Synthesis of electroactive tetraaniline grafted polyethylenimine for tissue engineering

Shilei Dong¹, Lu Han¹³, Muhang Cai¹, Luhai Li¹ and YanWei¹²

¹BeijingEngineering Research Center of Printed Electronics, Beijing Institute of Graphic communication, Beijing 102600, China
²Department of Chemistry and Key Laboratory of Bioorganic Phosphorus Chemistry and Chemical Biology (Ministry of Education), Tsinghua University, Beijing 100084, China

E-mail: hanlu@bigc.edu.cn

Abstract. Tetraaniline grafted polyethylenimine (AT–PEI) was successfully synthesized in this study. Proton Nuclear Magnetic Resonance (1H NMR) Spectroscopy was used to determine the structure of carboxyl-capped aniline tetramer (AT-COOH) and AT–PEI. UV-Vis spectroscopy and Fourier transform infrared (FT-IR) spectroscopy were employed to characterize the absorption spectrum of the obtained AT–PEI samples. The morphology of AT–PEI copolymers in aqueous solution was determined by Scanning electron microscope (SEM). Moreover, AT–PEI copolymers demonstrated excellent solubility in aqueous solution and possessed electroactivity by cyclic voltammogram (CV) curves, which showed its potential application in the field of tissue engineering.

1. Introduction
Polyaniline is widely used in many fields such as metal anti-corrosion [1], gas sensor [2], and artificial muscle [3], owing to its good conductivity and reversible reoxidation. However, due to its rigid structure, polyaniline is generally insoluble in aqueous solution, which limits its practical application [4, 5]. As compared to polyaniline, aniline tetramer has well-defined structure, homogeneous molecular weight and various end groups for easy functionalization [6-8]. What is more important, it reserves almost the same electrical conductivity as polyaniline [9]. Recently, lots of literatures reported that the solubility, biocompatibility and biodegradability of aniline tetraaniline were dramatically improved by grafting with biocompatible molecule [10]. Wei and his colleagues [11] prepared electroactive and biocompatible triblock copolymer, i.e. (poly (L-glutamicacid)-graft-tetraaniline/poly (L-lysine)-graft-tetraaniline)n, (PGA-g-TA/PLL-g-TA)n, which could be used for bone tissue scaffold. Besides, they synthesized electroactive copolymer, i.e. PEG-aniline tetramer, which self-assembled into micelles in aqueous phase [12]. In addition, Ma [13] et al. fabricated nanofibrous electroactive scaffolds by grafting aniline tetramer to chitosan and using electricity spinning.

Polyethylenimine (PEI) is a cationic polymer containing amine groups, which can deliver genes into cells via endocytosis [14, 15]. Herein, aniline tetramer (AT) was conjugated with PEI (Mw=600),
which not only improve the solubility of AT, but also endow the synthesized copolymer, AT-PEI, with electroconductibility and gene transfection ability. Thus AT-PEI could be blended with hydrogel to form 3D scaffolds for facilitating gene delivery efficiency inside stem cells. In addition, Wei et al. [16] founded that, the poly (esteramide) s-graft-tetraaniline copolymers (PEA-g-TA) could promote adhesion and proliferation of mouse preosteoblastic MC3T3-E1 cells exposed to electrical stimulation. Therefore, AT-PEI may have the great potential application in stem cell gene therapy for bone and cartilage tissue defect.

2. Experimental section

2.1. Materials

N-phenyl-1, 4-phenylenediamine (aniline dimmer, AD, GR), succinic anhydride (GR) were Sigma-Aldrich (Steinheim, Germany). PEI (Mw=600, AR), N-(3-Dimethylaminopropyl)-N-ethylcarboimidide hydrochloride (EDC·HCl, AR), N-hydroxysuccinimide (NHS, AR) were obtained from Aladdin Reagent Company (China). Dimethylformamide (DMF, AR), Dimethyl Sulphoxide (DMSO, AR) and Ammonium persulfate (AR) were received from Shanghai Reagent Corporation (China).

2.2. Synthesis of AT-COOH and AT-PEI copolymers

AT-COOH was prepared according to the reported method [17] with minor modification. Briefly, AD (1.84 g, 0.01 mol) was allowed to react with succinic anhydride (1.05 g, 10.5 mM) in DCM to obtain AD-COOH (figure 1). 0.01 mol AD and 0.01mol AD-COOH were dissolved in 130 ml of mixture solution containing 100 ml DMF, 15 ml HCl and 15ml deionized water. Ammonium persulfate (4.56 g, 0.2 mol) was dissolved in 50 ml of 1M HCl and added dropwise into the above AD suspension with stirring. After 4 h reaction, the obtained suspension was filtered under vacuum. The fully reduced leucoemeraldine (LM) of AT was prepared by reduction with N\textsubscript{2}H\textsubscript{4}·H\textsubscript{2}O and then doping with NH\textsubscript{3}·H\textsubscript{2}O for overnight. The LMAT product was filtered under vacuum and washed thoroughly with deionized water and finally dried in vacuum at 45°C for 48 h.

The 0.928 g of AT (2 mM), 1.151 g of NHS (10 mM) and 1.917 g of EDC·HCl (10 mM) were dissolved in 20 ml DMF, which was distilled over CaH\textsubscript{2} before use. The mixture was stirred at room temperature for 24 h. PEI 600 (2.56 g, (C\textsubscript{2}H\textsubscript{5}N, 60 mM) was dissolved in 20 ml of DMSO: DMF (1:1). Then PEI solution was added dropwise into the above mixture with stirring for 24 h at RT. After that, the final product was washed using deionized water and subsequently anhydrous ether by centrifugation. The collected product was dried in vacuum at 45°C.

2.3. Characterization

\textsuperscript{1}H NMR spectra of AT and AT-PEI were obtained on a Bruker Avance 300 MHz NMR instrument using DMSO-d\textsubscript{6} as solvent. FT-IR spectra of AT and AT-PEI were recorded on a Nicolet 6700 FT-IR spectrometer (Thermo Scientific Instrument). The absorption spectra of AT, AT-COOH and AT-PEI were signified by UV-Vis spectrophotometer (UV-2501PC, Shimadzu). The morphology of AT-PEI copolymer was investigated via Scanning Electron Microscopy (SEM, Quanta 400, FEI) and the sample was prepared by depositing a drop of solution on a silicon slice. The particle size distributions were obtained by Dynamic light scattering (DLS, Malvern Zetasizer Nano ZS). Cyclic voltammetry (CV) of AT and AT-PEI were conducted on an Electrochemical Workstation (PGZ 402, VoltaLab, France) employing a three electrode system (DropSens, Spain) with a carbon disk as working electrode, and carbon and silver electrode as the auxiliary and reference electrodes, respectively.

3. Result and discussion

3.1. Characterization of AT-COOH and AT-PEI
In this work, AT-PEI was synthesized by amidization reaction between amine groups on PEI and carboxyl group of AT-COOH. The preparation procedure of AT-PEI was depicted in figure 1. In brief, succinic anhydride was reacted with N-phenyl-1, 4-phenylenediamine to obtain carboxyl-capped aniline dimmer (AD-COOH), which was then conjugated with AD to obtain AT-COOH by oxidative coupling. The AT-COOH was fully reduced by doping with NH₃·H₂O. Subsequently, the carboxyl groups of AT-COOH were activated by NHS before grafting with PEI by amidization reaction.

\[ \text{(NH}_3\text{)}_2\text{SO}_4 \text{DMF/HCl} \rightarrow \text{NH}_3\text{H}_2\text{O} \text{DMSO:DMF=1:1} \rightarrow \text{PEI} \]

Figure 1. Synthetic route of AT-PEI.

\(^{1}\text{H NMR and FT-IR spectra were used to characterize AT-PEI copolymer, the results of which was plotted in figures 2 and 3, respectively. As shown in figure 2, the proton signals between 7.94 and 6.63 ppm (multiplet) were ascribed to hydrogen of benzene ring. Proton signals at 3.9, 2.6 and 1.6 ppm were assigned to amino and alkyl groups of PEI, illustrating that AT was successfully grafted on PEI molecule. Furthermore, the signals of –NH– groups of AT segment between 7.77 and 7.64 ppm are still present, indicating that the synthesized copolymer remained structural integrity of AT. As displayed in figure 3, the peaks at 1572 cm\(^{-1}\) and 1501 cm\(^{-1}\) were ascribed to the vibrations of quinoid and benzene rings, respectively. The stretching vibration of amide group (\(-\text{CO-NH}\)–) appeared at 1649 cm\(^{-1}\). We also see that, the peak at 1720cm\(^{-1}\) corresponding to –COOH group was absent in the FT-IR curve of AT, demonstrated that AT was chemically grafted with PEI main chains by amidization reaction between carboxyl groups of AT and amine groups of PEI.}

3.2. Self-assembling behavior of PEI-AT in water

According to self-assembling theory of the polyelectrolyte complexes [18], a hydrophilic cationic polymer, i.e. PEI in our study, fully stretched in aqueous solution due to the strong electrostatic repulsion. After conjugation with hydrophobic AT, the AT-PEI copolymer self-assembled into micelles with AT segments as nuclear and PEI molecular bone as a shell in aqueous phase. As shown in figure 4(A), nanoscale particles with the mean size of around 200 nm were observed by SEM, suggesting polyelectrolyte PEI-graft-AT formed nanoparticles owing to hydrophobic/hydrophilic interaction. However, the inhomogeneous size of particles may attribute to the broad range of molecular weight of PEI. The method of dynamic light scattering was further used to measure the hydrous size of assembled particles in water, as shown in figure 4(B). The mean diameter of 483 nm was detected with PDI of 0.463, demonstrating excellent dispersity. The discrepancy between the DLS...
and SEM results was ascribed to that, high molecular weight, PEI expanded in the aqueous phase and shrunk after being dried.

![Figure 2. 1H NMR spectrum of AT-COOH and AT-PEI.](image)

![Figure 3. FTIR spectra of AT-COOH and AT-PEI.](image)
Figure 4. SEM image of spherical aggregates of AT-PEI (A) and Size distribution histograms of AT-PEI aggregate in water (B).

3.3. Electrochemical properties of AT-PEI

The electrochemical properties of synthesized products were characterized by UV-Vis spectrophotometer and electrochemical workstation. The UV-Vis spectra of AD-COOH, AT-COOH, and AT-PEI in DMF are shown in figure 5(A). The spectrum of AD-COOH exhibits an absorption peak at 298 nm, which was assigned to the absorption of benzene ring. The reduced state of AT-COOH and AT-PEI was observed only at 327 nm, which was ascribed to the π-π*transition of aromatic ring [19]. Figure 5(B) displayed the UV-Vis spectra of AT-PEI during in-situ oxidation by (NH4)2S2O8. We can see that two absorption bands of AT-PEI were observed. Band was attributed to the absorption of conjugated phenyl ring. Band b was assigned to the benzenoid-to-quinoid excitonic transition with no blue-shift. Moreover, AT-PEI can only be tuned between fully reduced leucoemeraldine (LM) and emeraldine (EM) state under common oxidation conditions [20], as shown in figure 6. In figure 7, we observed only one oxidation peak at 0.559 V, which could be attributed to the transition of AT-PEI from LM to EM state of tetraaniline (AT) segment. This result demonstrated that, AT-PEI synthesized in our study was endowed with electrical conductivity form AT and excellent solubility because of PEI conjugation.

Figure 5. UV-Vis spectra of AD-COOH, AT-COOH and PEI-AT (A); AT-PEI(B) oxidized with ammonium persulfate dissolve in DMF.
4. Conclusion
A novel electroactive AT-PEI copolymer was successfully synthesized by the amidization reaction between the carboxyl group of AT-COOH and amine group of PEI. The structure of AT-PEI was confirmed by $^1$H NMR and FI-IR. UV-Vis and cyclic voltammetry was also employed to verify its electrochemical properties. The morphology of AT-PEI copolymer by SEM clearly revealed that, the copolymer could self-assemble into nanoparticles. AT-PEI showed one oxidation peak in 1.0 M sulfuric acid solution, suggesting AT-PEI still remained electroactivity in aqueous solution. All these results indicated that the electroactive copolymer, AT-PEI, might have potential applications in stem cell gene therapy for bone and cartilage tissue defect.

Acknowledgments
This work was financially supported in part by grants from the Natural Science Foundation of China (No. 31300820), Beijing Municipal Commission of Education Project (No. KM20140015001), a project founded by Beijing Institute of Graphic Communication (No. 27170115004/025), a grant from Beijing Municipal Commission of Education (No. 08150115/153) and Beijing Institute of Graphic Communication (No. 25000115006/009).

References
[1] Lu W, Elsenbaumer R L and Westling B 1995 Corrosion protection of mild steel by coatings containing polyaniline Synthetic Metals 71 2163-6
[2] Virji Huang, Kaner J, et al. 2004 Polyaniline nanofiber gas sensors: Examination of response mechanisms Nano Letters 4 491-6
[3] Kaneto K, Kaneko M, Min Y, et al. 1995 Artificial muscle: Electromechanical actuators using polyaniline films Synthetic Metals 71 2211-2
[4] Li S, Dong H and Cao Y 1989 Synthesis and characterization of soluble polyaniline Synthetic Metals 71 329-36
[5] Huang J, Virji S, Weiller B H, et al. 2003 Polyaniline nanofibers: Facile synthesis and chemical sensors Journal of the American Chemical Society 125 314-5
[6] Wei Z and Faul C F J 2008 Aniline oligomers–architecture, function and new opportunities for nanostructured materials Macromolecular Rapid Communications 29 280-92
[7] Stejskal J and Trchová M 2012 Aniline oligomers versus polyaniline Polymer International 61 240-51
[8] Mullen K and Wegner G 1998 Electronic Materials: the Oligomer Approach (New Jersey: Wiley)
[9] Lu F, Wudl F, Nowak M, et al. 1986 Phenyl-capped octaaniline (COA): An excellent model for polyaniline J. Am. Chem. Soc. 108 8311-3
[10] Guo B, Lidija G and Ann-Christine A 2013 Biodegradable and electrically conducting polymers for biomedical applications Progress in Polymer Science 38 1263-86
[11] Cui H, Liu Y, Cheng Y, et al. 2014 In vitro study of electroactive tetraaniline-containing thermosensitive hydrogels for cardiac tissue engineering Biomacromolecules 15 1115-23
[12] Yang Z, Wang X, Yang Y, et al. 2010 Synthesis of electroactive tetraaniline-PEO-tetraaniline triblock copolymer and its self-assembled vesicle with acidity response Langmuir 26 9386-92
[13] Ma X, Ge J, Li Y, et al. 2014 Nanofibrous electroactive scaffolds from a chitosan-grafted-aniline tetramer by electrospinning for tissue engineering Rsc Advances 26 13652-61
[14] Pollard H, Remy J S, Loussouarn G, et al. 1998 Polyethylenimine but not cationic lipids promotes transgene delivery to the nucleus in mammalian cells Journal of Biological Chemistry 273 7507-11
[15] Boussif O, Lezoualc'h F, Zanta M A, et al. 1995 A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine Proceedings of the National Academy of Sciences 92 7297-301
[16] Cui H, Liu Y, Deng M, et al. 2012 Synthesis of biodegradable and electroactive tetraaniline grafted poly (ester amide) copolymers for bone tissue engineering Biomacromolecules 13 2881-9
[17] Chen L, Yu Y, Mao H, et al. 2005 Synthesis of parent aniline tetramer and pentamer and redox properties Materials Letters 59 2446-50
[18] Jiang M, Eisenberg A, Liu G, et al. 2006 Macromolecular Self-Assembly (Beijing: Science Press)
[19] Chen L. 2005 Synthesis of Monodisperse Oligoanilines and Their Properties (Changchun: Jilin University)
[20] Wang H, Guo P, Han Y 2006 Synthesis and surface morphology of tetraaniline–block–poly (L-lactate) diblock oligomers Macromolecular Rapid Communications 27 63-68