utilized to trigger the unharmful cell detachment.