Facile Synthesis of Multiamino Vinyl Poly(amino acid)s for Promising Bioapplications

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We presented a general and facile strategy to prepare biocompatible multiamino polymers. Series of new monomers were synthesized by esterification of 2-hydroxyethyl methacrylate (HEMA) and Boc-amino acids, such as Boc-L-phenylalanine, Boc-glycine, Boc-L-alanine, Boc-L-valine, and Boc-L-lysine. Subsequent vinyl polymerization of monomers gave rise to vinyl poly(amino acid)s with a side primary amino group at each unit if deprotected. Both atom transfer radical polymerization (ATRP) and conventional free radical polymerization (FRP) were employed to prepare the multiamino polymers. A well-controlled effect upon molecular weight with the standard first-order kinetics was achieved in cases of ATRP, and high molecular weight polymers were obtained via FRP. MTT assay showed that cell survival rates for the multiamino polymers were almost maintained above 90% and that their cytotoxicities were much lower than that of linear PEI (PEI 25000). Zeta potential measurements demonstrated that the vinyl poly(amino acid)s are electropositive, and AFM measurements showed that the vinyl poly(amino acid)s could tightly condense DNA into granular structures at a suitable concentration. The combination of facile availability, controlled productivity, low cytotoxicity and strong binding ability with DNA promises the great potential of the novel multiamino polymers in bioapplications.

Introduction

Polyelectrolytes (PEs), a class of polymers with ionogenic groups, play crucial roles in nature and have found diversiform applications in industrial processes and daily life, including the fields of food additives,1 paper strengthening technique,2 drug delivery,3 and oil field,4 because of their unique attributes such as high osmotic pressure, good water solubility, and electric response effect. Based on the different nature of the ionogenic groups, PEs could be divided into three sorts: cationic polyelectrolyte (CPE), anionic polyelectrolyte (APE), and zwitterionic polyelectrolyte (ZPE). Specially, CPE with amino groups, PEs could be divided into three sorts: cationic polyelectrolyte (CPE), anionic polyelectrolyte (APE), and zwitterionic polyelectrolyte (ZPE). Specially, CPE with amino groups has been treated with durative attention during the last decades for its application in gene delivery and other fields.5,6 Several preparation methods for CPEs have been developed, for instance, (1) ring-opening polymerization for polyethyleneimine (PEI),7 (2) repeated Michael addition and condensation reactions for poly(amido amine) (PAMAM) dendrimers8 and one-pot polycondensation for hyperbranched PAMAMS,9 and (3) polycondensation of amino acids for polypeptides.10 In addition to the synthesis methods, the modification and application of CPEs have also been widely addressed. It is no doubt that the synthesis of CPE with controlled structures is the basis for the further advancement of CPE applications. However, there are insuperable drawbacks associated with the conventional methodologies such as uncontrollability over molecular weights, tedious procedures and harsh reaction conditions. Therefore, it is eagerly expected to develop an alternative controlled methodology for facile preparation of CPEs.

Recently, Mori et al.11 have employed reversible addition–Fragmentation chain transfer (RAFT) polymerization,12 a kind of controlled radical polymerization (CRP), to synthesize APE with carboxylic groups from protected amino acids. Armes and co-workers13 have reported that biocompatible ZPE could be accessed readily through another CRP technique, atom transfer radical polymerization (ATRP).14 On the other hand, because the discovery of polylysine (PLL) used to mediate the transfection of cells by Wu et al.15 in 1987, much efforts have been explored in the synthesis of amino acid based polymers by conventional free radical polymerization (FRP) of vinyl monomers carrying amino acid residues.16 As far as we know, however, no one had ever tried yet to synthesize CPE with primary amino groups through CRP that has been widely demonstrated as a powerful technique in the material design and production.17,18

To meet such a big challenge, we present a general and facile strategy to prepare a series of biocompatible CPEs with amino acid moieties in the side chains. As shown in Scheme 1, various vinyl monomers are first synthesized by the esterification of Boc-amino acids and 2-hydroxyethyl methacrylate (HEMA), subsequent ATRP of monomers affords desired polymers with controlled structures. Our synthesis strategy possesses at least three advantages over the reported ones: (1) the commercially available Boc-amino acids and HEMA are used as the raw materials, paving the way for the large-scale production of vinyl monomers; (2) the well-established CRP techniques can be simply applied to prepare CPEs, laying the foundation for the controlled synthesis of CPEs with primary amino groups; (3) versatile CPEs can be easily obtained by either random or block copolymerization of different vinyl monomers, providing an efficient approach to mimic biomacromolecules such as proteins constructed with different amino acids. Although the main chain of our new CPEs is not constructed with C=N amido bonds but with C–C bonds, there is amino acid moiety and a primary amino group on each unit which is the source of its electrochemical parameters and can be expediently modified further. So we can term these new CPEs as vinyl polypeptides or vinyl poly(amino acid)s (Note: poly(amino acid)s normally mean the
products having amino acid moiety in the main chain, which are generally prepared by ring-opening polymerization of N-carboxy-α-amino acid anhydrides (NCAs) or polycondensation of amino acids. The CPEs presented in this paper are not the conventional poly(amino acid)s but vinyl polymers with side amino acid moiety. Furthermore, we demonstrated that the vinyl poly(amino acid)s showed low cytotoxicity, electropositive, and strong binding effect with DNA, promising their great potential in bioapplications.

Experimental Section

Materials. Boc-L-phenylalanine (Boc-Phe-OH, 98.5%), Boc-glycine (Boc-Gly-OH, 98.5%), Boc-L-alanine (Boc-Ala-OH, 99.2%), Boc-L-valine (Boc-Val-OH, 99.6%), Boc-L-lysine (Boc-Lys-OH, 98%), N,N-dicyclohexylcarbodiimide (DCC, 98%) were purchased from J&K Chemical. Ethyl 2-bromoisobutyrate (EBIB, 98%) was purchased from Sigma-Aldrich. 1,1,4,7,7-Pentamethyldiethylenetriamine (PMDETA) and CuBr (98%, purified according to ref 19 before use) were purchased from Sinopharm Chemical Reagent Co. Ethyl 2-bromoisobutyrate (EBIB, 98%) was purchased from GL Biochem (Shanghai) Ltd. 4-Dimethylaminopyridine (DMAP, 99%) was purchased from Alfa Aesar. 2,2'-Bipyridyl (Bpy, 99%) were purchased from Sigma-Aldrich. 1,1,4,7,7-Pentamethyldiethylenetriamine (PMDETA, 99%) was the product of J&K Chemica. Ethyl 2-bromoisobutyrate (EBIB, 98%) and CuBr (98%, purified according to ref 19 before use) were purchased from Sigma-Aldrich. 1,1,4,7,7-Pentamethyldiethylenetriamine (PMDETA, 98%) and 2,2'-bipyridyl (Bpy, 99%) were purchased from Alfa Aesar. 2,2'-Azobis (2-methyl propionitrile) (AIBN) was employed after twice recrystallization. Ethyl acetate and dichloromethane were dried with MgSO4 overnight prior to use. Tris[2-(dimethylamino)ethyl]amine (Me6TREN) was synthesized in our laboratory, according to the previous procedures.30 Trifluoroacetic acid (TFA, 99%) was purchased from Sinopharm Chemical Reagent Co.

Instrument. Gel permeation chromatography (GPC) was recorded on a PL, PL-GPC220, using THF as the eluent at a flow rate of 1.0 mL/min and polystyrene as standards at 40 °C. 1H NMR and 13C NMR measurements were carried out on a Varian NMR/300 Hz spectrometer using CDCl3 or D2O as solvent. AFM images were taken in the tapping mode by carrying out on a NIKON EPI3800, with samples prepared by spin-coating sample solutions onto freshly cleaved mica substrates at 1000 rpm. ESI-mass spectrometry analysis was carried out on Bruker esquire 3000 plus liquid chromatography—mass spectrometry and Bruker Daltonics DataAnalysis 3.1. Thermal gravitational analysis (TGA) was carried out using a Perkin-Elmer Pyris 6 TGA instrument with a heating rate of 20 °C/min under a nitrogen flow (30 mL/min).

Zeta potential measurements were performed by laser Doppler electrophoresis using the Zeta-Nanosizer (ZEN3600, Malvern Instruments Ltd., Worcestershire, U.K.), which was routinely calibrated with a −50 mV Zeta-potential standard (Malvern Instruments). The attenuator was set at 9 and the F (Ka) value was set at 1.5. Measurements were carried out in a folded capillary electrophoresis cell (Malvern Instruments) at 25 °C. The average values of Zeta potential were calculated with the data obtained from three runs.

Synthesis of Monomer Boc-Amino-HEMA. Taking the Boc-Gly-OH as an example, DCC (23.6585 g, 112.32 mmol) was added to a 250 mL Schlenk flask containing 100 mL ethyl acetate and 13.80 mL (112.32 mmol) HEMA under nitrogen. The flask was immersed in an ice–water bath. When DCC was dissolved, Boc-Gly-OH (20.0002 g, 112.45 mmol) dissolved in 80 mL ethyl acetate was added dropwise over a period of 20 min under magnetic stirring. Then 1.4053 g (11.25 mmol) of DMAP dissolved in 10 mL of ethyl acetate was added into the mixture within 10 min. The reaction mixture was allowed to react in the ice–water bath for 0.5 h and then at room temperature for 24 h. After the removing of insoluble N,N-dicyclohexylurea (DCU) by suction filtration, it was weighed to determine whether the reaction was complete. The filtrate was concentrated and then further purified by silica gel column chromatography using hexane/ethyl acetate as mobile phase (2/3 vol/vol) to get a colorless liquid with a yield of 76%. 1H NMR (CDCl3, δ, ppm): 6.09 and 5.57 (C=CH2, 2H, s), 5.05 (NHCOO, 1H, s), 4.34 (OCH2CH2O, 4H, m), 3.91 (NHCOOCON, 2H, s), 1.91 (C=CH3, 3H, s), 1.44 (CH2CH3, 9H, s), IR: 3395 (N–H), 2982 and 2931 (C–H), 1757 and 1722 (C=O), 1636 (C=C), 1514 (N–H), 1452, 1369, 1295, 1250, 1158 (C–O–C), 1056, 950, 862, 814, 784, 653 cm−1.

Likewise, Boc-Phe-OH was prepared from Boc-Phe-OH and HEMA (yield 85%). 1H NMR (CDCl3, δ, ppm): 7.26–7.12 (C6H5, 5H, m), 6.11 and 5.57 (C=CH2, 2H, s), 5.02 (NHCOO, 1H, s), 4.58 (C6H5CH2CH2, 1H, s), 4.28 (OCH2CH2O, 4H, m), 3.06 (C6H5CH2, 2H, s), 1.92 (C=CH3, 3H, s), 1.38 (CH2CH3, 9H, s). ESI-MS: [M + Na+] = 400 m/z.

Boc-Val-HEMA was prepared from Boc-Val-OH and HEMA (yield 79%). 1H NMR (CDCl3, δ, ppm): 6.10 and 5.57 (C=CH2, 2H, s), 5.02 (NHCOO, 1H, s), 4.46–4.38 (CH2CH2CH2, 1H, m), 4.36–4.33 (OCH2CH2O, 4H, m), 4.30–4.20 (CH2CH2CH2, 1H, m), 1.92 (C=CH3, 3H, s), 1.42 (CH2CH3, 9H, s), 0.94 (CH2CH3CH3, 6H, m). ESI-MS: [M + Na+] = 352 m/z.

Boc-Ala-OH was prepared from Boc-Ala-OH and HEMA (yield 66%). 1H NMR (CDCl3, δ, ppm): 6.12 and 5.59 (C=CH2, 2H, s), 5.05 (NHCOO, 1H, s), 4.49–4.29 (OCH2CH2O and CH2CHCOO, 5H, m), 1.94 (C=CH3, 3H, s), 1.42 (CH2CH3, 9H, s), 1.26 (CH2CH2COO, 3H, m).

Synthesis of Poly(Boc-amino-HEMA)s by Atom Transfer Radical Polymerization (ATRP). Typically, a solution of Boc-Gly-HEMA (1.815 g, 2.58 mmol) with 1.8 mL of ethyl acetate was added to a 25
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Synthesis of Poly(Boc-amino-HEMA)s by Free Radical Polymerization (FRP). A representative example is as follows. A solution of Boc-Val-HEMA (2.4963 g, 7.6 mmol) with 6 mL ethyl acetate was added to a 25 mL Schlenk flask with a magnetic stir bar under continuous flow of N₂. Then AIBN (12.4 mg, 0.076 mmol) was added. The mixture was stirred at 60 °C for 2 h. The monomer structures were confirmed by 1H NMR, FTIR, and mass spectroscopy measurements. As shown in Figure 1A, for the monomer Boc-Gly-HEMA as an example, the protons of vinyl group were observed in the 1H NMR spectrum as two peaks at 6.5 and 7.2 ppm, respectively.

Cellular Cytotoxicity Evaluation. According to ref 23, the cytotoxicity of the polymers was measured by MTT assay. SPCA1 cell and cos-7 cell were seeded in 96-well tissue culture plates with a density of approximately 1 × 10⁴ cells per well and cultured to subconfluent monolayer for 24 h at 37 °C and 5% of CO₂ by weight. Then, the medium was changed into culture medium and incubated again for 20 h. Then, 10 µL of MTT solution (5 mg/mL in phosphate buffer solution, pH 7.4) was added to each well followed by incubation for 4 h at 37 °C. And 100 µL of solution (10% sodium dodecyl sulfonate, 1% mol/L hydrochloric acid, 5% isopropyl alcohol) was added. At last, the absorbance was recorded at 570 and 650 nm to assay for cell viability.

Results and Discussion

Synthesis of Vinyl Monomers. Scheme 1 depicts the synthetic route of vinyl monomers and vinyl poly(amino acid)s. The monomers of Boc-amino-HEMA are synthesized via the classic esterification condensation of Boc-amino acids and HEMA in the presence of DCC and DMAP. The immediate appearance of white precipitate of DCU indicated the condensation between hydroxyl and carboxyl groups, and the collected DCU quantity showed that this esterification was conducted quantitatively, and the conversion of Boc-amino acids was always higher than 95%. This efficient one-step process from commercial and inexpensive starting materials makes large-scale production of the vinyl monomers readily accessible. The monomer structures were confirmed by 1H NMR, FTIR, and mass spectroscopy measurements. As shown in Figure 1A, for the monomer Boc-Gly-HEMA as an example, the protons of vinyl group were observed in the 1H NMR spectrum as two peaks at 6.09 and 5.57 ppm, the acylamide proton of the original Boc-amino acid at 5.05 ppm remained intact during the esterification reaction. The characteristic peaks at 4.34
Monomers of Boc-Amino Acids via ATRP or FRP

Table 1. Polymerization Conditions and Results of Different Monomers of Boc-Amino Acids via ATRP or FRP

<table>
<thead>
<tr>
<th>Monomers</th>
<th>[M]/[I]₀</th>
<th>Temp (°C)</th>
<th>Time (min)</th>
<th>Yield (%)</th>
<th>Mₙ,GPC</th>
<th>Mₙ,TH</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boc-Phe-HEMA</td>
<td>100</td>
<td>23</td>
<td>25</td>
<td>58</td>
<td>22100</td>
<td>21700</td>
<td>1.40</td>
</tr>
<tr>
<td>Boc-Gly-HEMA</td>
<td>175</td>
<td>23</td>
<td>9</td>
<td>74</td>
<td>30800</td>
<td>37300</td>
<td>1.77</td>
</tr>
<tr>
<td>Boc-Lys-HEMA</td>
<td>175</td>
<td>23</td>
<td>5</td>
<td>10</td>
<td>12400</td>
<td>6230</td>
<td>1.64</td>
</tr>
<tr>
<td>Boc-Ala-HEMA</td>
<td>100</td>
<td>23</td>
<td>226</td>
<td>55</td>
<td>13800</td>
<td>16600</td>
<td>1.71</td>
</tr>
<tr>
<td>Boc-Val-HEMA</td>
<td>100</td>
<td>23</td>
<td>110</td>
<td>89</td>
<td>29200</td>
<td>29200</td>
<td>1.41</td>
</tr>
<tr>
<td>Boc-Ala-HEMA*</td>
<td>100</td>
<td>23</td>
<td>1740</td>
<td>80</td>
<td>28800</td>
<td></td>
<td>2.10</td>
</tr>
<tr>
<td>Boc-Val-HEMA*</td>
<td>100</td>
<td>60</td>
<td>540</td>
<td>19</td>
<td>38900</td>
<td></td>
<td>2.08</td>
</tr>
</tbody>
</table>

*The polymers are prepared by FRP, initiated with AIBN at 60 °C.

Yield of the resultant polymer. Mₙ is calculated by Mₙ,th = [M]/[I]₀ × M₀ × Y (M₀ is the molecular weight of monomer, Y is the yield of polymer) for the polymers made by ATRP.

(methylene protons in HEMA), 3.91 (methylene protons in Boc-Gly-OH), 1.91 (methyl protons), 1.44 (methyl protons of the Boc group) are clearly seen. The exact single condensation between HEMA and Boc-amino acids was further demonstrated by mass spectroscopy. For instance, the structures of Boc-Phe-HEMA and Boc-Val-HEMA were confirmed by ESI-MS with molecular masses [M + Na⁺] = 400 m/z and [M + Na⁺] = 352 m/z, respectively. The mass spectrum exhibits a series of peaks, which are exactly equal to the mass of the residual group of the monomer (the detailed mass spectrum analysis is shown in Supporting Information, Figures S1 and S2). In addition, FTIR spectra also gave clear evidence for the formation of targeted vinyl monomers. For the case of Boc-Gly-HEMA, after esterification of Boc-Gly-OH with HEMA, the characteristic absorption peaks of amide groups of Boc-Gly-OH can still be observed at 3395 and 1514 cm⁻¹, respectively (Figure S3A). The band located at 1158 cm⁻¹ is attributed to the stretching vibrations of C=O. In the region 1650–1800 cm⁻¹, two strong and sharp peaks at 1757 and 1722 cm⁻¹ are assigned to the stretching vibrations of carbonyl (C=O) groups, demonstrating the presence of C=O (ester) in different chemical environments. The peaks at 2982 and 2931 cm⁻¹ are associated with the symmetric and antisymmetric C–H stretching vibrations of CH₂ and CH₃ groups, and the C=CH stretching vibrations are located at 1636 cm⁻¹. All these characteristics proved the successful synthesis of Boc-amino-HEMA.

Controlled Polymerization of Vinyl Amino Acids by ATRP. We have obtained the pure vinyl monomers through an efficient one-step process. ATRP, as one of the most important methods to achieve controlled/living polymerization, was subsequently employed to yield a series of multiamino polymers with controlled molecular weight and low polydispersity, such as poly(Boc-Phe-HEMA), poly(Boc-Gly-HEMA), poly(Boc-Ala-HEMA), poly(Boc-Val-HEMA), and poly(Boc-Lys-HEMA).

Generally, the catalyst/ligand system has obvious influence on the controllability of ATRP. It was reported that CuBr/PMDETA can be used for the ATRP of methacrylates such as methyl methacrylate (MMA) with good control over both molecular weight and polydispersity (polydispersity index, PDI, 1.15–1.35) under mild condition (e.g., 30 °C). Herein, in order to optimize reaction conditions, we tried a variety of catalysts, such as Me₆TREN, Bpy, and PMDETA, and found that PMDETA performed better than Me₆TREN and Bpy in the polymerizations (data not shown). So we selected PMDETA as the ligand to investigate the details of ATRP in this work. The typical ATRP conditions and the results were summarized in Table 1. It is found that the number-average molecular weight determined by GPC (Mₙ,GPC) for the resulting polymers prepared by ATRP is closed to the corresponding theoretical value (Mₙ,th), and PDI is relatively narrow (1.4–1.77) compared with the previous report, indicating that the functional vinyl monomers of amino acids are active in the ATRP reactions.

The structures of as-prepared polymers were further confirmed by ¹H NMR, ¹³C NMR, and TGA measurements. In the ¹H NMR spectrum of poly(Boc-Gly-HEMA) in Figure 1B, the vinyl signals of the methacrylate monomer completely disappeared, while the new obtuse peaks attributed to the methylene group (a) and methyl (b) are clearly observed at δ 0.86–1.24 and 1.83 ppm, respectively. The isotactic (iso), heterotactic (het), and syndiotactic (syn) α-methyl signals (b) in poly(Boc-Gly-HEMA) were observed in Figure 1B. The peaks at 4.36 and 4.17 ppm are assigned to methylene protons (OCH₂CH₂O). The peak of amide proton at δ 5.05 ppm is shifted to 5.54 ppm after polymerization. For other polymers, the ¹H NMR spectra also confirmed their structures with similar peak, as shown in Figure S4. Besides, IR spectra further confirm our successful synthesis of poly(Boc-Gly-HEMA). In the FT-IR spectrum of poly(Boc-Gly-HEMA) (Figure S3B), the peak at 1636 cm⁻¹ for C=O disappeared.

The thermal stability of poly(Boc-Gly-HEMA) was investigated by TGA with a heating rate of 20 °C/min under a nitrogen flow. As can be seen from Figure 2A, poly(Boc-Gly-HEMA) is thermally decomposed in three steps: the weight loss of 35.9% from 200 to 275 °C is likely attributed to the degradation of Boc groups (I); the weight loss of 57.7% from 275 to 385 °C is ascribed to ester degradation (II); the straightforward weight loss beginning from 385 °C is due to the degradation of polymer backbone (III).

To investigate the controllability of the ATRP process in details, we selected a representative monomer, Boc-Phe-HEMA, to do a series of experiments in which the molar feed ratio of monomer to initiator was varied from 100:1 to 250:1, while the ratio of monomer/CuBr/PMDETA was kept at 80:1:1. In the polymerization process, 0.1 mL of the reaction mixture was removed with a degassed syringe at different reaction times and analyzed using ¹H NMR spectroscopy and GPC to monitor monomer conversion and molecular weight variations. It is interesting to note that the GPC analysis against linear polystyrene standards of all samples resulted in typical symmetric Gaussian distributions (Figure 3). Indeed, it clearly shows that the polymer molecular weight increases with the reaction time, accompanying with narrow polydispersities (PDI < 1.35). The influence of the initiator amount on the polymerization rate is shown in Figure 4a. Generally, polymerization rate decreases with increasing the ratio of [M]/[I]₀, as expected. The two kinetics ln([M]/[M]) time plots are linear passing through zero point, which indicates that (1) the polymerizations are approximately first order and (2) the number of active species
remains constant during the polymerization process. The $M_{n,\text{GPCs}}$ increase linearly with the monomer conversion (Figure 4b) and are close to corresponding $M_{n,\text{obs}}$, indicating that EBIB is an efficient initiator for the polymerization of the Boc-amino-HEMA at 23 °C.

To assess the tolerance of ATRP of the vinyl amino-acids toward temperature, a series of polymerizations of Boc-Phe-HEMA were performed at different temperatures such as 0, 10, 23, 60 °C, and so on. The polymerization carried out at 60 °C was too fast to be controlled (PDI = 2.3), so we run polymerizations at relatively low temperature to prepare the CPEs. Detailed kinetic study of the polymerizations also shows a linear increase of monomer conversion $\ln([M]_0/[M])$ versus reaction time at different temperatures (Figure 5a). The results indicated that lower polymerization rate was associated with lower temperature, as expected. In addition, what encouraged is that the $M_{n,\text{GPC}}$ conversion plots for all of the reactions are in a line orderly (see Figure 5b), suggesting a fast initiation with negligible terminations, and thus revealing a living/controlled polymerization process at a variety of temperatures.

**Synthesis of Vinyl Poly(amino acid)s with High Molecular Weight by FRP.** We have observed controlled/“living” polymerization in the ATRP protocol mentioned above, obtaining polymers with controlled molecular weights and low polydispersities. This lays the foundation for molecular-weight-dependent structure–property investigation and application exploration. On the other hand, sometimes we need CPE with high molecular weights and broad polydispersities. Hence, we also tried the FRP of the new monomers initiated by AIBN. The molecular weight approached 388000 with PDI 2.08, demonstrating that high molar mass poly(amino acid)s can be achieved by the convenient RFP technique. Interestingly, we found that the polymerization rate for the FRP was much lower than that for the ATRP. The monomer conversion could approximately reach up to 89% at 110 min of reaction in ATRP at 23 °C, while only 19% of conversion was observed after 9 h reaction for FRP at an elevated temperature 60 °C. These results declared that the new monomers of vinyl amino-acids are highly active for both conventional and controlled free radical polymerizations, providing more choices for the practical applications with different purposes.

**Deprotection of Poly(Boc-amino-HEMA)s.** In this paper, the synthesis of CPE involves classical protection/deprotection chemistry, using Boc group, as shown in Scheme 1. Poly(Boc-amino-HEMA)s could be feasibly deprotected by the trifluoroacetic acid, resulting water-soluble CPE poly(HEMA-amino)s. Figure 1C shows the $^1$H NMR spectrum of poly(HEMA-Gly) in D$_2$O. After deprotection, the signal at 1.40 ppm, methyl protons of the Boc group, disappeared without a trace, indicating the Boc groups can be completely deprotected to afford primary-amino polymers. Correspondingly, the proton peaks at 1.83 (CH$_2$-CCH$_3$) and 1.24–0.86 (C–CH$_3$) for poly(Boc-Gly-HEMA) shift to 1.75 and 0.83–0.73 ppm, respectively. In the
FT-IR spectrum of poly(HEMA-Gly) (Figure S3(C)), the absorption peak at 1514 cm\(^{-1}\) attributed to N–H disappears, while the broad peak at 1595 cm\(^{-1}\) appears due to the conversion of Boc group into NH\(_2\) in the deprotection reaction.

In addition, the thermal stability of poly(HEMA-Gly) was also investigated by TGA. According to the TGA curves depicted in Figure 2B, we find that all of these polymers remain stable at 150 °C, and that the temperature of losing 50% original weight for poly(HEMA-Gly) is slightly higher than that of poly(Boc-Gly-HEMA) due to the detaching of Boc groups.

Cellular Cytotoxicity Evaluation of Vinyl Poly(amino acid)s.

CPE is employed for various of applications, including gene vector as one of the most important domains. It is well-known that the most essential indicator for a good gene vector is low toxicity or even nontoxicity. So far, many different CPEs have been developed for gene delivery applications. In this regard, the most intensively addressed one would be linear or branched PEI. It is the standard polymer-based gene delivery systems against which new delivery systems should generally be compared. The high transfection efficiency of PEI as well as its high toxicity have much to do regarding the molecular weight and geometry, thus, it requires careful modification when used.\(^{31-33}\) The PAMAM dendrimers are another kind of candidate molecules for gene delivery. Despite their perfect structure and generation-controlled molecular weight, PAMAM dendrimers seem difficult to be widely used because of their laborsome synthesis and considerable toxicity.\(^{34-36}\) Recently, Gao et al. reported a facile one-pot strategy to scalably synthesize hyperbranched PAMAMs which showed high gene-delivery efficiency but very low toxicity.\(^{37,38}\) Shedding a light for using dendritic/highly branched polymers in biomedicine. However, it is still highly expected to develop new linear cationic polymers with good performance for future gene therapy and relevant applications.

The above synthesis protocol paves the way for obtaining CPE with primary amino groups from commercial materials. Herein, we attempted to investigate the cytotoxicity of these new vinyl poly(amino acid)s in cells. As an example, the cytotoxicity of poly(HEMA-Val), synthesized by both ATRP ($M_n = 29200$, PDI = 1.43) and FRP ($M_n = 388000$, PDI = 2.08) was evaluated by using MTT assay against COS-7 and SPCA-1 cells. As shown in Figure 6, low molecular weight poly(HEMA-Val) showed very little cytotoxicity, with about 90% cell viability observed even at a concentration up to 250 µg/mL, which is greatly high for human body in the clinical practice. By comparison, only a very slight downside is observed in the case of high molecular weight poly(HEMA-Val), also with 80% or more cell viability in the experimental concentration range. The results suggest that poly(HEMA-Val) is highly biocompatible, promising its potential bioapplications. What’s more, we could select the polymer with appropriate molecular weight according to the transfection efficiency and other targets in practice, neglecting the molecular weight effect on the toxicity. In sharp contrast, the cytotoxicity of linear PEI (PEI 25000) is generally much higher than that of poly(HEMA-Val) in the same concentration range, only 45% of the cells survived, especially in the case of cells SPCA-1. It has been demonstrated that the cytotoxicity induced by CPEs depends not only on the concentration or exposure time but also on the density of cationic residues interacting with the cell.\(^{39}\) Generally, polymers with degradable bonds and biomolecular moiety such as amino acid showed better biocompatibility with cells or lower cytotoxicity.\(^{40,41}\) Our results based on vinyl poly(amino acid)s also confirmed such an understanding. Nevertheless, more detailed tests and evaluations shall be done to check the possible influence of residual catalyst, polymer end groups, molecular weight, polydispersity, and other parameters on the cellular cytotoxicity.

DNA Binding of Vinyl Poly(amino acid)s. Although cellular cytotoxicity evaluation has shown the as-prepared polymers with low cytotoxicity, the efficiency of binding DNA is another key issue to be investigated for a promising gene vector. Moreover, the transfection efficiency of gene vector depends on the particle size and zeta potential.\(^{42}\) Therefore, we conducted Zeta potential measurements to assess the overall charge of the DNA/
Furthermore, as a sign, indicating that the new CPE may be used as a promising chain, which could tie DNA more closely. This is a very good diameters in the AFM image (Figure 8B). The granular complexes have achieved, DNA was bound into the granular structure with a nm, as shown in Figure 8A. Once charge compensation was DNA takes on a planar net-like structure with height scale efficency.

Besides the surface charge of DNA/polymer complexes, particle between poly(HEMA-Val) with DNA approaches saturation. (6 43,44 So we could synthesize the vinyl poly(amino acid)s with appropriate fraction of side chains to promote a high uptake through cell endocytosis, which is the first barrier of gene expression. The optimization and in-depth work is currently in progress and will be reported later.

poly(HEMA-Val) complexes (Figure 7) and employed AFM to image DNA and DNA/poly(HEMA-Val) complexes preliminarily (Figure 8). DNA/polymer complexes was formed by mixing different concentrations of poly(HEMA-Val) aqueous solution with 0.03 mg/mL DNA aqueous solution by equal volume. The polyplexes were equilibrated at RT for 30 min prior to analysis of Zeta potential and AFM image. As shown in Figure 7, when the mass ratio of poly(HEMA-Val) to DNA \( R_{\text{w/w}} \) is less than 0.2, the complexes remain electronegative, indicating that complexes at this stage are not fully formed, and when \( R_{\text{w/w}} \) is greater than 0.4, Zeta potentials are positive. Furthermore, as \( R_{\text{w/w}} \) increases gradually to a certain value (6–25), Zeta potential tends to a constant, and the complexing between poly(HEMA-Val) with DNA approaches saturation. Besides the surface charge of DNA/polymer complexes, particle size is another important parameter determining the transfection efficacy.

At moderate concentrations (0.015 mg/mL), the naked cycle DNA takes on a planar net-like structure with height scale ~1.0 nm, as shown in Figure 8A. Once charge compensation was achieved, DNA was bound into the granular structure with a uniform height scale ~2.2 nm, which could be visually observed in the AFM image (Figure 8B). The granular complexes have diameters ~150 nm due to the primary amine closer to the main chain, which could tie DNA more closely. This is a very good sign, indicating that the new CPE may be used as a promising gene vector for clinical application.

Conclusions

We have demonstrated that a series of new monomers of vinyl amino acids based on HEMA and Boc-amino acids could be prepared by the esterification reaction in the presence of DCC and DMAP. The facile synthesis process as well as the commercially available materials ensured the large-scale availability of vinyl monomers. The polymerization of these monomers using ATRP proceeded in a controlled manner and was consistent with the standard first-order kinetics. The average molecular weigh of final polymers was well consistent with the theoretical value. The polymerization using FRP yielded polymers with broad polydispersity and high molecular weight, which provided a choice for the application requiring CPE with high molecular weight. Excitingly, we also found that the cytotoxicity of deprotected multiamino polymers was low, and far below that of linear PEI (PEI 25000) in MTT assays. Zeta potential measurements demonstrated the electropositive of the CPEs, and AFM images directly proved that the new CPEs could condense DNA into granular structure at a suitable concentration, indicating that this type of polymers may be used as a promising gene vectors for clinical application.

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Supporting Information Available. ESI-MS spectra of monomers, FT-IR spectra of monomer and polymer, the photographs of product, and additional \(^1\)H NMR spectra of monomers and polymers. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes


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