

## Supporting Information

### **Post-synthetic Modification of Polyvinyl Alcohol with a Series of N Alkyl Substituted Carbamates towards Thermo and CO<sub>2</sub>-Responsive Polymers**

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## SI. Materials

Poly(vinyl alcohol) (PVA;  $M_w$ :146000-186000) were purchased from Sigma Aldrich Co. , *n*-Propylamine (nPA), *N,N*-Dimethylethylenediamine (DMEDA), *N,N*-Diethylethylenediamine (DEEDA), *N,N*-Dimethyl-1,3-propanediamine (DMPDA), *N,N*-Diethyl-1,3-propanediamine (DEPDA) and *N,N'*-Carbonyldiimidazole (CDI) were supplied by Energy Chemical, China. and were used without further purification. Other chemicals were analytical-grade reagents and were used as received. Deionized (DI) water from a Explorer-45s water purification system (Orangeins Inc.) had a resistivity of 18.2 M $\Omega$  cm. All aqueous solutions were prepared with DI water. High purity Carbon dioxide(99.99% and nitrogen (99.999%) were used as received.

## SII. Synthesis of PVA derivatives

**Synthesis of PVA-C2-Me.** PVA (2 g) was dissolved by 80 mL dry DMSO in a 250 mL round bottom flask by heating. The obtained PVA solution was cooled down to room temperature, then 2.24g CDI (13.6 mmol, 0.3 equiv/hydroxyl) was added and kept stirring for 3h at room temperature. Afterwards, 1.12 mL of *n*-propylamine (nPA) (13.6 mmol) was added to CDI activated PVA drop-by-drop. After additional stirring for 80 h, 20 mL of 25% ammonia solution was added and the reaction mixture was stirred for 2h to hydrolyze all CDI activated hydroxyls which did not react with *n*-propylamine. The product, namely PVA-C2-Me, precipitated out by adding 10-fold acetone and resolved in DI water by three times. The precipitate was collected by centrifugation and dried in a vacuum oven at room temperature. <sup>1</sup>H-NMR(D<sub>2</sub>O, 400 MHz): 3.79-4.12 ppm (-CH- in the backbone), 3.09 ppm (-NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 1.37-1.94 ppm (-NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub> and -CH<sub>2</sub>- in the backbone) and 0.90 ppm (-NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>).

**Synthesis of PVA-C2-NMe.** PVA (2g) was dissolved by 80 mL dry DMSO in a 250 mL round bottom flask by heating. The obtained PVA solution was cooled down to room temperature, then 3.73g CDI (22.7 mmol, 0.5 equiv/hydroxyl) was added and kept stirring for 3h at room temperature. Afterwards, 2.48 mL of *N,N*-Dimethylethylenediamine (DMEDA) (22.7 mmol) was added to CDI activated PVA drop-by-drop. After additional stirring for 90 h, 20 mL of 25% ammonia solution was added and the reaction mixture was stirred for 2 h to hydrolyze all CDI activated hydroxyls which did not react with *N,N*-Dimethylethylenediamine (DMEDA). The

product, namely PVA-C2-NMe, precipitated out by adding 10-fold acetone and resolved in DI water by three times. The precipitate was collected by centrifugation and dried in a vacuum oven at room temperature.  $^1\text{H-NMR}(\text{D}_2\text{O}, 400 \text{ MHz})$ : 3.65-4.12 ppm ( $-\underline{\text{CH}}-$  in the backbone), 3.23 ppm ( $-\text{NH}-\underline{\text{CH}}_2-\text{CH}_2-\text{N}(\text{CH}_3)_2$ ), 2.47 ppm ( $-\text{NH}-\text{CH}_2-\underline{\text{CH}}_2-\text{N}(\text{CH}_3)_2$ ), 2.22 ppm ( $-\text{NH}-\text{CH}_2-\text{CH}_2-\text{N}(\underline{\text{CH}}_3)_2$ ) and 1.25-2.00 ppm ( $-\underline{\text{CH}}_2-$  in the backbone).

**Synthesis of PVA-C3-NMe.** PVA (2g, mol) was dissolved by 80 mL dry DMSO in a 250 mL round bottom flask by heating. The obtained PVA solution was cooled down to room temperature, then 3.73g CDI (22.7 mmol, 0.5 equiv/hydroxyl) was added and kept stirring for 3h at room temperature. Afterwards, 2.89 mL of *N,N*-Dimethyl-1,3-propanediamine (DMPDA) (22.7 mmol) was added to CDI activated PVA drop-by-drop. After additional stirring for 90 h, 20 mL of 25% ammonia solution was added and the reaction mixture was stirred for 2h to hydrolyze all CDI activated hydroxyls which did not react with *N,N*-Dimethyl-1,3-propanediamine (DMPDA). The product, namely PVA-DMPDA, precipitated out by adding 10-fold acetone and resolved in DI water by three times. The precipitate was collected by centrifugation and dried in a vacuum oven at room temperature.  $^1\text{H-NMR}(\text{D}_2\text{O}, 400 \text{ MHz})$ : 3.59-4.10 ppm ( $-\underline{\text{CH}}-$  in the backbone), 3.11 ppm ( $-\text{NH}-\underline{\text{CH}}_2-\text{CH}_2-\text{CH}_2-\text{N}(\text{CH}_3)_2$ ), 2.35 ppm ( $-\text{NH}-\text{CH}_2-\text{CH}_2-\underline{\text{CH}}_2-\text{N}(\text{CH}_3)_2$ ), 2.19 ppm ( $-\text{NH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{N}(\underline{\text{CH}}_3)_2$ ) and 1.28-2.00 ppm ( $-\text{NH}-\text{CH}_2-\underline{\text{CH}}_2-\text{N}(\text{CH}_3)_2$  and  $-\underline{\text{CH}}_2-$  in the backbone).

**Synthesis of PVA-C2-NEt.** PVA (2g, mol) was dissolved by 80 mL dry DMSO in a 250 mL round bottom flask by heating. The obtained PVA solution was cooled down to room temperature, then 3.73g CDI (22.7 mmol, 0.5 equiv/hydroxyl) was added and kept

stirring for 3 h at room temperature. Afterwards, 3.2 mL of *N,N*-Diethylethylenediamine (DEEDA) (22.7 mmol) was added to CDI activated PVA drop-by-drop. After additional stirring for 90 h, 20 mL of 25% ammonia solution was added and the reaction mixture was stirred for 2h to hydrolyze all CDI activated hydroxyls which did not react with *N,N*-Diethylethylenediamine (DEEDA). The product, namely PVA-C2-NEt, precipitated out by adding 10-fold acetone and resolved in DI water by three times. The precipitate was collected by centrifugation and dried in a vacuum oven at room temperature. <sup>1</sup>H-NMR(D<sub>2</sub>O, 400 MHz): 3.62-4.12 ppm (-CH- in the backbone), 3.23 ppm (-NH-CH<sub>2</sub>-CH<sub>2</sub>-N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>), 2.59 ppm (-NH-CH<sub>2</sub>-CH<sub>2</sub>-N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>), 1.36-2.06 ppm (-CH<sub>2</sub>- in the backbone) and 1.04 ppm (-NH-CH<sub>2</sub>-CH<sub>2</sub>-N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>).

**Synthesis of PVA-C3-NEt.** PVA (2g) was dissolved by 80 mL dry DMSO in a 250 mL round bottom flask by heating. The obtained PVA solution was cooled down to room temperature, then 3.73g CDI (22.7 mmol, 0.5 equiv/hydroxyl) was added and kept stirring for 3h at room temperature. Afterwards, 3.65 mL of *N,N*-Diethyl-1,3-diaminopropane (DEPDA) (22.7 mmol) was added to CDI activated PVA drop-by-drop. After additional stirring for 90 h, 20 mL of 25% ammonia solution was added and the reaction mixture was stirred for 2h to hydrolyze all CDI activated hydroxyls which did not react with *N,N*-Diethyl-1,3-diaminopropane (DEPDA). The product, namely PVA-C3-NEt, precipitated out by adding 10-fold acetone and resolved in DI water by three times. The precipitate was collected by centrifugation and dried in a vacuum oven at room temperature. <sup>1</sup>H-NMR(D<sub>2</sub>O, 400 MHz): 3.60-4.12 ppm (-CH- in the backbone), 3.12 ppm (-NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>), 2.54 ppm (-NH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>), 1.31-2.07 ppm (-CH<sub>2</sub>- in the backbone) and 1.02 ppm (-NH-CH<sub>2</sub>-CH<sub>2</sub>-

CH<sub>2</sub>-N(CH<sub>3</sub>)<sub>2</sub>).

### **SI. Characterization Methods**

***Nuclear Magnetic Resonance Spectroscopy (NMR).*** All NMR spectra of the PVA and PVA derivatives were recorded by using D<sub>2</sub>O or H<sub>2</sub>O/D<sub>2</sub>O (9:1, v/v) as the solvent were recorded on a Bruker 400 MHz spectrometer. <sup>13</sup>C, <sup>13</sup>C distortionless enhancement by polarization transfer (DEPT)135, <sup>1</sup>H-<sup>1</sup>H nuclear Overhauser effect spectroscopy (NOSEY) spectra were recorded on a Bruker 400 MHz spectrometer by using 2,500 Hz spectral windows and 150 ms mixing time. (150 ms is chosen so that artificial signals caused by spin-spin diffusion can be avoided, allowing the NOESY of different cross peaks to be compared.) Variable temperature NMR (VT-NMR) were acquired in the temperature range of 23 °C to 60 °C with 10 min of thermostatic time before each acquisition. The solution of polymers were bubbling N<sub>2</sub> overnight at room temperature to avoid the influence of CO<sub>2</sub> present in air.

***Attenuated total reflectance Fourier transformed infrared (ATR-FTIR) spectroscopy*** spectra were recorded using a Bruker Vector 22 FT-IR spectrometer with an ATR unit.

***Powder X-ray Diffraction (PXRD)*** X-ray diffraction (XRD) patterns were obtained on a Bruker Advance D8 diffractometer with a lynxeye detector at 40 kV and 40 mA with a scanning rate of 4 °/min.

***Thermal gravimetric analysis (TGA) and differential scanning calorimeter (DSC).*** The thermal behavior of PVA and PVA derivatives were investigated by using TGA (TA, Q50) and DSC (TA, Q20). TGA was tested in the temperature from 25 °C to 800 °C under air flow at a ramping rate of 10 °C/min. DSC was recorded from 25 °C to 100 °C

under N<sub>2</sub> flow at a ramping rate of 10 °C/min.

**UV-Vis Spectroscopy.** UV-Vis spectroscopic measurements were conducted on a Hewlett-Packard 8453 spectrophotometer coupled with a circulator temperature controller. The thermally-responsive soluble-insoluble phase transition of the PVA derivatives aqueous solution was characterized by measuring the % transmittance (%T) at  $\lambda=600$  nm. The heating rate was 0.5 °C per minute during measurement. The cloud temperature point of PVA derivatives aqueous solution was defined as the transmittance was decreased to 50%. To evaluate the CO<sub>2</sub> effect on turbidity, the turbidity of PVA derivatives vs. temperature was monitored during the CO<sub>2</sub> bubbling at a flow rate ca. 10 mL/min.

**Dynamic light scattering (DLS).** DLS was performed on a ZETASIZER-3000HSa (Marvern) using 633 nm HeNe laser. The automatic mode was adopted for identifying the best number of subruns and measurement time (n = 3). Hydrodynamic diameter ( $D_h$ ) was estimated by using the value of cumulant (z-average) size. The scattering angle used is 90°. The concentration of polymers was 1 mg/mL and filtered through a 0.45  $\mu$ m hydrophilic syringe filter before measurement

**Cell culture.** HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and recommended working concentrations of antibiotics (penicillin and streptomycin) for the next step usage.

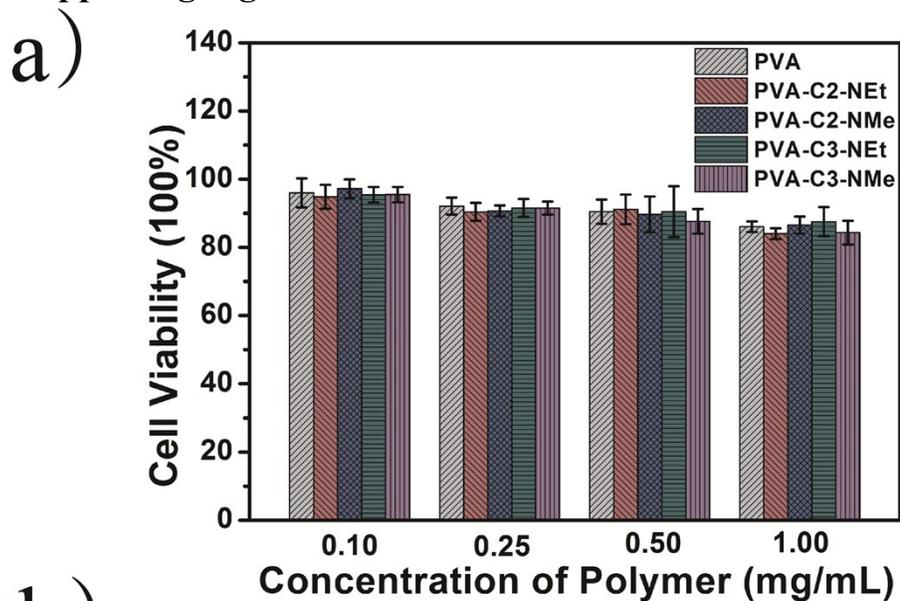
**Cell viability assay.** Approximately  $1 \times 10^5$  cells were seeded in a 96-well plates per well. After 24 hour incubation under 5% CO<sub>2</sub> and 95% air at 37 °C, the cells were treated with different concentrations of polymers (0, 0.1, 0.25, 0.5, 1.0 mg/mL) at 37 °C for 24 hour and then added MTS (120  $\mu$ L, 0.1 mg/mL) for another 4 h incubation in the dark. Cells

treated with nothing were used as a control. The absorbance of MTS was detected using a microplate reader at 490 nm to determine the cell viability.

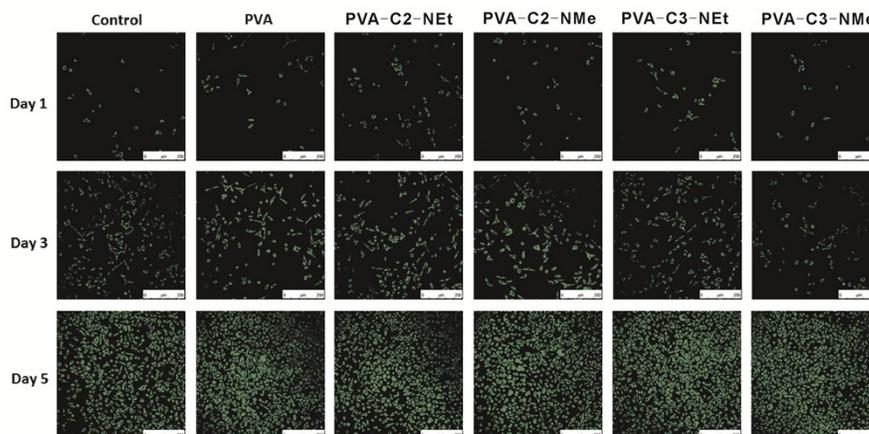
***Cell proliferation assay.*** Approximately  $3 \times 10^3$  cells were seeded in a 96-well plates per well. After 24 hour incubation under 5% CO<sub>2</sub> and 95% air at 37 °C, the cells were treated with different concentrations of polymer (0, 0.1, 0.25, 0.5, 1.0 mg/mL) at 37 °C. After 1 day, 3 day and 5 day incubation, MTS assay were performed to determine the cell proliferation using the same method provided in cell viability assay.

***Live/dead viability assay.*** Approximately  $3 \times 10^3$  cells were seeded in a 8-well ibidi® glass bottom plates and then incubated 24 hour under 5% CO<sub>2</sub> and 95% air at 37 °C. Then, different polymers (1 mg/mL) were added into the wells for the incubation. After 1 day, 3 day and 5 day incubation, the cells were washed with PBS buffer and added Calcein AM (2 μM) and Ethidium Homodimer (4 μM) together for another 30 mins incubation. After washing with PBS buffer, fluorescence images were then taken using a Leica TCS SP5 Confocal Scanning Microscope. For Calcein AM, excitation was 488 nm and emission range was 500-550 nm which showed green color in the imaging. For Ethidium Homodimer, excitation was 543 nm and emission range was 600-650 nm which showed red color in the imaging.

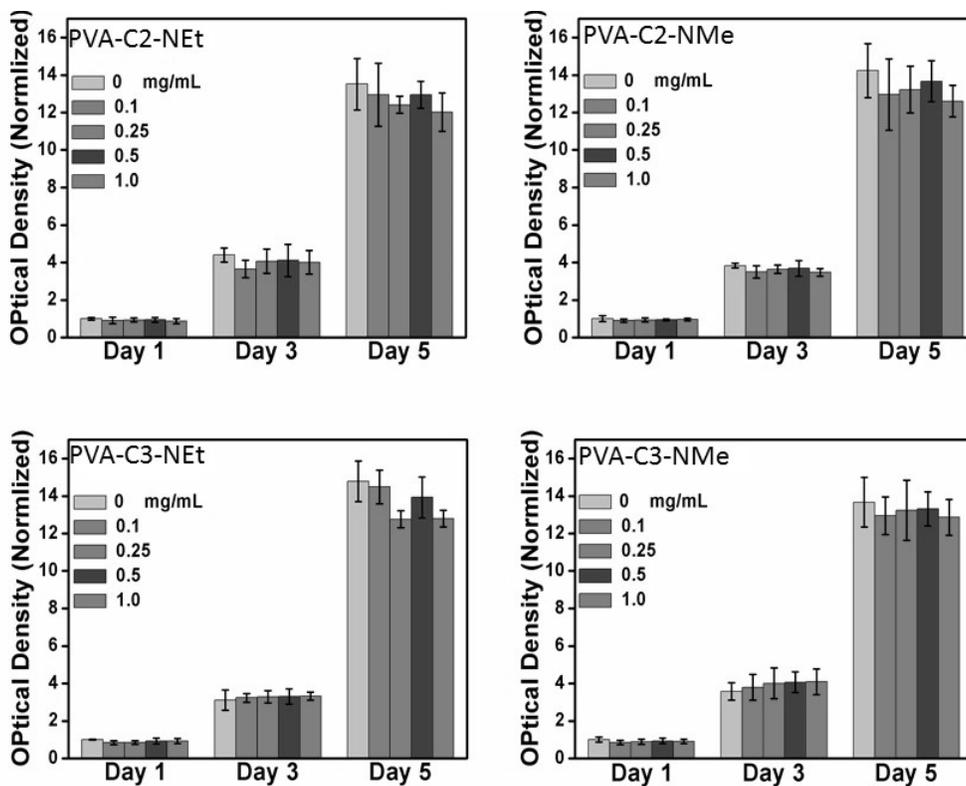
### SIII. Supporting Figures



b)



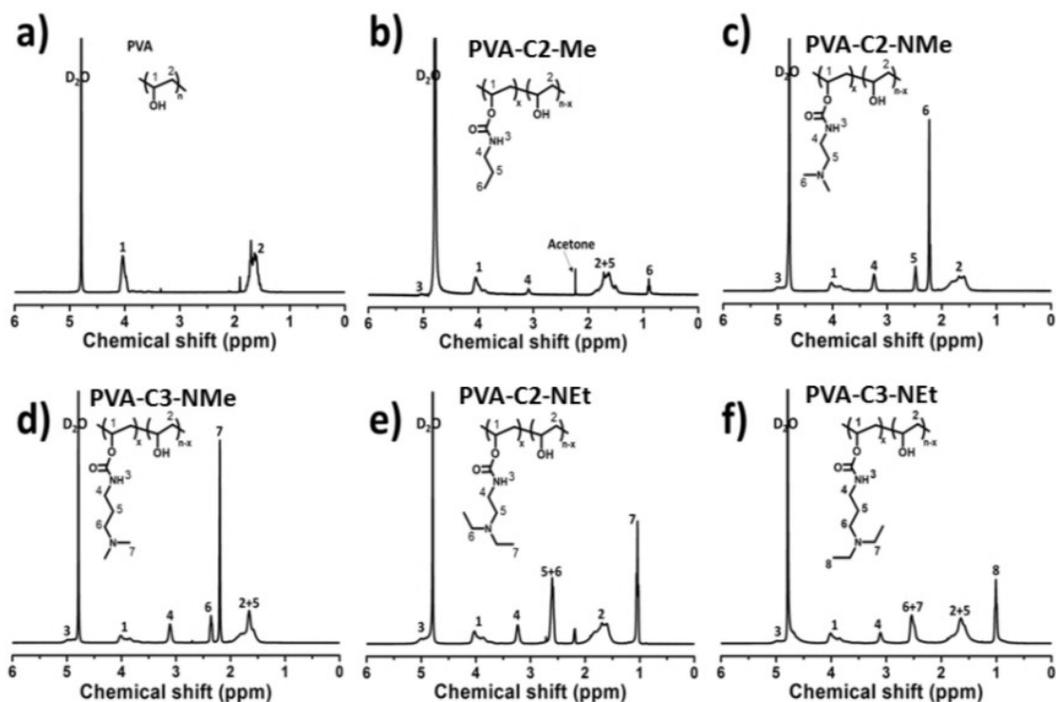
**Figure S1.** (a) MTS assay with different concentrations of related polymer in HeLa cells after 24 h of incubation; (b) Cell proliferation assay with live/dead assay for the related polymer (1 mg/mL) in HeLa cells after incubation for 1, 3 and 5 days by confocal fluorescence microscopy with magnification at 40 $\times$ .



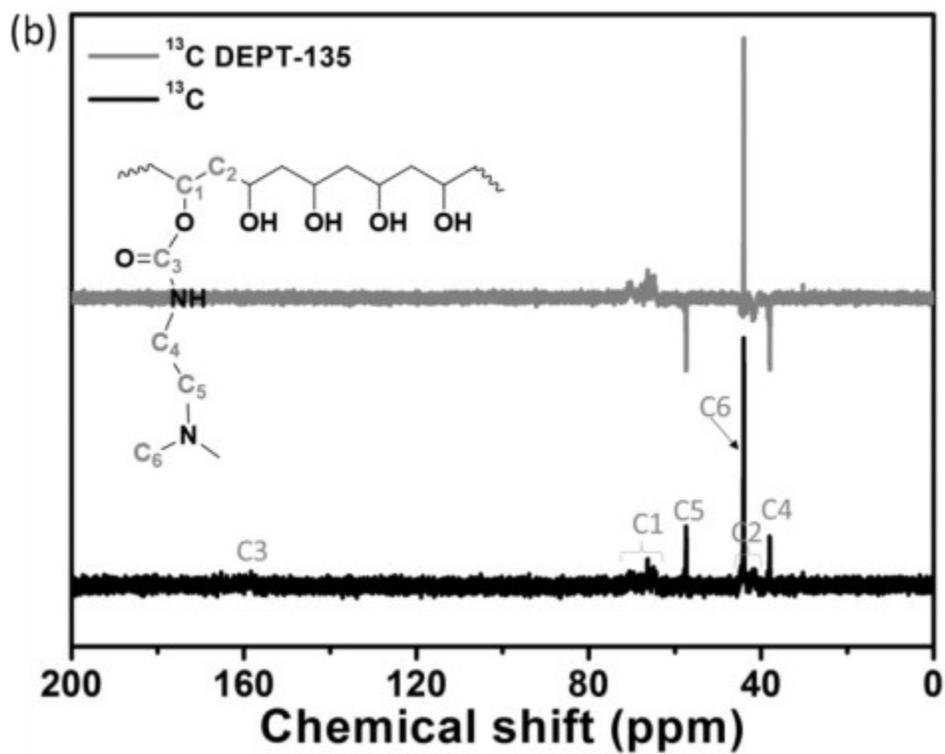
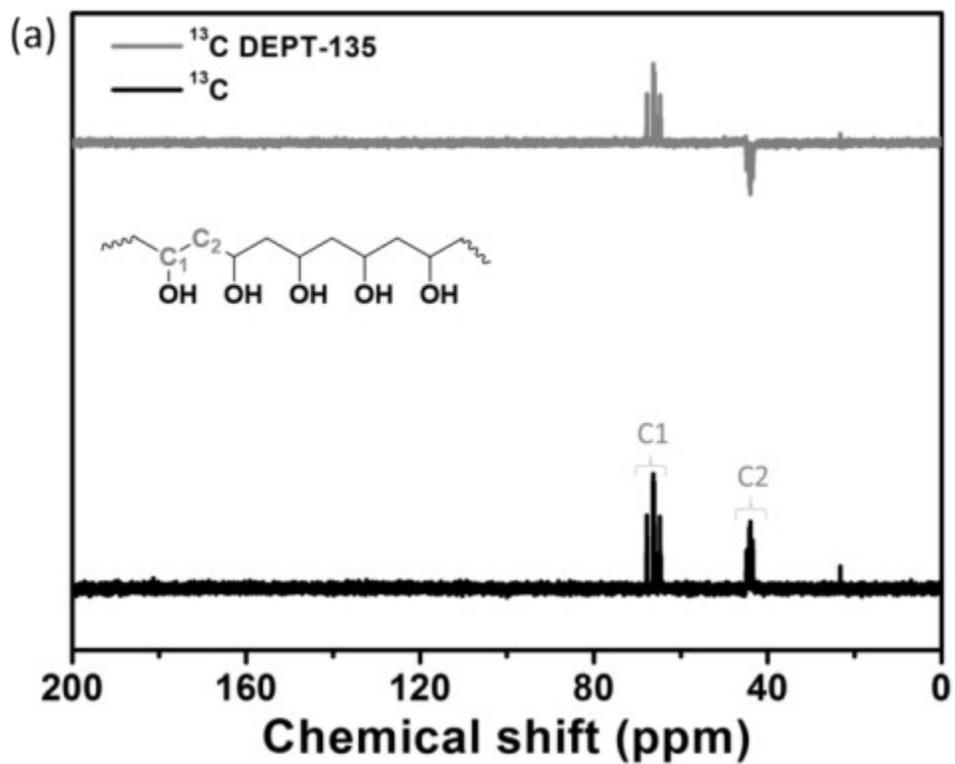
**Figure S2.** The cell proliferation assay for the related polymers with different concentration in HeLa cells after incubation for 1, 3 and 5 days.

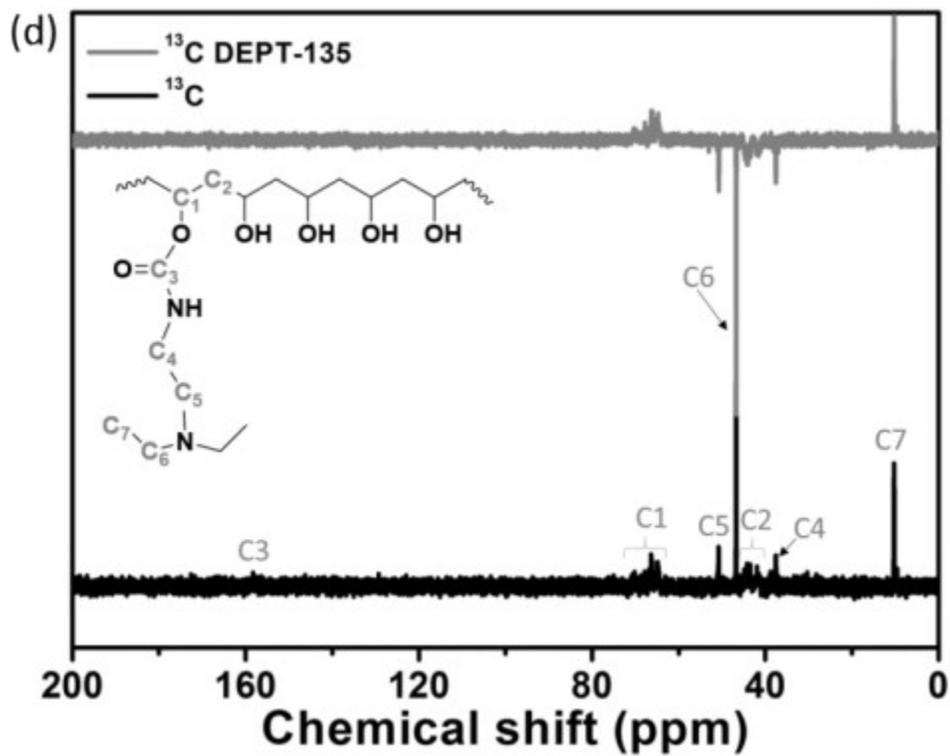
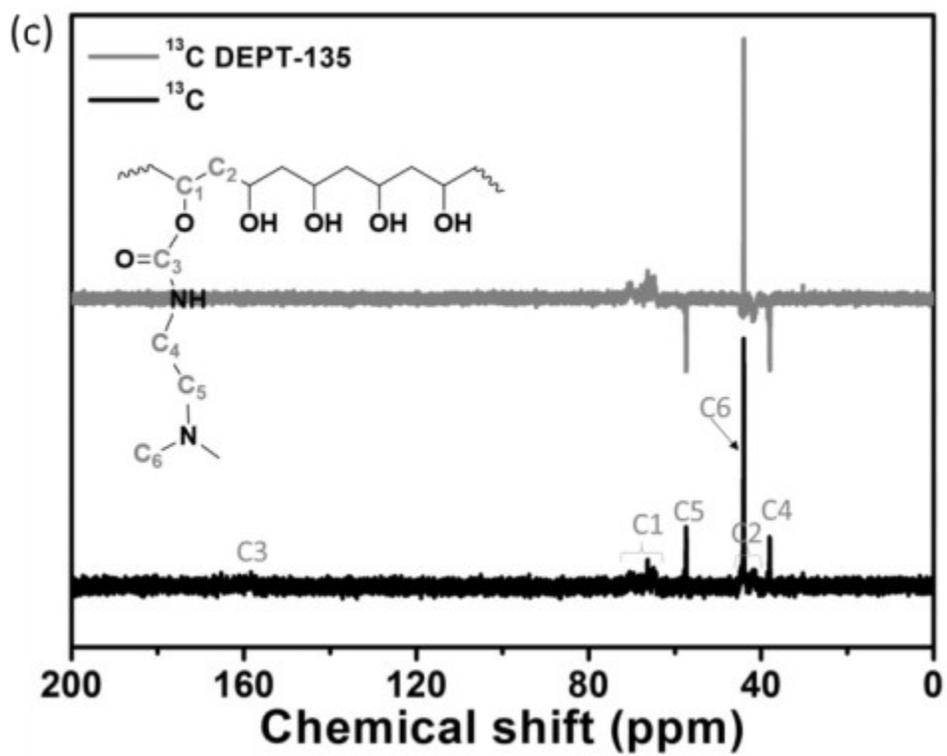
Although the neat PVA has been widely recognized to be safe for biological system, the influences of appendant *N*-alkyl substituted carbamates on the cytocompatibility should be further verified. Therefore, the cytocompatibility of PVA derivatives with various *N* alkyl substituted carbamate is evaluated by in vitro culture of HeLa cervical adenocarcinoma epithelial (HeLa) cells (see Experimental Section). Cells seeded without polymers extracts served as controls. As shown in **Figure S1**, the MTS assay for PVA derivatives with various appendant *N* alkyl substituted carbamate shows the excellent cell viability as high as ~85-98%, indicating their non-cytotoxicity. To further investigate the cell compatibility and visualize the cell viability of the composite hydrogels, cell proliferation and live/dead viability assays as a function of time are respectively employed on these PVA derivatives. **Figure S2** shows the cell proliferation assay for the

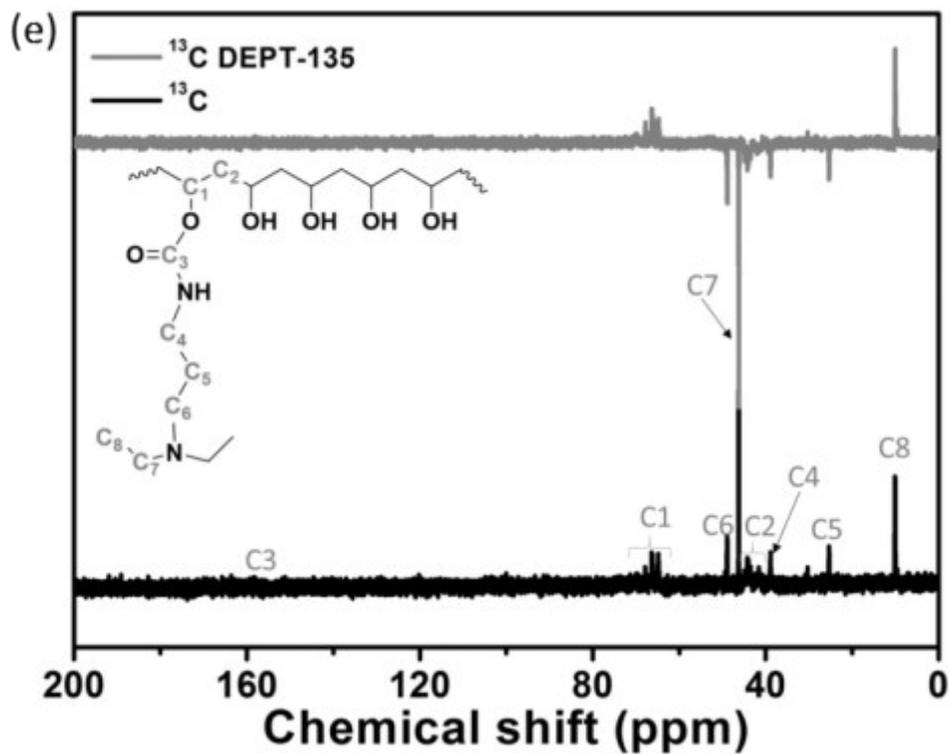
related polymers with different concentration in HeLa cells after incubation for 1, 3 and 5 days. After the first day's culture, all the control and PVA derivatives show similar cell density. The cell density increases dramatically during the following culture. After culture for 5 days, no noticeable variation in cell density can be observed among the various samples. The live/dead cell is further visualized by using live/dead fluorescence images are shown in **Figure S1b**. After culture for 1 day, similar live cell (green) density is observed for all the control and PVA derivatives without dead cells (red) could be found, indicating the excellent biocompatibility of *N* alkyl substituted carbamate groups on PVA derivatives. This result is consistent with the abovementioned MTS results. Along with the culture time, the cells continuously proliferate and spread uniformly for all of PVA derivatives until almost full coverage after 5 days, and dead cells are barely observed during this extended culture. This indicates that outstanding cell viability of the PVA is perfectly maintained after the adding of *N*-alkyl substituted carbamates.



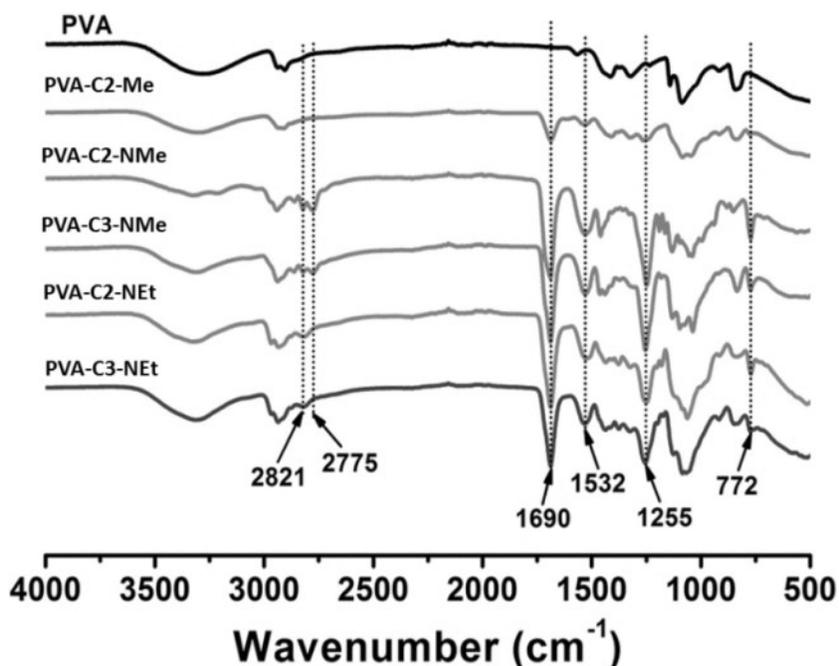
**Figure S3.**  $^1\text{H}$  NMR spectra of a) PVA, b) PVA-C2-Me, c) PVA-C2-NMe, d) PVA-C3-NMe, e) PVA-C2-NEt and f) PVA-C3-NEt in  $\text{D}_2\text{O}$  recorded at room temperature. Conc. 5 mg/mL, Solvent.  $\text{D}_2\text{O}$  Degree of substitution ( $\text{DS} = [\text{pendant group}]/[-\text{OH}]_0$ ) for PVA-C2-Me, PVA-C2-NMe, PVA-C3-NMe, PVA-C2-NEt and PVA-C3-NEt are 0.05, 0.24, 0.25, 0.23 and 0.23, respectively.



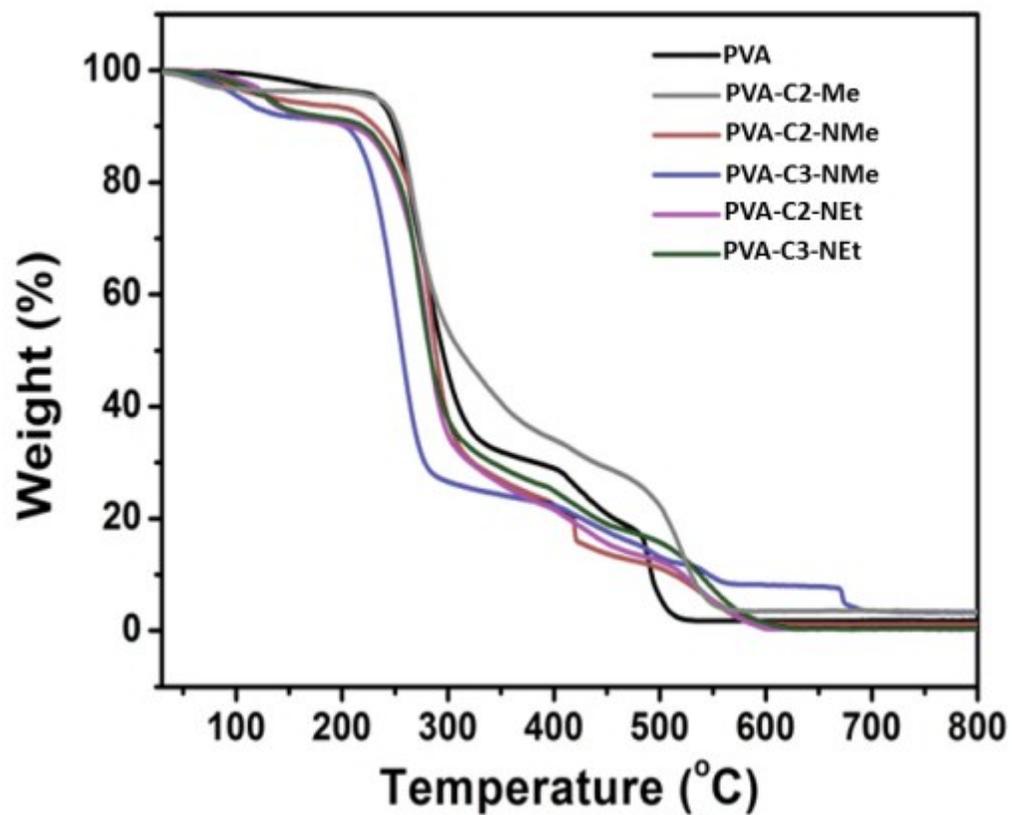




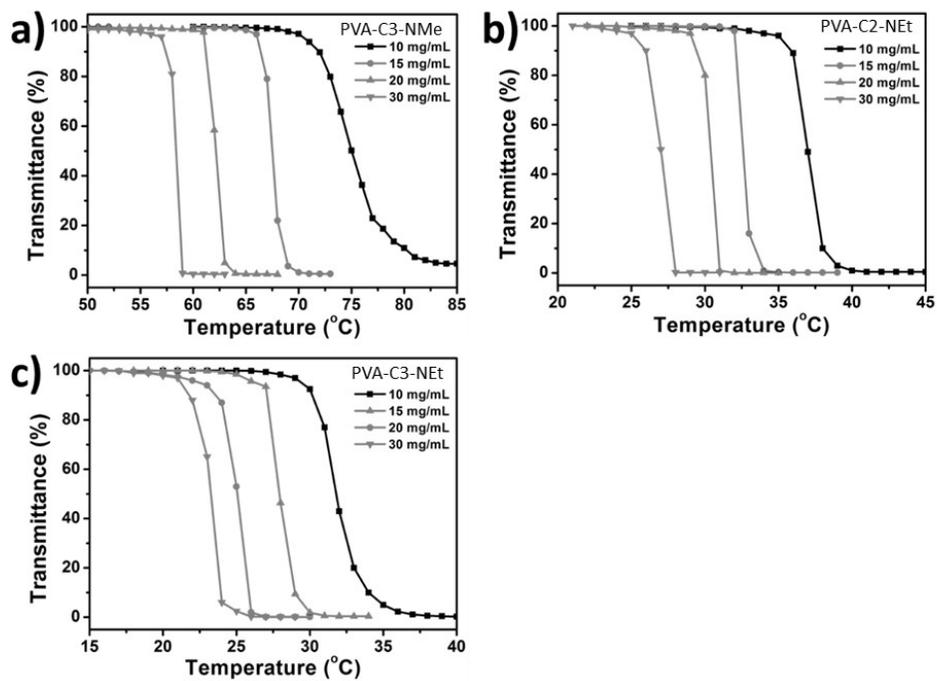
**Figure S4.**  $^{13}\text{C}$  and  $^{13}\text{C}$  DEPT-135 spectra for the for PVA-C2-Me, PVA-C2-NMe, PVA-C3-NMe, PVA-C2-NEt and PVA-C3-NEt.



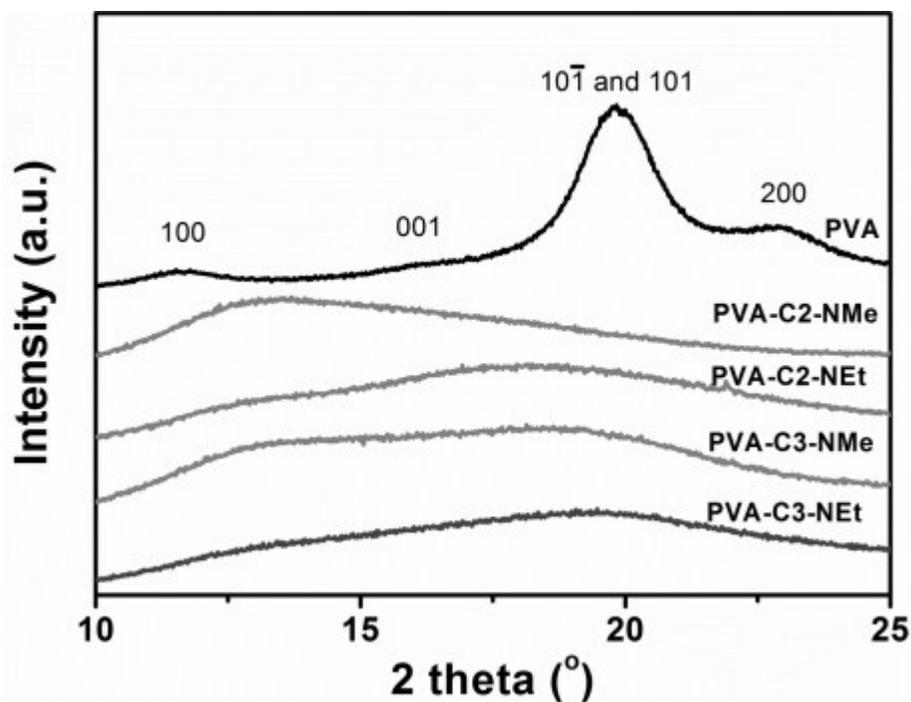
**Figure S5.** ATR-FTIR spectra of bulk PVA, PVA-C2-Me, PVA-C2-NMe, PVA-C3-NMe, PVA-C2-NEt and PVA-C3-NEt. In finger print region, all PVA derivatives present four strong peaks at 1690  $\text{cm}^{-1}$ , 1532  $\text{cm}^{-1}$ , 1255  $\text{cm}^{-1}$  and 778  $\text{cm}^{-1}$ , which does not present for the neat PVA. The peak at 1690  $\text{cm}^{-1}$  should be assigned to the C=O stretching vibration of the carbamate group (-O-(CO)-NH-). The asymmetric -C(O)-O-C stretching vibration is also observed at 1255  $\text{cm}^{-1}$ . The peaks at 1532  $\text{cm}^{-1}$  should be assigned to NH bending. These information suggests that all PVA derivatives contain carbamate groups. In addition, several weak peaks in the region of 2500-3000  $\text{cm}^{-1}$  provide information for us to distinguish the *N* alkyl carbamate groups. A new peak at 2775 is observed for PVA-C2-Me, PVA-C2-NMe, while no such peak is present for PVA-C3-NMe, PVA-C2-NEt and PVA-C3-NEt. According to the literatures, the peak can be assigned to the -CH<sub>3</sub> directly bonded to the N, therefore, PVA-C2-NEt and PVA-C3-NEt with terminal ethyl group does not exhibit this peak.



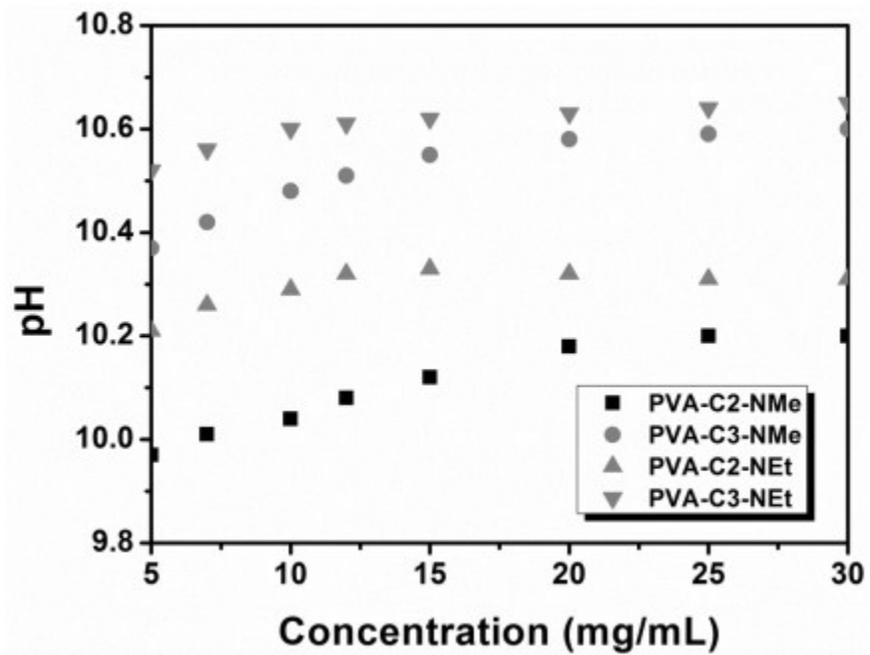
**Figure S6.** TGA curves for PVA and various PVA derivatives. Addition of functional groups to PVA barely adversely affects the thermal stability of PVA backbone. The loss of the adsorbed water contributes the weight loss before thermal decomposition at 160 °C.



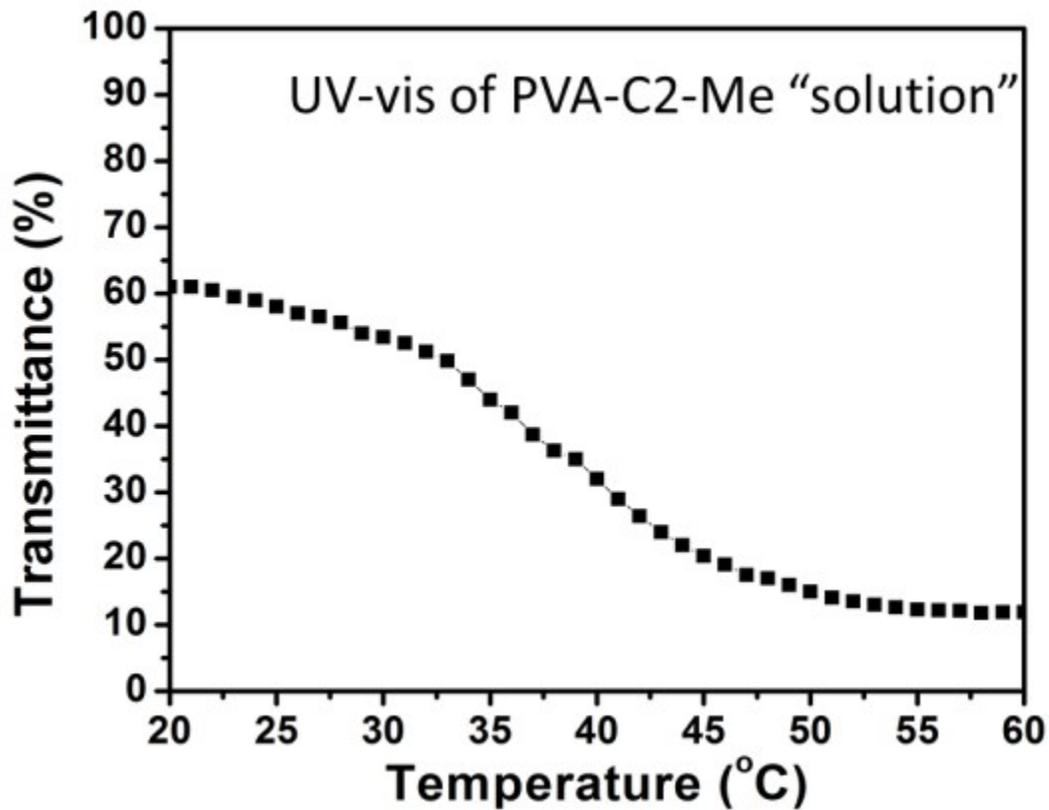
**Figure S7.** The changes on transmittance of (a) PVA-C3-NMe; (b) PVA-C2-NEt and (c) PVA-C3-NEt.



**Figure S8.** XRD patterns for PVA and various PVA derivatives. The peaks are indexed according to the prior work.[Crystallinity in polyvinyl alcohol 1. An X-ray diffraction study of atactic PVOH, *Polymer*, 1998, 39, 4295-4302.] The pristine PVA has semi-crystal nature. After being appended with responsive groups, both of these peaks become weak and broaden, indicating the significantly decrease semi-crystallinity. This breakage of crystallinity can be caused by perturbation of hydrogen bonds of PVA chains due to the addition of the bulky responsive groups. One of important consequences for the decrease in crystallinity is the improved solubility of polymers. Unlike the pristine PVA which can only be dissolved in hot water, the functionalized polymers with responsive groups can be readily dissolved in cold water. It is also notable that the major peak experience small-angle shifting upon the appending of responsive groups. This can be generally explained by the reduction in packing density, increasing the lattice parameters for the crystal region of all PVA derivatives. Among all of PVA derivatives, PVA-C2-NMe should have the weakest hydrophobic interaction and loose packing structure, therefore, the most pronounced small-angle shifting is observed .

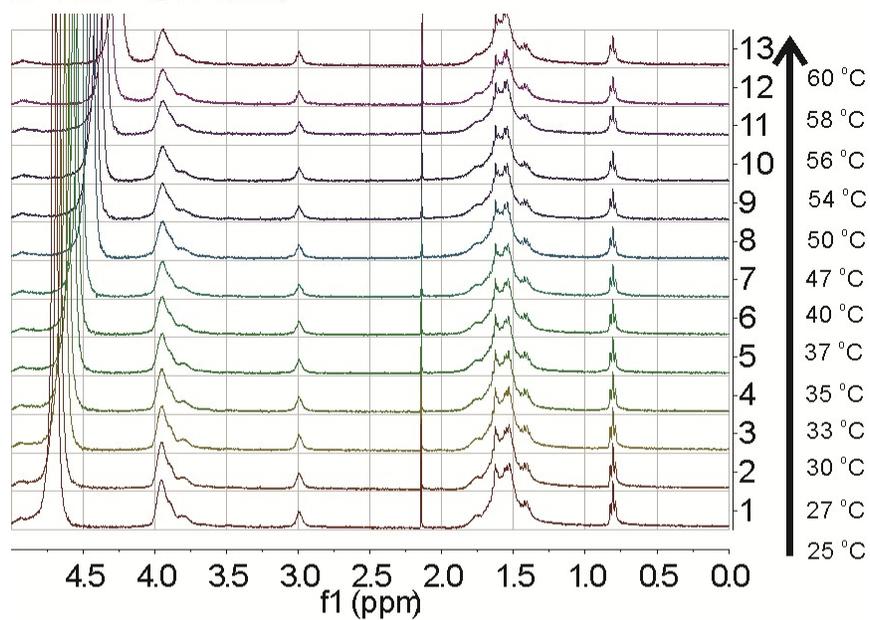


**Figure S9.** *pH* value of amine modified PVA as a function of concentration. (25 °C), solvent: H<sub>2</sub>O

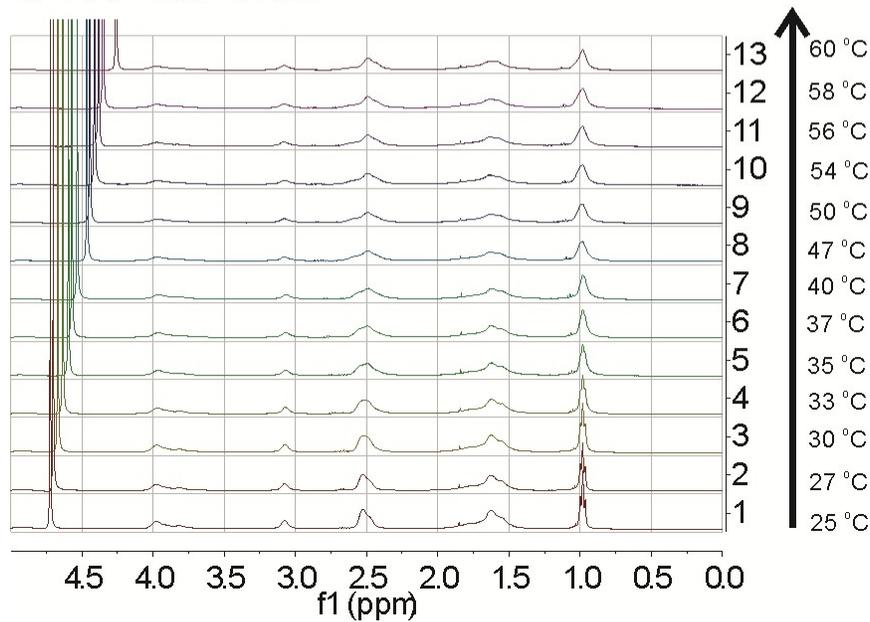


**Figure S10.** The transmittance of PVA-C2-Me solution as a function of temperature. (5 mg/mL), solvent: H<sub>2</sub>O.

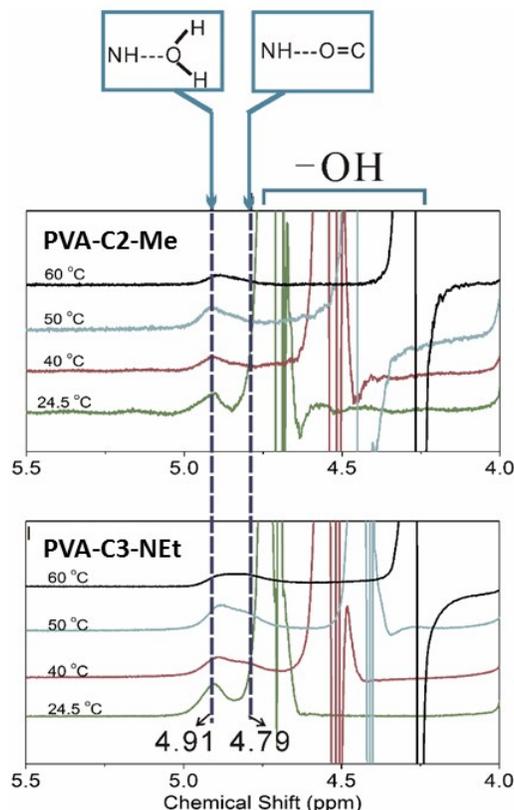
### PVA-C2-Me



### PVA-C3-NEt

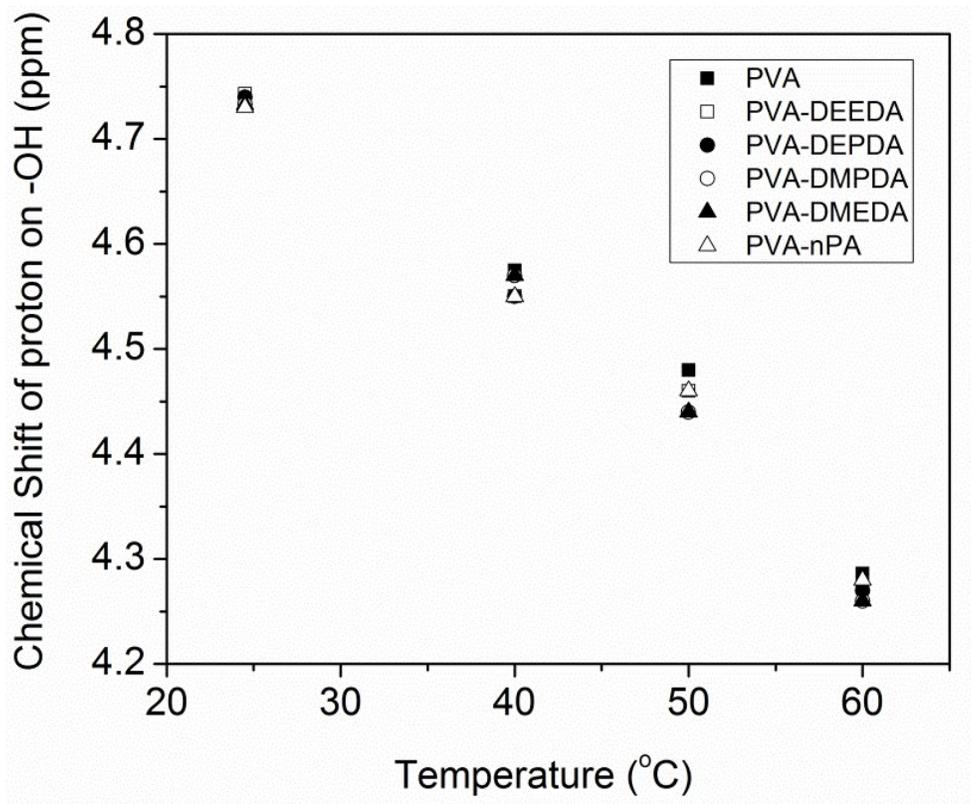


**Figure S11.**  $^1\text{H}$  NMR spectra of a) PVA-C2-Me b) PVA-C3-NEt in  $\text{D}_2\text{O}$  (5 mg/mL) recorded at 25-60 °C.

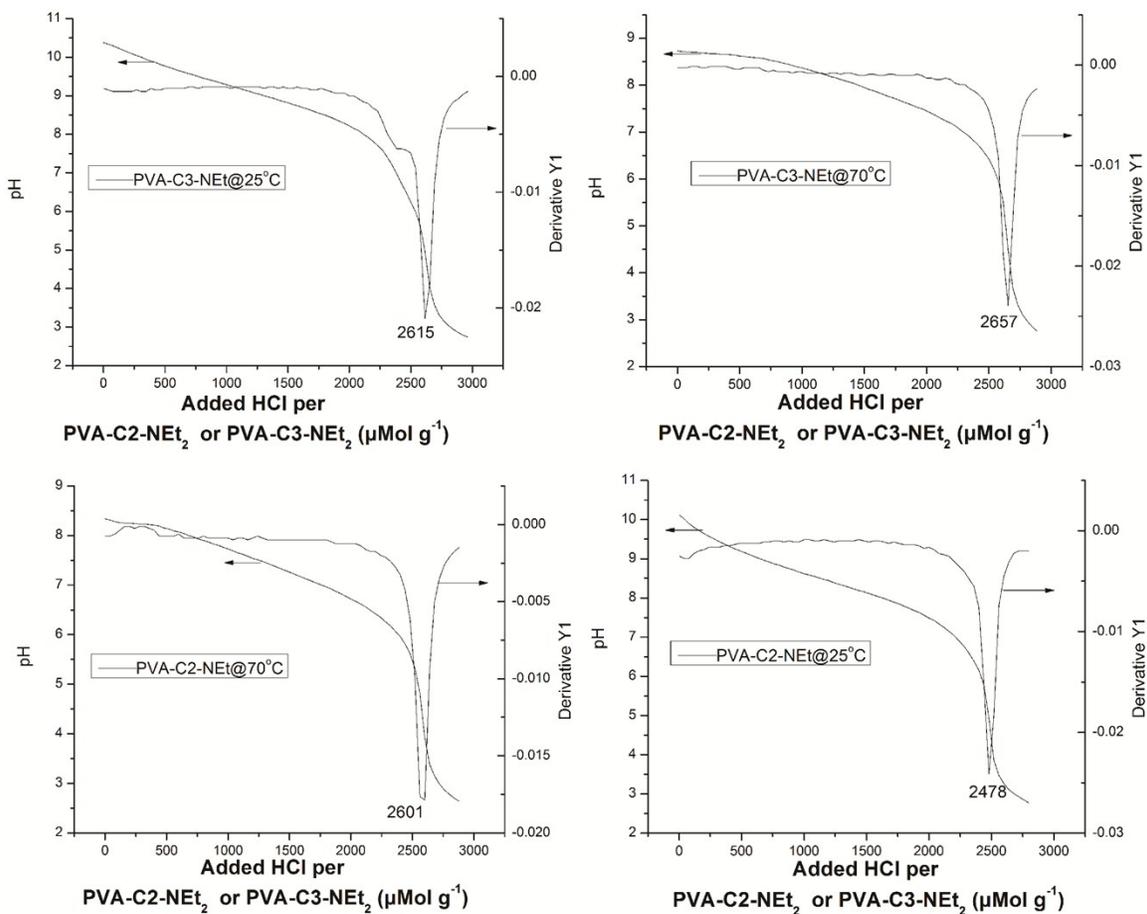


**Figure S12.** VT-NMR spectra for PVA-C2-Me and PVA-C3-NEt. Solvent: H<sub>2</sub>O/D<sub>2</sub>O (9:1, v/v) with water suppression; Conc. 5 mg/mL. With temperature increasing over LCST, the dehydration of -NH- group results in the present of new peak at ca. 4.79 ppm. Note the intensity of proton peak is not directly comparable. Chemical shifting of protons on NMR is well known to be highly sensitive to the hydrogen bonding, therefore VTNMR is employed here to provide another pieces of evidence. In addition, one especially interesting question is whether the hydroxyl groups of these PVA derivatives take part in the formation of hydrogen bonding that is related to the LCST behavior. To unveil this, we performed NMR by using 9:1 H<sub>2</sub>O/D<sub>2</sub>O (9:1, v/v, water suppression) as solvent, so that the proton signal on -NH- and -OH is detectable. Otherwise, these protons can be exchanged and their signal fall into noisy. We focus on the region of chemical shifts assigned to -NH- and -OH (**Figure S12**). As shown in **Figure S12**, the -OH peak of PVA on NMR shows upfield shifting, which is positively proportional to the changes of temperature. This is because the -OH hydrogen bond bridge of the neat PVA gradually breaks with temperature increasing, weakening the electron shielding. Interestingly, the exactly same linear temperature dependence occurs to the chemical shifts of all of the responsive PVA derivatives (**Figure S12**). This result suggests that the LCST behavior of these PVA derivatives is evidently not related to the -OH. Therefore, close attention is paid to examine the -NH- segment. The proton on -NH- for all of the PVA derivatives is present at around 4.91 ppm. Upon heating, a shoulder at higher field (4.79 ppm) appears, implying the increases in the electron density of the -NH- motif. One reasonable explanation for this phenomenon should be related to the dehydration of PVA-C3-NEt caused by the partial loss of hydrogen bonding between NH and water. On the other hand,

the -NH- peak of PVA-C2-ME at 4.91 ppm only undergoes very slight broadening, and no new peak presents at higher field. These results reinforce the conclusion drawn from the FTIR spectra in **Figure 5**, suggesting that different mechanism should be responsible to the temperature-induced turbidity increase for PVA-C2-Me and PVA-C3-NEt solution. It is important to note that the formation of hydrogen bonding is not sufficient to offer LCST property, because PVA-C2-NMe, which also shows the similar hydrogen bonding effect according to the NMR spectra, does not exhibit detectable volume phase transition in the temperature range of 20 °C to 80 °C.



**Figure S13.** Chemical shift of -OH on PVA and PVA derivatives as a function of temperature. Conc. 5 mg/mL, Solvent: D<sub>2</sub>O/H<sub>2</sub>O.



**Figure S14.** *pH* titration of PVA-C2-NEt and PVA-C3-NEt at 25 °C and 70 °C. When the addition of HCl yielded sharpest change of *pH*, tertiary amines are regarded to be 100% protonated. Conc. 20 mg/mL, Solvent. H<sub>2</sub>O.