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Amphiphilic Block Copolymers Bearing Hydrophobic \( \gamma \)-Tocopherol Groups with Labile Acetal Bond

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1. Introduction

Amphiphilic diblock copolymers in water form polymer micelles consisting of a hydrophobic core and hydrophilic shell [1–3]. This serves as an effective route to encapsulate hydrophobic anticancer drugs into the core, thereby improving the solubility of the reagents while limiting side effects [4]. As such, the passive targeting of drug-loaded polymer micelles of 10–100 nm average size can enhance the permeability and retention (EPR) effect [5,6]. In general, polymer micelles with a size of several hundred nanometers are unable to penetrate normal vascular walls, while polymer micelles around tumor tissue can penetrate through defected vascular walls (enhanced permeability). This is due to incomplete neovascularization around the tumor and gaps that form between vascular endothelial cells. In addition, polymer micelles cannot be completely removed from the lymphatic tissue around the tumor due to the immaturity of the lymphatic tissue. Therefore, the leaked polymer micelles from tissue vesicular walls tend to accumulate around tumor tissue (enhanced retention). Such a property generated by the accumulation of polymer micelles around tumor tissue is the EPR effect [7]. However, in the passive targeting mechanism, the amount of drugs for delivery to the affected area is low, making the evaluation of the side effects of anticancer drugs non-negligible [8,9].

Recently, cancer-cell apoptosis has been reported using food-derived natural products [10,11]. \( \gamma \)-tocopherol (\( \gamma \)TCP) is a type of vitamin E, contained in vegetable oils such as canola, soybean,
and corn oils. This substance possesses antioxidative and anti-inflammatory effects, and demonstrates the potential to improve cardiovascular disease and prostate cancer \[12,13\]. According to the literature, an increase in γTCP concentrations in plasma may decrease the risk of prostate cancer \[14\]. However, it is well established that the accumulation of excess amounts of vitamin E in the body by a normal diet is impossible due to the low absorption rate of vitamin E that is in the range of 21%–29% \[15\]. Nevertheless, by local administration of more highly concentrated vitamin E, cancer-cell apoptosis can be induced without the use of anticancer drugs.

According to previous studies, the pH of cancer and healthy cells was determined as 5 and 7.4, respectively \[16\]. Acetal bonds under acidic conditions can also be readily cleaved into suitably functional moieties \[17\]. pH-responsive nanoparticles containing acetal linkage within the chemical structure for the controlled release of drugs under acidic conditions have been developed in the last two decades \[18–21\]. Simo et al. \[22\] reported that acetal linkage-containing hydrophilic N-(2-(tetrahydoro-2H-pyna-2-yl)oxy)ethyl acrylamide (HEAmTHP) was polymerized via reversible addition-fragmentation chain transfer (RAFT) radical polymerization using a hydrophobic poly(2-hydroxyethyl acrylate) (PHEA) macro-chain transfer agent (CTA) to prepare an amphiphilic diblock copolymer (PHEA-PHEAmTHP). In a subsequent study \[23\], the encapsulation of a hydrophobic antibiotic substance in the hydrophobic domain of formed PHEA-PHEAmTHP micelles in water was performed. Another study by Gold et al. investigated the effects of attached Amphotericin B to the cell membrane of micro-organisms, which showed antibiotic property by breaking membrane structures \[24\]. Under acidic conditions, Amphotericin B was released from the polymer-micelle duet that cleaved the acetal linkages. Feng et al. \[25\] prepared hyperbranched polyesters (S-hbPE) containing multiple acetal linkages via condensation of 2,2'-{(propane-2,2-diylbis(oxy)) diethanol, phosphoryl chloride, and poly(ethylene glycol) monomethyl ether (m-PEG\(_{45}\)-OH). Chlorin e6 a photosensitizer for photodynamic therapy (PDT) was encapsulated into S-hbPE to prepare nanoparticles. Chlorin e6 was then released under acidic conditions around tumor tissue attributed to the decomposition of the acetal linkages. As expected, the release of chlorin e6 under acidic conditions around tumor tissue showed the high efficiency of PDT with reduced side effects. Thus, nanocarriers containing acid labile acetal linkages for delivery systems via endosome/lysosome possess advantages such as solubilization of hydrophobic guest molecules, and controlled release in the acidic conditions around tumor tissue.

In this study, we first prepared a precursor diblock copolymer (PEG\(_{54}\)-PAA\(_{140}\)) composed of biocompatible poly(ethylene glycol) (PEG) and poly(acrylic acid) (PAA) blocks. This was followed by the preparation of a diblock copolymer (PEG\(_{54}\)-P(AA/VE35)\(_{140}\)) via incorporation of vinyl ether (VE) groups to the pendant carboxylic acid groups of the PAA block through esterification. Finally, a diblock copolymer (PEG\(_{54}\)-P(AA/VE6/γTCP29)\(_{140}\)) was prepared via introduction of hydrophobic γTCP groups onto the pendant VE groups by acetal linkage (Figure 1). The plane and subscript numbers express the content (mol%) and degree of polymerization (DP), respectively. In addition, aqueous solutions of PEG\(_{54}\)-P(AA/VE6/γTCP29)\(_{140}\) were formulated to produce polymer micelles via hydrophobic interactions of pendant γTCP (Figure 2). Under acidic conditions, partial release of γTCP occurred due to the decomposition of the pendant acetal linkages. This was also attributed to the slight swelling of the polymer micelles since hydrophobicity of the micelles decreased with the release of hydrophobic γTCP. This suggests that the release of highly concentrated γTCP by the polymer micelles under acidic conditions may lead to cancer-cell apoptosis without the use of anticancer drugs.
Figure 1. Synthesis of PEG54-PAA140, PEG54-P(AA/VE35)140, and PEG54-P(AA/VE6/\(\gamma\)TCP29)140.

Figure 2. (a) Micelle formation and release of \(\gamma\)TCP in acidic aqueous solution; (b) chemical structure of PEG54-P(AA/VE6/\(\gamma\)TCP29)140.

2. Materials and Methods

2.1. Materials

Poly(ethylene glycol) methylether (4-cyano-4-pentanoate dodecyltrithiocarbonate) (PEG54, \(M_n = 2.40 \times 10^3\) g/mol) and \(\gamma\)-tocopherol (\(\gamma\)TCP, 96%) were purchased from Sigma Aldrich (St. Louis, MO, USA); 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 98%) and \(N, N\)-dimethyl-4-aminopyridine (DMAP, 99%) were procured from Fujifilm Wako Pure Chemicals (Osaka, Japan). Ethylene glycol mono-vinyl ether (EGVE, 98%) and \(p\)-toluenesulfonic acid (\(p\)-TSA, 98%) were supplied by Tokyo Chemical Industry (Tokyo, Japan). All reagents above were used as received without further purification. We used 2,2′-azobisisobutyronitrile (AIBN, 98%) from Sigma Aldrich (St. Louis, MO, USA) after recrystallization using methanol. Acrylic acid (AA, 98%), 1,4-dioxane
We have used the conventional esterification method using EDC [26]. PEG54-PAA140 (134 mg, 1.50 mmol of COOH unit), DMAP (91.8 mg, 0.751 mmol), and EDC (860 mg, 4.49 mmol) were dissolved in 1, 4-dioxane (50 mL). The solution was deoxygenated by purging argon gas for 30 min. The reaction was complete, the solution was dialyzed against pure water for 4 days under an argon atmosphere, and then EGVE (0.667 mL, 7.55 mmol) was added to the solution. The solution was stirred at 30 °C for 24 h. The exchange ratio from the VE to TCP was calculated as 82.9% from "H NMR.  

2.4. PEG54-P(AA/VE6/γTCP29)140 Synthesis  

Conjugation of γTCP onto PEG54-P(AA/VE35)140 via an acid-labile acetal bond was prepared according to a previously reported method [17]. PEG54-P(AA/VE35)140 (62.5 mg, 180 µmol of VE unit), γTCP (25.0 mg, 60.0 µmol), and p-TSA (0.56 mg, 2.94 µmol) were dissolved in DMF (10 mL). Molecular sieves 4A (1.00 g) was added to the solution. The reaction was carried out at 50 °C for 4 days under argon atmosphere. Molecular sieves were removed by filtration, and the solution was dialyzed against pure water for 4 days. The polymer (PEG54-P(AA/VE6/γTCP29)140) was isolated by a freeze-drying method using GPC. The exchange ratio from the VE to γTCP was calculated as 82.9% from 1H NMR.  

2.5. Measurements  

1H NMR spectra were measured with a Bruker DRX-500 (Billerica, AM, USA) at room temperature. The block copolymer sample solutions for the 1H NMR measurements were prepared in dimethylsulfoxide-d6 (DMSO-d6). GPC measurements were performed using Shodex (Tokyo, Japan) Asahipak GF-1G guard column and GF-7M HQ column at 40 °C with a refractive index detector and yielded a phosphate buffer solution (PBS) of pH 9 containing 10% v/v of acetonitrile at 0.6 mL/min in the developing solvent. Determination of Mn and Mw/Mn according to GPC were calibrated using standard sodium poly(styrene sulfonate) (2). Hydrodynamic radius (Rg) and scattered light intensity (LSI) were estimated using a Malvern (Malvern, UK) Zetasizer Nano ZS-ZEN3600 equipped with a He–Ne laser source (4 mW at 632.8 nm) at 25 °C. The samples solutions for light-scattering measurements were filtered through a filter with pore-size diameter of 0.8 µm. Static light scattering (SLS) measurements were carried out with Otsuka Electronics (Osaka, Japan) DLS 7000 at 25 °C. The He–Ne laser (10 mW at 632.8 nm) was used as a light source. Weight average molecular weight (Mw) and radius of gyration (Rg) were calculated from a Debyel plot at 1 polymer concentration. The Rayleigh ratio of toluene was estimated using GPC were determined as 1.02 ± 0.07, 1.36, respectively, using gel-permeation chromatography (GPC).
used in instrument calibration. Refractive index increment against the polymer concentration \((\text{dn/dC}_p)\) at 633 nm was determined using Otsuka Electronics DRM-3000 differential refraction meter at 25 °C. TEM observations were performed with a JEOL (Tokyo, Japan) JEM-2100 at an accelerating voltage of 160 kV. Prior to TEM analysis, the sample was prepared by placing 1 drop of the aqueous solution on a copper grid coated with Formvar. Excess water was blotted using filter paper. Samples were stained by sodium phosphotungstate and dried under vacuum for a period of 1 day.

3. Results and Discussion

3.1. PEG\(_{54}\)-P(AA/VE6/\(\gamma\)TCP29\(_{140}\)) Preparation

The conversion of AA by RAFT polymerization using PEG\(_{54}\) macro-CTA was determined as 98.2% estimated from the decrease of \(^1\)H NMR integral area intensity ratio of the vinyl group in AA after polymerization. The DP of the PAA block was calculated to be 140 from the area integral intensity ratio of peaks attributed to ethylene oxide protons in PEG\(_{54}\) at 3.7 ppm and the main chain protons in PAA at 2.3 ppm (Figure 3a). The introduction of the pendant vinyl groups in PEG\(_{54}\)-P(AA/VE35)\(_{140}\) were confirmed by the observed \(^1\)H NMR signal at 6.5 ppm attributed to VE (Figure 3b). The reaction rate from the pendant carboxylic acid to the vinyl groups, calculated as 34.8%, was estimated from the integral intensity ratios of the attributed peaks to the main chain protons of PAA\(_{140}\) at 6.3 ppm and the vinyl protons in EGVE at 6.5 ppm. This result indicated that about 49 pendant vinyl groups were introduced into one polymer chain of PEG\(_{54}\)-P(AA/VE35)\(_{140}\). The protons attributed to the terminal methyl at 0.8 ppm, benzyl at 2.0 ppm, and phenyl protons at 6.3 ppm in the pendant \(\gamma\)TCP could be observed from a PEG\(_{54}\)-P(AA/VE6/\(\gamma\)TCP29)\(_{140}\) \(^1\)H NMR spectrum (Figure 3c). The introduction rate of \(\gamma\)TCP in PEG\(_{54}\)-P(AA/VE6/\(\gamma\)TCP29)\(_{140}\) was calculated as 83.3% estimated from the integral intensity ratio of the unreacted pendant vinyl protons in PEG\(_{54}\)-P(AA/VE35)\(_{140}\) at 6.5 ppm and phenyl protons in \(\gamma\)TCP at 6.3 ppm [27]. This result indicated that about 41 pendant \(\gamma\)TCPs were introduced into a single polymer chain of PEG\(_{54}\)-P(AA/VE6/\(\gamma\)TCP29)\(_{140}\). The theoretical \(M_n\) of each polymer was calculated using the following equation:

\[
M_n(\text{theo}) = \frac{[M]_0}{[\text{CTA}]_0} \times \frac{p}{100} \times M_m + M_{\text{CTA}},
\]

where \([M]_0\) and \([\text{CTA}]_0\) are the initial molar concentrations of monomer and CTA, respectively, \(p\) is the conversion of the monomer, and \(M_m\) and \(M_{\text{CTA}}\) are the molecular weights of the monomer and CTA, respectively.

The GPC-determined \(M_n\) values of PEG\(_{54}\)-PAA\(_{140}\), PEG\(_{54}\)-P(AA/VE35)\(_{140}\), and PEG\(_{54}\)-P(AA/VE6/\(\gamma\)TCP29)\(_{140}\) were \(1.25 \times 10^4\), \(7.02 \times 10^3\), and \(1.02 \times 10^4\) g/mol, respectively (Figure 4). Due to the unexpected interactions between polymers within the GPC column, and the difference between structures of the measured polymer and the standard polymer, accurate molecular weight could not be estimated [28,29]. The \(M_w/M_n\) values of PEG\(_{54}\)-PAA\(_{140}\), PEG\(_{54}\)-P(AA/VE35)\(_{140}\), and PEG\(_{54}\)-P(AA/VE6/\(\gamma\)TCP29)\(_{140}\) were relatively narrow, obtained as 1.36, 1.38, and 1.47, respectively. Table 1 summarizes the molecular weight and \(M_w/M_n\) of each polymer.
Figure 3. $^1$H NMR spectra of (a) PEG$_{54}$-PAA$_{140}$, (b) PEG$_{54}$-P(AA/VE$_{35}$)$_{140}$, (c) PEG$_{54}$-P(AA/VE$_{6}$/γTCP$_{29}$)$_{140}$, and (d) γTCP in DMSO-$d_6$.

Figure 4. Gel-permeation chromatography (GPC) elution curves of PEG$_{54}$-PAA$_{140}$ (—), PEG$_{54}$-P(AA/VE$_{35}$)$_{140}$ (– –), and PEG$_{54}$-P(AA/VE$_{6}$/γTCP$_{29}$)$_{140}$ (—-) at 40 °C using a phosphate buffer solution (PBS) at pH 9 containing 10% v/v acetonitrile as the eluent.
was estimated as 53.4 nm, assuming that the lengths of the vinyl monomer unit and the PEG chain
γ polymeric micelles. These polymer micelles were formed due to the hydrophobic interaction between

\[ \text{pH} 7.4 \text{ at } 25^\circ \text{C} \]

of the micelles, for instance, hard sphere as 0.775, monodisperse sphere as 1.0, and rodlike shape as

\[ \text{more than 2.0} \]

The number-average molecular weight (\(M_n\)) determined by SLS. Theoretically, \(R_h\) and LSI of the polymers were measured at

\[ 11, 36 \]

Table 1. Number-average molecular weight (\(M_n\)) and \(M_n\) distribution (\(M_w/M_n\)).

| Sample                  | Theoretical \(M_n \times 10^4\) (g/mol) | \(^1\)H NMR \(M_n \times 10^4\) (g/mol) | GPC \(M_n \times 10^4\) (g/mol) | \(M_w/M_n\) |
|-------------------------|----------------------------------------|---------------------------------------|---------------------------------|-------------|
| PEG54-PAA140            | 1.30                                   | 1.26                                  | 1.25                            | 1.36        |
| PEG54-P(AA/VE35)140     | 1.54                                   | 1.67                                  | 0.702                           | 1.38        |
| PEG54-P(AA/VE6/γTCP29)140 | 1.83                                 | 1.96                                  | 1.02                            | 1.47        |

3.2. PEG54-P(AA/VE6/γTCP29)140 Micelle Formation

The \(R_h\) and LSI of the polymers were measured at \(C_p = 0.10\) g/L in a PBS buffer of pH 7.4 at 25 °C to confirm aggregation-state changes for PEG54-PAA140, PEG54-P(AA/VE35)140, and PEG54-P(AA/VE6/γTCP29)140 (Figure 5). The \(R_h\) values of PEG54-PAA140 and PEG54-P(AA/VE35)140 were 4.4 and 5.8 nm, respectively. The LSI values of PEG54-PAA140 and PEG54-P(AA/VE35)140 were estimated as 40.4 and 83.0 Kcps, respectively. These polymers may dissolve in PBS to form unimers due to their small \(R_h\) and LSI values. On the other hand, the \(R_h\) and LSI of PEG54-P(AA/VE6/γTCP29)140 increased to 84.1 nm and 709 Kcps, suggesting the formation of polymer micelles. These polymer micelles were formed due to the hydrophobic interaction between pendant γTCP groups, which were composed of a hydrophobic P(AA/VE6/γTCP29)140 core and hydrophilic PEG54 shells. The end-to-end distance of the PEG54-P(AA/VE6/γTCP29)140 polymer chain was estimated as 53.4 nm, assuming that the lengths of the vinyl monomer unit and the PEG chain were 0.25 and 18.4 nm, respectively [30–32]. The \(R_h\) value of the polymer micelle was calculated as 84.1 nm, which was larger than the end-to-end distance of PEG54-P(AA/VE6/γTCP29)140 (=53.4 nm). Therefore, polymers could not form simple spherical core–shell structures. The polymers may form intermicellar aggregates or large compound micelles.

![Figure 5](image_url)

**Figure 5.** Hydrodynamic-radius \((R_h)\) distributions for (a) PEG54-PAA140, (b) PEG54-P(AA/VE35)140, and (c) PEG54-P(AA/VE6/γTCP29)140 in PBS at \(C_p = 0.10\) g/L.

To characterize the polymer micelles formed from PEG54-P(AA/VE6/γTCP29)140 in the PBS buffer, SLS measurements were performed (Figure 6). Using SLS, the apparent \(M_w\) and \(R_g\) were estimated from the Debye plot (Table 2). The measured \(dn/dC_p\) (=1.70 mL/g) of the polymer micelles were then used to estimate the apparent \(M_w\). The aggregation number \((N_{agg})\), which is the number of polymer chains to form one micelle, was estimated using the following equation:

\[ N_{agg} = \frac{M_w (\text{SLS})}{M_w/M_n \times M_n (\text{NMR})} \]  

\(N_{agg}\) was estimated as 248 using \(M_w\) (=7.15 × 10\(^6\) g/mol), \(M_w/M_n\) (=1.47), and \(M_n (\text{NMR})\) (=1.96 × 10\(^6\) g/mol) determined by SLS. Theoretically, \(R_g/R_h\) depends on the shape and polydispersity of the micelles, for instance, hard sphere as 0.775, monodisperse sphere as 1.0, and rodlike shape as...
more than 2.0 [33–35]. The $R_g/R_h$ of the polymer micelles formed from PEG$_{54}$-P(AA/VE$_{6}/\gamma$TCP$_{29}$)$_{140}$ was calculated as 1.32, which is close to 1.0. Therefore, the polymers formed spherical micelles.

Figure 6. Debye plot for PEG$_{54}$-P(AA/VE$_{6}/\gamma$TCP$_{29}$)$_{140}$ in PBS at fixed $C_p$ (=0.10 g/L). $K$, optical constant; $C_p$, polymer concentration; $R_0$, difference between Rayleigh ratio of solution and solvent; and $\theta$, scattering angle.

Table 2. Dynamic and static light-scattering data of PEG$_{54}$-P(AA/VE$_{6}/\gamma$TCP$_{29}$)$_{140}$ in PBS.

| Sample                  | $SLS M_w \times 10^6$ (g/mol) | $N_{agg}$ | $R_g$ (nm) | $R_h$ (nm) | $R_g/R_h$ | $dn/dC_p$ (mL/g) |
|-------------------------|-------------------------------|-----------|------------|------------|-----------|-----------------|
| PEG$_{54}$-P(AA/VE$_{6}/\gamma$TCP$_{29}$)$_{140}$ | 7.15                          | 248       | 111        | 84.1       | 1.32      | 1.70            |

3.3. Micelle Decomposition in Acidic Condition

To evaluate the time dependence on micelle size and density formed from PEG$_{54}$-P(AA/VE$_{6}/\gamma$TCP$_{29}$)$_{140}$ in acetate buffer of pH 5.2 (0.01 M), $R_h$ and LSI changes were monitored at 25 °C (Figure 7). At constant particle size, density depended on the LSI. As a reference experiment, the time dependence on $R_h$ and LSI of the polymer micelles in a PBS buffer of pH 7.4 (0.01 M) were also monitored. $R_h$ increased with increasing time at pH 5.2. $R_h$ values just after preparation and after 60 h at pH 5.2 were determined as 81.0 and 107.0 nm, respectively. LSI values of just after preparation and after 60 h at pH 5.2 were estimated as 693 and 321 Kcps, respectively. The LSI were observed to decrease with increasing time. At pH 5.2, the pendant acetal bonds decomposed to release the hydrophobic $\gamma$TCP from the polymer micelles. As a result, hydroxyl groups were generated at the pendant groups. However, not all pendant acetal bonds were decomposed at pH 5.2. However, an increase in $R_h$ and decrease of the LSI was due to increasing densities observed at pH 5.2, attributed to the hydration and then swelling of the core of the polymer micelles. Oho et al. [36] reported that $\gamma$TCP is relatively stable against acidic conditions. The released $\gamma$TCP from the polymer in a buffer at pH = 5.2 may not be decomposed.

At pH 7.4, the $R_h$ values of the micelles just after preparation and after 60 h were calculated as 81.5 and 83.8 nm, respectively. The LSI of the micelles slightly decreased with increasing time. The values of LSI just after preparation and after 60 h were obtained as 695 and 492 Kcps. These observations suggest that the pendant acetal groups slightly decomposed at pH 7.4. Compared to the time dependence of $R_h$ and LSI at pH 5.2 and 7.4, $\gamma$TCP was effectively released due to the decomposition of the pendant acetal bonds.

The micelles of PEG$_{54}$-P(AA/VE$_{6}/\gamma$TCP$_{29}$)$_{140}$ in PBS of pH 7.4 were observed with Transmission electron microscopy (TEM, Figure S1). The radius ($R_{TEM}$) estimated from the TEM image was 72.1 ± 12 nm. $R_{TEM}$ was calculated from a randomly selected 50 particles ($N = 50$). $R_{TEM}$ was smaller than the $R_h$ values determined by light-scattering measurements because the aggregates shrank during the drying process in preparation for TEM measurements. The micelles of PEG$_{54}$-P(AA/VE$_{6}/\gamma$TCP$_{29}$)$_{140}$ in the acetate buffer of pH 5.2 just after preparation and after 60 h were observed using TEM to confirm
were demonstrated to dissolve in water, forming polymer micelles composed of hydrophobic

The amphiphilic block copolymers of PEG54-P(AA/VE6/γTCP29)140 bearing hydrophobic γTCP via acetal bond linkage were prepared using RAFT radical polymerization. PEG54-P(AA/VE6/γTCP29)140 were demonstrated to dissolve in water, forming polymer micelles composed of hydrophobic P(AA/VE6/γTCP29)140 block cores and hydrophilic PEG45 shells. At pH 5.2, a gradual increase

4. Conclusions

The amphiphilic block copolymers of PEG54-P(AA/VE6/γTCP29)140 bearing hydrophobic γTCP via acetal bond linkage were prepared using RAFT radical polymerization. PEG54-P(AA/VE6/γTCP29)140 were demonstrated to dissolve in water, forming polymer micelles composed of hydrophobic P(AA/VE6/γTCP29)140 block cores and hydrophilic PEG45 shells. At pH 5.2, a gradual increase

Figure 7. Time dependence on (a) hydrodynamic radius ($R_h$) and (b) light-scattering intensity (LSI) for PEG54-P(AA/VE6/γTCP29)140 at $C_p = 0.10$ g/L at 25 °C in acetate buffer of pH 5.2 (○) and in PBS buffer of pH 7.4 (△).

Figure 8. Transmission electron microscopy (TEM) images of PEG54-P(AA/VE6/γTCP29)140 at $C_p = 0.10$ g/L in acetate buffer of pH 5.2 (a) just after preparation and after (b) 60 h.
Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4360/12/1/36/s1: Figure S1. (a) TEM images of PEG54–P(AA/VE6/γTCP29)140 at Cp = 0.10 g/L in PBS buffer of pH 7.4, in acetate buffer of pH 5.2 (b) just after preparation and (c) after 60 h.

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Conflicts of Interest: The authors declare no conflict of interest.

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