PEGylation of Carbon Nanotubes via Mussel Inspired Chemistry: Preparation, Characterization and Biocompatibility Evaluation

Xiaoyong Zhang^{a,*}, Guangjian Zeng^a, Jianwen Tian^a, Qing Wan^a, Qiang Huang^a, Ke Wang^b, Qingsong Zhang^b, Meiyong Liu^a, Fengjie Deng^{a,*}, and Yen Wei^{b,*}

^a Department of Chemistry, Nanchang University, 999 Xuefu Avenue, Nanchang 330031, China. ^b Department of Chemistry and the Tsinghua Center for Frontier Polymer Research, Tsinghua University, Beijing, 100084, P. R. China.

Abstract

A novel strategy for surface modification of multi-walled carbon nanotubes (MWCNT) was developed via combination of mussel inspired chemistry and Michael addition reaction. In this procedure, pristine MWCNT were first coated with polydopamine (PDA) through self polymerization of dopamine. The PDA functionalized CNT (CNT-PDA) were further functionalized with amino-terminated polymers (polyPEGMA), which were synthesized via free radical polymerization using cysteamine hydrochloride as the chain transfer agent and poly(ethylene glycol) monomethyl ether methacrylate as the monomer. The successful modification of CNT was ascertained by a series of characterization techniques including transmission electron microscopy, Fourier transform infrared spectroscopy, thermal gravimetric analysis and X-ray photoelectron spectrometry. The polymer modified CNT showed enhanced dispersibility in aqueous and organic solution. Cytotoxicity evaluation of polymers modified CNT showed that these modified CNT are biocompatible with cells. Finally, due to the universal adhesive of PDA and chain transfer free radical polymerization, this strategy developed in this work can also be extended for surface modification of many other nanomaterials with different functional polymers.

Keywords: Mussel inspired chemistry, carbon nanotubes, surface modification, PEGylation; Biocompatibility

Introduction

Carbon nanotubes (CNT) are one of the most popular **one-dimensional** nanomaterials, which have attracted broad research interest from all over the world since their first discovery in 1991.[1] Because of their unique structure and excellent physical, chemical, electrical, thermal and mechanical properties.[2, 3] CNT have been intensively investigated for applications in a variety of fields, such as electronic devices, composites hydrogen storage, catalysis and drug delivery *et al.*[4-6] however, practical applications of CNT have been severely hindered by their agglomeration. It is well known that pristine CNT tended to agglomerate into large clusters for their large specific surface areas and strong hydrophobic interaction between the individual CNT.[7-9] Therefore, appropriate surface modification and particle stabilization has become critical challenges for achieving better performance of CNT.

Over the past few decades, great efforts have been devoted to surface modification of CNT via both covalent and non-covalent strategies.[10-15] For example, oxidation of CNT with strong acid could introduce oxygen functional groups (e.g. -OH, C-O-C and COOH) to the surface of CNT.[16-18] On the one hand, these functional groups could enhance the dispersibility of CNT in aqueous solution in some extent. On the other hand, they could serve as active sites for further surface modification of CNT with other functional components. However, the covalent strategy will inevitably destroy the structure of CNT and influence their properties. Furthermore, covalent strategy often involves hazardous chemical agents, harsh reaction condition and complex reaction procedure. Non-covalent strategy is relied on self assembly of CNT and amphiphilic polymers, which can preserve the structure and electrical properties of CNT. However, thus obtained CNT-polymer composites can only stable in specific solution due to the relative weak and reversible interaction between the CNT and polymers. Therefore the development of novel strategy, which could overcome the drawbacks of both covalent and non-covalent **methods** is still of great research interest.

Mussel inspired chemistry is a recent emerged surface modification strategy, which

was inspired by the strong **adhesion** of mussel feet to various substrates.[19-30] Previous studies have suggested that the strong adhesion of mussel is associated with the dopamine-like components in mussel foot proteins. Since the pioneer work by Lee *et al* in 2007, mussel inspired chemistry has attracted increasing interest and been intensively explored for a wide range of applications.[31-40] For example, Lee *et al* demonstrated that dopamine could self-polymerize under alkaline environment to form a polymer coating on a variety of organic and inorganic materials, including polymers, noble metals, oxides, semiconductors and ceramics.[41] More importantly, thus obtained polydopamine (PDA) coating could further react with amino- or thiol-contained molecules via Michael addition or Schiff base reaction.[42, 43] Therefore, we could therefore functionalize any material surfaces with various amino- or thiol-contained molecules in theoretically *via* combination of mussel inspired chemistry and Michael addition reaction.[44] **Furthermore, surface modification of carbon nanomaterials and membranes with different macromolecules such as bovine serum albumin and heparin through mussel inspired chemistry has also reported by Zhao et al recently.[45-48] They demonstrated that biocompatible materials and membranes with better biological properties and performance could be facilely fabricated independent of the substrates. These results suggested that mussel inspired chemistry should be a general and effective route for surface modifications of materials for biomedical applications. However, to the best of our knowledge, surface modification CNT with synthetic biocompatible polymers such as polyethylene glycol (PEG) through mussel inspired chemistry has been seldom reported.**

In this work, amino-terminated polymers **were** synthesized via free radical polymerization under a rather mild reaction conditions using cysteamine hydrochloride as the chain transfer agent (CTA) and poly(ethylene glycol) monomethyl ether methacrylate (PEGMA) as the monomer **(Fig. 1)**. And then amino-terminated polyPEGMA was further linked with PDA functionalized CNT (CNT-PDA) through Michael addition reaction. These functionalized CNT

(CNT-PDA-PEGMA) were characterized by a series of techniques. We demonstrated that CNT could be facilely functionalized with polyPEGMA through the strategy developed in this work. On the other hand, CNT-PDA-PEGMA showed enhanced dispersibility in both aqueous and organic solution. Due to the universality of mussel inspired chemistry, this strategy could also be expanded for surface modification of many other nanomaterials. Therefore the method developed in this work should be a novel, simple and effective strategy for preparation of CNT-polymer composites.

Fig. 1 Schematic representation for the preparation of CNT-PDA-PEGMA composites via a combination of mussel inspired chemistry and a Michael addition reaction. **Step 1:** amino-terminated polymers (polyPEGMA) was synthesized via free radical polymerization under a rather mild reaction conditions using cysteamine hydrochloride as CTA and PEGMA as the monomer. **Step 2:** the PEGMA was further linked with PDA functionalized CNT (CNT-PDA) in deionized water for 6 h at room temperature (pH value is about 8.5).

2. Experiment

2.1 Materials and characterization

CNT with diameter of 30-50 nm were purchased from Sino Nanotech Ltd. (Beijing, China). Dopamine hydrochloride was purchased from Sangon Biotech. Co., Ltd. (Shanghai, China.) The PEGMA was obtained from Aladdin (Shanghai, China). Tris (hydroxymethyl) aminomethane (Tris) was obtained from Sinopharm Chemical

Reagent Co., Ltd. All the other reagents **were** of analytical grade and used without further purification.

^1H NMR spectra **were** recorded on Bruker Avance-400 spectrometer with D_2O as the solvent. Transmission electron microscopy (TEM) images were recorded on a Hitachi 7650B microscope operated at 80 kV; the TEM specimens were made by placing a drop of the nanoparticle ethanol suspension on a carbon-coated copper grid. The Fourier transform infrared (FT-IR) spectra were obtained by using a Nicolet 380 Fourier transform spectrometer with a resolution of 2 cm^{-1} . The samples were pressed with KBr into a pellet before measuring the infrared absorption spectra. Thermal gravimetric analysis (TGA) was conducted on a TA instrument Q50 with a heating rate of $20\text{ }^\circ\text{C min}^{-1}$. Samples weight between 10 and 20 mg were heated from 25 to $600\text{ }^\circ\text{C}$ in air flow (60 mL min^{-1}), N_2 as the balance gas (40 mL min^{-1}). Each sample was ultrasonicated for 30 min prior to analysis. The reported values are the mean values of three measurements. The X-ray photoelectron spectra (XPS) were performed on a VGESCALAB 220-IXL spectrometer using an Al $\text{K}\alpha$ X-ray source (1486.6 eV). The energy scale was internally calibrated by referencing to the binding energy (E_b) of the C1s peak of a carbon contaminant at 284.6 eV. The size distribution of PEGylated CNT in water was determined using a zeta Plus apparatus (ZetaPlus, Brookhaven Instruments, Holtsville, NY).

2.2 Synthesis of polyPEGMA

The amino-terminated polyPEGMA was synthesized via free radical polymerization using cysteamine hydrochloride as CTA and PEGMA as monomer. In a typical experiment, PEGMA (40 mmol), cysteamine hydrochloride (1 mmol), ammonium persulphate (0.5 mmol) and deionized water (30 mL) or PEGMA (20 mmol), Cysteamine hydrochloride (1 mmol), ammonium persulphate (0.5 mmol) and deionized water (30 mL) were introduced in a polymerization bottle with a magnetic stir bar and purged by nitrogen flow at $38\text{ }^\circ\text{C}$ for 48 h. The final reaction mixture was put into deionized water for overnight. Then soak 6 h in methanol, take out the product, and dried for further characterization and experiments.

2.3 Synthesis of CNT-PDA

CNT-PDA was prepared according to our previous method.[49] Dopamine solution with 5 g/L was prepared by dissolving dopamine in Tris buffer solution (10 mM, pH = 8.5). The 500 mg of CNT were suspended in the 100 mL of dopamine solution under stirring for 2 h. After the polymerization, the CNT coated with PDA were separated by centrifugation at 8000 rpm for 10 min and washed with deionized water and methanol several times. The product were finally collected and dried in the oven at 60 °C for 12 h. These as-synthesized CNT-PDA composites were collected and stored in vials for further characterization and experiments.

2.4 Synthesis of CNT-PDA-PEGMA

To preparation of CNT-PDA-PEGMA, 200 mg of CNT-PDA was first dispersed in 60 mL of Tris buffer solution (10 mM, pH = 8.5). Then 200 mg of polyPEGMA was added into the mixture and further reacted for overnight at room temperature. These as-synthesized CNT-PDA-PEGMA composites were collected by repeated centrifugation and washing to remove unreacted polymers. Both the pristine and functionalized CNT were characterized by a series of techniques including FT-IR, TEM *et al.*

2.5 Biocompatibility evaluation of CNT-PDA-PEGMA

The biocompatibility of CNT-PDA-PEGMA composites to human alveolar basal epithelial (A549) cells was first evaluated by cell morphology observation.[50] Briefly, cells were seeded in 6-well microplates at a density of 1×10^5 cells per mL in 2 mL of the respective media containing 10% heat-inactivated fetal bovine serum (FBS). After cell attachment, plates were washed with phosphate buffer solution (PBS) and the cells were treated with complete cell culture medium, or different concentration of PEGylated CNT prepared in 10% FBS containing media for 24 h. Then all samples were washed with **PBS** three times to remove the uninternalized CNT. The morphology of cells was observed by an optical microscopy (Leica, Germany).

The cell viability was further used to quantitatively evaluate the biocompatibility of CNT-PDA-PEGMA using the WST assay as described in our previous reports.[51-53] Briefly, cells were seeded in 96-well microplates at a density of 5×10^4 cells per mL in 160 μL of the respective media containing 10% FBS. After 24 h of cell attachment, the cells were incubated with 10, 20, 40, 80, 120 $\mu\text{g mL}^{-1}$ of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40 for 12 and 24 h. Then the cells were washed with PBS for three times to remove the uninternalized nanoparticles. After that, 10 μL of CCK-8 dye and 100 μL of Dulbecco's Modified Eagle's Medium (DMEM) cell culture media was added to each well and incubated for 2 h at 37 °C. Plates were then analyzed with a microplate reader (VictorIII, Perkin-Elmer). Measurements of dye absorbance were carried out at 450 nm, with the reference wavelength at 620 nm. The values were proportional to the number of live cells. The percent reduction of WST was compared to the control (cells not exposed to nanoparticles), which represented 100% WST reduction. Three replicate wells were used for each control and test concentrations per microplate, and the experiment was repeated three times. Cell survival was expressed as absorbance relative to that of untreated controls. Results are presented as mean $\pm$ standard deviation (SD).

3. Results and discussion

The polyPEGMA, CNT-PDA and CNT-PDA-PEGMA in D_2O were first characterized by ^1H NMR spectroscopy. A series of characteristic signals of polyPEGMA with chemical shift at 4.04 ppm (COOCH_2), 3.59-3.38 ppm ($\text{OCH}_2\text{CH}_2\text{O}$), 3.25-3.23 ppm (OCH_3), 1.81 ppm (CCH_2C), 0.93-0.80 ppm (CCH_3) were observed in the ^1H NMR (Fig. S1). It should be noted that the vinyl protons of PEGMA at 5.7 and 6.1 ppm are no longer discernible after polymerization, suggesting that successful synthesis of aminated polyPEGMA through chain transfer free radical polymerization. Fig. S2 showed the ^1H NMR spectrum of CNT-PDA. Apart from the obvious peaks with chemical shift at 3.49–3.47 and 1.03–1.01 ppm, small peaks with chemical shift at 6.72-6.59 ppm can be found in CNT-PDA. As compared with the spectrum of CNT-PDA, the signals with chemical shift at

6.72-6.59 ppm were disappeared. These results suggested that aminated polyPEGMA was conjugated on the CNT-PDA successfully (Fig. S3).

The morphology of CNT **samples** before and after surface modification has been characterized by TEM (Fig. 2). Consistent with the information from manufacture, the diameter of pristine CNT between 30-50 nm can be clearly identified. After modification with PDA and PEGMA, thin polymer films coated on the surface of CNT can be clearly observed (as indicated by the red arrows in Fig. 2B-D), demonstrating that CNT have been successfully modified with PDA and polyPEGMA. More importantly, no obvious structure destroy was observed from the TEM images, suggesting that the electronic characteristic of CNT was preserved after surface modification. On the other hand, the method described in this work is rather simple and effective. It is therefore more suitable for surface modification of CNT as compared with the covalent methods.

Fig. 2 Representative TEM images of pristine CNT (A), CNT-PDA (B), CNT-PDA-PEGMA-20 (C) and PEGMA-40 (D), scale bar = 200 nm. Thin polymer films coated on CNT were clearly observed by TEM observation after they were functionalized with PDA and polymers (see the arrows in Fig. B-D). The TEM images confirmed the successful modification of PEGMA through mussel inspired chemistry and Michael addition reaction.

Many other techniques such as FT-IR, TGA and XPS have also been utilized to characterize the CNT samples. As shown Fig 3, the broad band closed to 3450 cm^{-1} which can be attributed to N-H or O-H stretching vibration mode was observed in CNT-PDA, evidencing the successful modification of CNT by PDA. Furthermore, an intensive peak located at 1636 cm^{-1} , which can be attributed to the stretching vibration band of C=O was observed both in the sample of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40, implying the successful linkage of CNT-PDA with polyPEGMA. Moreover, two peaks located at 1384 and 1108 cm^{-1} are due to C-H bending vibration and C-O stretching vibration mode, further confirming the successful fabrication CNT-polymer composites.

Fig. 3 FT-IR spectra of MWCNT and PEGMA functionalized MWCNT. Characteristic IR peaks located at 1636 , 1384 and 1108 cm^{-1} were observed in CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40, suggesting that the functional groups such as C=O, C-H and C-O were existed in CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40.

The successful modification of CNT with PDA and PEGMA was also confirmed by TGA. As shown in Fig. 4, the weight loss of pristine CNT is about 2.7% when the temperature is $600\text{ }^{\circ}\text{C}$. After modification with PDA, the weight loss of CNT-PDA

calculated from the temperature 114 to 600 °C is about 25.2%, which suggested that the PDA was coated on the CNT *via* mussel inspired chemistry. Therefore, the weight percentage of PDA on the CNT is 22.5% based on TGA results. On the other hand, no sharp weight loss was observed based on the TGA curve of CNT-PDA, suggesting that the composition of PDA is rather complex. After the polyPEGMA was linked on the CNT-PDA, much more weight loss was observed in the samples of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40. It can be seen that the weight loss of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40 was increased to 38.0% and 36.6%, respectively. It is therefore, the mass percentages of polymer conjugated on the CNT-PDA were calculated to be 15.5% and 14.1 % for CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40, respectively. On the other hand, a significant mass decrease can be observed in the samples of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40 when the temperature is located at 270-380 °C, which is the decomposition temperature of PEGMA.[54] Therefore, TGA results also confirmed that CNT were successfully modified with PDA and polyPEGMA through mussel inspired chemistry and Michael addition reaction.

Fig. 4 TGA curves of CNT, CNT-PDA and CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40. Significant mass decrease was observed in the samples of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40 when the temperature is located at 270-380 °C, indicating the PEGMA was attached on CNT via Michael addition reaction successfully.

XPS was further utilized to analyze the chemical compositions of the CNT samples. As indicated by Fig. 5A, the elements including carbon (C), oxygen (O) and nitrogen (N) were found in CNT samples. The detailed information of XPS spectra including C1s, O1s and N1s was shown in Fig. 5B-D. It can be seen that the photoelectron peak of C in the pristine CNT is located at 284.78 eV, which can be assigned to graphitic C on CNT (Fig. 5B and Fig. S4). After their surface was coated with PDA and modified with polyPEGMA, novel peaks between 286.03-288.83 eV were emerged, which can be ascribed to the C-N, C-O and C=O bands of PDA and polyPEGMA (Fig. S4B-D). More importantly, the signals of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40 of C1s at 289.30 eV can be ascribed to the O-C=O, which indicating that polyPEGMA can be conjugated with CNT-PDA through Michael addition reaction. Fig. 5C showed the N1s spectra of CNT samples. Obvious N1s peak located at 396.2-405.2 eV can be found in the sample of CNT-PDA, suggesting that PDA was successfully coated on CNT. After further conjugated CNT-PDA with polyPEGMA, the signals of N1s decreased correspondingly in the samples of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40. From O1s spectra (Fig. 5D), we found that signals of O1s at 528.1-537.6 eV in the samples of CNT-PDA and polyPEGMA functionalized CNT are much greater than that of pristine CNT, which is well consistent with the results of C1s and N1s. Surprisingly, no sulfur (S) signals are found in the samples of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40 although there are theoretically in present of these samples. We speculated that possible reason is content of S is too low to be detected by XPS.

Fig. 5 XPS spectra of CNT, CNT-PDA, CNT-PDA-PEGMA-20, CNT-PDA-PEGMA-40. (A) Survey scan curves range from 0-1200 eV. (B) The C1s region, (C) N1s region and (D) O1s region.

Furthermore, based on XPS analysis, the weight percentages of C, N, O were also calculated (Table 1). Two elements C (97.13%) and O (2.87%) were found in pristine CNT. After surface coating with PDA, the weight percentages of C and O were changed to 84% and 12.08%, respectively. More importantly, a new element N (3.92%) was found in the sample of CNT-PDA. These results evidenced the successful coating CNT with PDA. After further conjugation of CNT-PDA with polyPEGMA, the oxygen contents were further increased to 14.47% and 15.73% in the sample of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40, respectively. Well consistent with the above results, the percentages of N was decreased from 3.92% for CNT-PDA to 0.45% for CNT-PDA-PEGMA-20 and 1.02% for CNT-PDA-PEGMA-40, respectively. These results further demonstrated that these aminated polyPEGMA can be used for surface modification of CNT through combination of mussel inspired chemistry and Michael addition reaction.

Table 1 Element contents (%) of CNT nanoparticles based on XPS analysis

Sample	C	O	N
CNT	97.13	2.87	0
CNT-PDA	84.00	12.08	3.92
CNT-PDA-PEGMA-20	85.08	14.47	0.45
CNT-PDA-PEGMA-40	83.25	15.73	1.02

The dispersibility of CNT samples in organic and water was further evaluated. As shown in Fig. S5, pristine CNT were quickly precipitated within 5 min in acetone (Fig. S5B). As compared with pristine CNT, the dispersibility of CNT-PDA in acetone was improved in some extent, which will be also precipitated within 2 h (Fig. S5C). After CNT-PDA was further modified with polyPEGMA, the dispersibility of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40 is obviously enhanced. No perceivable precipitation was found in the sample of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40 even they were deposited for 24 h (Fig. S5D). Water dispersibility is a very important parameter which should be first considered for the biomedical applications of nanomaterials. Therefore, the water dispersibility of CNT and modified CNT was further investigated. Similar to the dispersibility in acetone, CNT-PDA also exhibited enhanced dispersibility in water as compared with pristine CNT (Fig. 6B). However, due to only limited hydrophilic groups are existed on CNT-PDA, they will also be precipitated within 2 h. After the CNT-PDA was further conjugated with aminated polyPEGMA, the water dispersibility of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40 was significantly improved. It can be seen that these polyPEGMA functionalized CNT can deposit in water for more than 24 h (Fig. 6D). The size distribution of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40 was also investigated by dynamic laser scattering. We found that the hydrodynamic size distribution of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40 is 242.7 ± 78.1 and 222.8 ± 92.2 nm with polydispersity index of 0.160 and 0.231, respectively (Fig. S6). These results also implied that aminated polyPEGMA were successfully conjugated on CNT. Furthermore, the effect

of surface modification on the dispersibility of CNT samples in PBS and normal saline solution was also evaluated. It can be seen that CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40 exhibited rather good stability in both PBS and saline solution (Fig. S7). No obvious precipitation was found in these PEG modified CNT samples after they were dispersed in PBS and saline solution for more than 24 h. These above results implied that these PEG modified CNT samples are of great potential for biomedical applications.

Fig. 6 Dispersion of CNT samples (Bottles from left to right are the samples of CNT, CNT-PDA, CNT-PDA-PEGMA-20, and CNT-PDA-PEGMA-40, respectively) in water for different time. (A) Just dispersed, and dispersed in water for (B) 5 min, (C) 2 h and (D) 24 h.

PEG is a commonly used polymer for surface modification. Different strategies for PEGylation of nanomaterials with different shape, composition and properties have also been developed.[55-58] It has been reported that PEGylated nanomaterials could exhibit remarkable enhanced dispersibility, good biocompatibility, which make these nanomaterials better for biomedical applications.[59] For example, we have previously demonstrated that PEGylation of nanodiamonds could be achieved through both graft to method and graft from method.[54] These PEGylated nanodiamonds showed high water dispersibility and can be used for intracellular delivery of anticancer drug (DOX) into cells with good performance.[60] More

importantly, PEGylation is also an important strategy for avoiding nanomaterials swallowed by reticuloendothelial system *in vivo*, which is very useful for improving the therapeutic index and decreasing the adverse effects of drugs.[59] Therefore, the biocompatibility of CNT-PDA-PEGMA composites to cells was subsequently investigated.

Fig. 7 Cell viability of CNT-PDA-PEGMA-20 (A) and CNT-PDA-PEGMA-40 (B). Cells were incubated with different concentrations (10-120 $\mu\text{g mL}^{-1}$) of PEGylated CNT for 12 and 24 h.

The effects of CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40 to A549 cells were first evaluated by optical microscopy observation. It can be seen that both CNT-PDA-PEGMA-20 and CNT-PDA-PEGMA-40 showed no obvious adverse effects on cells. Cells still adhered to cell plates and showed no significant cell

morphology change when they were incubated with different concentrations of CNT samples (Fig. S8). Based on the optical microscopy observation, we could conclude that the PEGylated CNT are biocompatible with cells. Furthermore, cell viability was subsequently measured to quantitatively evaluate the biocompatibility of CNT-PDA-PEGMA. As shown in Fig. 7, the cell viability of CNT-PDA-PEGMA-20 is still greater than 89% after cells were incubated with CNT-PDA-PEGMA-20 for 12 and 24 h. The half maximal inhibitory concentration (IC_{50}) value of CNT-PDA-PEGMA-20 at 24 h is about $231.9 \mu\text{g mL}^{-1}$. As compared with CNT-PDA-PEGMA-20, CNT-PDA-PEGMA-40 also showed excellent biocompatibility with IC_{50} value of $287.1 \mu\text{g mL}^{-1}$ after 24 h incubation, which is well consistent with optical microscopy observation. These results demonstrated that these PEGylated CNT are biocompatible for biomedical applications. More importantly, these PEGylated CNT is relative good biocompatible as compared with the pristine CNT.[16] We speculated the possible reasons are PDA and polyPEGMA coated on the CNT could prevent CNT being recognized by cells and thus decrease the nonspecific interaction between cells and PEGylated CNT.[61]

Conclusion

Surface modification of CNT with polyPEGMA via combination of mussel inspired chemistry and chain transfer free radical polymerization was developed for the first time. The successful conjugation of aminated polyPEGMA with CNT was evidenced by a series of characterization techniques including FT-IR spectroscopy, TGA, Raman spectroscopy and XPS spectroscopy. Thus obtained PEGylated CNT showed remarkable enhanced dispersibility in both organic and aqueous solution. Furthermore, cell viability examination suggested CNT-PDA-PEGMA composites are biocompatible with A549 cells, implying their potential for biomedical applications. As compared with conventional methods, the method described in this work for surface modification of CNT is rather simple, effective and environmental friendly. On the other hand, apart from PEGMA, many other functional polymers can also be facilely used to modify CNT. Finally, this method described in this work can

be also expanded for surface modification of other nanomaterials and surface for the strong adhesion of PDA. Therefore this method should a novel and universal **strategy** for surface modification of nanomaterials for various applications.

Acknowledgments

This research was supported by the National Science Foundation of China (Nos. 21104039, 21134004, 21201108, 21474057), and the National 973 Project (Nos. 2011CB935700).

References

- [1] S. Iijima, Helical microtubules of graphitic carbon, *Nature* 354 (1991) 56-58.
- [2] A. Allaoui, S. Bai, H.-M. Cheng, J. Bai, Mechanical and electrical properties of a MWNT/epoxy composite, *Compos. Sci. Technol.* 62 (2002) 1993-1998.
- [3] Y. Zhu, W. Li, Q. Li, Y. Li, Y. Li, X. Zhang, Q. Huang, Effects of serum proteins on intracellular uptake and cytotoxicity of carbon nanoparticles, *Carbon* 47 (2009) 1351-1358.
- [4] X. Liu, L. Guo, D. Morris, A.B. Kane, R.H. Hurt, Targeted removal of bioavailable metal as a detoxification strategy for carbon nanotubes, *Carbon* 46 (2008) 489-500.
- [5] J. Zhu, N. Jia, J. Park, K. Gong, Carbon nanocylinders with high spectroscopic and electrocatalytic activities: An electrochemical route to superfill the gaps in vertically aligned carbon nanotubes for cutting them into specific lengths, *Carbon* 61 (2013) 270-277.
- [6] X. Geng, F. Li, D.-w. Wang, H.-m. Cheng, The influence of pretreatment on multi-wall carbon nanotubes for electrochemical capacitors, *Carbon* 60 (2013) 564.
- [7] A. Thess, R. Lee, P. Nikolaev, H. Dai, P. Petit, J. Robert, C. Xu, Y.H. Lee, S.G. Kim, A.G. Rinzler, Crystalline ropes of metallic carbon nanotubes, *Science* 273 (1996) 483-487.
- [8] A.M. Esawi, M.A. El Borady, Carbon nanotube-reinforced aluminium strips, *Compos. Sci. Technol.* 68 (2008) 486-492.
- [9] F. Gong, K. Bui, D.V. Papavassiliou, H.M. Duong, Thermal transport phenomena and limitations in heterogeneous polymer composites containing carbon nanotubes and inorganic nanoparticles, *Carbon* 78 (2014) 305-316.
- [10] P.-X. Hou, C. Liu, H.-M. Cheng, Purification of carbon nanotubes, *Carbon* 46 (2008) 2003-2025.
- [11] N. Karousis, N. Tagmatarchis, D. Tasis, Current progress on the chemical modification of carbon nanotubes, *Chem. Rev.* 110 (2010) 5366-5397.
- [12] X. Zhang, M. Liu, X. Zhang, F. Deng, C. Zhou, J. Hui, W. Liu, Y. Wei, Interaction of tannic acid with carbon nanotubes: enhancement of dispersibility and biocompatibility, *Toxicol. Res.* 4 (2015) 160-168.
- [13] X. Zhang, S. Wang, M. Liu, J. Hui, B. Yang, L. Tao, Y. Wei, Surfactant-dispersed nanodiamond: biocompatibility evaluation and drug delivery applications, *Toxicol. Res.* 2 (2013) 335-342.
- [14] B. Yang, Y. Zhao, X. Ren, X. Zhang, C. Fu, Y. Zhang, Y. Wei, L. Tao, The power of one-pot: a hexa-component system containing π - π stacking, Ugi reaction and RAFT polymerization for simple polymer conjugation on carbon nanotubes, *Polym. Chem.* 6 (2015) 509-513.
- [15] X. Ren, B. Yang, Y. Zhao, X. Zhang, X. Wang, Y. Wei, L. Tao, One-pot Polymer Conjugation on Carbon Nanotubes through Simultaneous π - π Stacking and the Biginelli Reaction, *Polymer* (2015) 10.1016/j.polymer.2015.1002.1033.

- [16] X. Zhang, W. Hu, J. Li, L. Tao, Y. Wei, A Comparative Study of Cellular Uptake and Cytotoxicity of Multi-walled Carbon Nanotube, Graphene Oxide, and Nanodiamond, *Toxicol. Res.* 1 (2012) 62-68.
- [17] X. Zhang, Y. Zhu, J. Li, Z. Zhu, J. Li, W. Li, Q. Huang, Tuning the Cellular Uptake and Cytotoxicity of Carbon Nanotubes by Surface Hydroxylation, *J. Nanopart. Res.* 13 (2011) 6941-6952.
- [18] X. Zhang, S. Wang, M. Liu, B. Yang, L. Feng, Y. Ji, L. Tao, Y. Wei, Size tunable fluorescent nano-graphite oxides: preparation and cell imaging applications, *Phys. Chem. Chem. Phys.* 15 (2013) 19013-19018.
- [19] Y. Liu, B. Yu, J. Hao, F. Zhou, Amination of surfaces via self-assembly of dopamine, *J. Colloid Interf. Sci.* 362 (2011) 127-134.
- [20] J. Ryu, S.H. Ku, H. Lee, C.B. Park, Mussel-Inspired Polydopamine Coating as a Universal Route to Hydroxyapatite Crystallization, *Adv. Funct. Mater.* 20 (2010) 2132-2139.
- [21] H. Hu, B. Yu, Q. Ye, Y. Gu, F. Zhou, Modification of carbon nanotubes with a nanothin polydopamine layer and polydimethylamino-ethyl methacrylate brushes, *Carbon* 48 (2010) 2347-2353.
- [22] S.M. Kang, S. Park, D. Kim, S.Y. Park, R.S. Ruoff, H. Lee, Simultaneous Reduction and Surface Functionalization of Graphene Oxide by Mussel-Inspired Chemistry, *Adv. Funct. Mater.* 21 (2011) 108-112.
- [23] B. Fei, B. Qian, Z. Yang, R. Wang, W. Liu, C. Mak, J.H. Xin, Coating carbon nanotubes by spontaneous oxidative polymerization of dopamine, *Carbon* 46 (2008) 1795-1797.
- [24] W. Lee, J.U. Lee, B.M. Jung, J.-H. Byun, J.-W. Yi, S.-B. Lee, B.-S. Kim, Simultaneous enhancement of mechanical, electrical and thermal properties of graphene oxide paper by embedding dopamine, *Carbon* 65 (2013) 296-304.
- [25] X. Zhang, M. Liu, Y. Zhang, B. Yang, Y. Ji, L. Feng, L. Tao, S. Li, Y. Wei, Combining mussel-inspired chemistry and the Michael addition reaction to disperse carbon nanotubes, *RSC Adv.* 2 (2012) 12153-12155.
- [26] Q. Wan, J. Tian, M. Liu, G. Zeng, Z. Li, K. Wang, Q. Zhang, F. Deng, X. Zhang, Y. Wei, Mussel Inspired Preparation of High Dispersible and Biocompatible Carbon Nanotubes, *RSC Adv.* 5 (2015) 25329-25336.
- [27] Q. Wan, M. Liu, J. Tian, F. Deng, G. Zeng, Z. Li, K. Wang, Q. Zhang, X. Zhang, Y. Wei, Surface Modification of Carbon Nanotubes via Combination of Mussel Inspired Chemistry and SET-LRP, *Polym. Chem.* 6 (2015) 1786-1792.
- [28] J. Tian, D. Xu, M. Liu, F. Deng, Q. Wan, Z. Li, K. Wang, X. He, X. Zhang, Y. Wei, Marrying mussel inspired chemistry with SET-LRP: A novel strategy for surface functionalization of carbon nanotubes, *J. Polym. Sci. Polym. Chem.* (2015) 10.1002/pola.27638.
- [29] Q. Wan, J. Tian, M. Liu, G. Zeng, Q. Huang, K. Wang, Q. Zhang, F. Deng, X. Zhang, Y. Wei, Surface Modification of Carbon Nanotubes via Combination of Mussel Inspired Chemistry and Chain Transfer Free Radical Polymerization, *Appl. Surf. Sci.* 346 (2015) 335-341.
- [30] Q. Wan, M. Liu, J. Tian, F. Deng, Y. Dai, K. Wang, Z. Li, Q. Zhang, X. Zhang, Y. Wei, Toward the Development of Versatile Functionalized Carbon Nanotubes, *RSC Adv.* (2015) 10.1039/C1034RA17292B.
- [31] Y. Lee, H. Lee, Y.B. Kim, J. Kim, T. Hyeon, H. Park, P.B. Messersmith, T.G. Park, Bioinspired surface immobilization of hyaluronic acid on monodisperse magnetite nanocrystals for targeted cancer imaging, *Adv. Mater.* 20 (2008) 4154-4157.
- [32] Q. Ye, F. Zhou, W. Liu, Bioinspired catecholic chemistry for surface modification, *Chem. Soc. Rev.*

40 (2011) 4244-4258.

- [33] M.-H. Ryou, Y.M. Lee, J.-K. Park, J.W. Choi, Mussel-Inspired Polydopamine-Treated Polyethylene Separators for High-Power Li-Ion Batteries, *Adv. Mater.* 23 (2011) 3066-3070.
- [34] S.H. Ku, C.B. Park, Human endothelial cell growth on mussel-inspired nanofiber scaffold for vascular tissue engineering, *Biomaterials* 31 (2010) 9431-9437.
- [35] K. Ai, Y. Liu, C. Ruan, L. Lu, G.M. Lu, Sp² C-Dominant N-Doped Carbon Sub-Micrometer Spheres with a Tunable Size: A Versatile Platform for Highly Efficient Oxygen-Reduction Catalysts, *Adv. Mater.* 25 (2013) 998-1003.
- [36] X. Zhang, S. Wang, L. Xu, Y. Ji, L. Feng, L. Tao, S. Li, Y. Wei, Biocompatible Polydopamine Fluorescent Organic Nanoparticles: Facile Preparation and Cell Imaging, *Nanoscale* 4 (2012) 5581-5584.
- [37] Y. Liu, K. Ai, J. Liu, M. Deng, Y. He, L. Lu, Dopamine-Melanin Colloidal Nanospheres: An Efficient Near-Infrared Photothermal Therapeutic Agent for In Vivo Cancer Therapy, *Adv. Mater.* 25 (2013) 1353-1359.
- [38] M. Liu, J. Ji, X. Zhang, X. Zhang, B. Yang, F. Deng, Z. Li, K. Wang, Y. Yang, Y. Wei, Self-polymerization of Dopamine and Polyethyleneimine: Novel Fluorescent Organic Nanoprobes for Biological Imaging Applications, *J. Mater. Chem. B* 3 (2015) 3476 - 3482.
- [39] X. Zhang, Q. Huang, M. Liu, J. Tian, G. Zeng, Z. Li, K. Wang, Q. Zhang, Q. Wan, F. Deng, Preparation of amine functionalized carbon nanotubes via a bioinspired strategy and their application in Cu²⁺ removal, *Appl. Surf. Sci.* 343 (2015) 19-27.
- [40] L. Xu, N. Liu, Y. Cao, F. Lu, Y. Chen, X. Zhang, L. Feng, Y. Wei, Mercury Ion Responsive Wettability and Oil/Water Separation, *ACS Appl. Mater. Inter.* 6 (2014) 13324-13329.
- [41] H. Lee, S.M. Dellatore, W.M. Miller, P.B. Messersmith, Mussel-inspired surface chemistry for multifunctional coatings, *Science* 318 (2007) 426-430.
- [42] H. Lee, J. Rho, P.B. Messersmith, Facile conjugation of biomolecules onto surfaces via mussel adhesive protein inspired coatings, *Adv. Mater.* 21 (2009) 431-434.
- [43] L.Q. Xu, W.J. Yang, K.-G. Neoh, E.-T. Kang, G.D. Fu, Dopamine-induced reduction and functionalization of graphene oxide nanosheets, *Macromolecules* 43 (2010) 8336-8339.
- [44] Y. Cao, X. Zhang, L. Tao, K. Li, Z. Xue, L. Feng, Y. Wei, Mussel-inspired chemistry and Michael addition reaction for efficient oil/water separation, *ACS Appl. Mater. Inter.* 5 (2013) 4438-4442.
- [45] C. Cheng, S. Nie, S. Li, H. Peng, H. Yang, L. Ma, S. Sun, C. Zhao, Biopolymer functionalized reduced graphene oxide with enhanced biocompatibility via mussel inspired coatings/anchors, *J. Mater. Chem. B* 1 (2013) 265-275.
- [46] C. Cheng, S. Li, S. Nie, W. Zhao, H. Yang, S. Sun, C. Zhao, General and biomimetic approach to biopolymer-functionalized graphene oxide nanosheet through adhesive dopamine, *Biomacromolecules* 13 (2012) 4236-4246.
- [47] C. Nie, L. Ma, Y. Xia, C. He, J. Deng, L. Wang, C. Cheng, S. Sun, C. Zhao, Novel heparin-mimicking polymer brush grafted carbon nanotube/PES composite membranes for safe and efficient blood purification, *J. Membrane Sci.* 475 (2015) 455-468.
- [48] C. Nie, L. Ma, C. Cheng, J. Deng, C. Zhao, Nanofibrous Heparin and Heparin-Mimicking Multilayers as Highly Effective Endothelialization and Antithrombogenic Coatings, *Biomacromolecules* 16 (2015) 992-1001.
- [49] X. Zhang, J. Ji, X. Zhang, B. Yang, M. Liu, W. Liu, L. Tao, Y. Chen, Y. Wei, Mussel Inspired Modification of Carbon Nanotubes Using RAFT Derived Stimuli-responsive Polymers, *RSC Adv.* 3 (2013)

21817-21823.

- [50] X. Zhang, S. Wang, C. Zhu, M. Liu, Y. Ji, L. Feng, L. Tao, Y. Wei, Carbon-dots derived from nanodiamond: Photoluminescence tunable nanoparticles for cell imaging, *J. Colloid Interf. Sci.* 397 (2011) 39-44.
- [51] H. Qi, M. Liu, L. Xu, L. Feng, L. Tao, Y. Ji, X. Zhang, Y. Wei, Biocompatibility Evaluation of Aniline Oligomers with Different End-functional Groups, *Toxicol. Res.* 2 (2013) 427-433.
- [52] X. Zhang, S. Wang, C. Fu, L. Feng, Y. Ji, L. Tao, S. Li, Y. Wei, PolyPEGylated nanodiamond for intracellular delivery of a chemotherapeutic drug, *Polym. Chem.* 3 (2012) 2716-2719.
- [53] J. Hui, X. Zhang, Z. Zhang, S. Wang, L. Tao, Y. Wei, X. Wang, Fluoridated HAp: Ln 3+(Ln= Eu or Tb) nanoparticles for cell-imaging, *Nanoscale* 4 (2012) 6967-6970.
- [54] X. Zhang, C. Fu, L. Feng, Y. Ji, L. Tao, Q. Huang, S. Li, Y. Wei, PEGylation and polyPEGylation of nanodiamond, *Polymer* 53 (2012) 3178-3184.
- [55] X. Zhang, X. Zhang, B. Yang, J. Hui, M. Liu, W. Liu, Y. Chen, Y. Wei, PEGylation and Cell Imaging Applications of AIE Based Fluorescent Organic Nanoparticles via Ring-opening Reaction, *Polym. Chem.* 5 (2014) 689-693.
- [56] X. Zhang, X. Zhang, B. Yang, M. Liu, W. Liu, Y. Chen, Y. Wei, Polymerizable Aggregation Induced Emission Dye Based fluorescent Nanoparticles For Cell Imaging Applications *Polym. Chem.* 5 (2014) 356-360.
- [57] X. Zhang, X. Zhang, B. Yang, S. Wang, M. Liu, Y. Zhang, L. Tao, Aggregation-Induced Emission Materials Based Fluorescent Organic Nanoparticles: Facile PEGylation and Cell Imaging Applications, *RSC Adv.* 3 (2013) 9633-9636.
- [58] X. Zhang, J. Hui, B. Yang, Y. Yang, D. Fan, M. Liu, L. Tao, Y. Wei, PEGylation of Fluoridated HAp: Ln3+ Nanorods for Cell Imaging, *Polym. Chem.* 4 (2013) 4120-4125.
- [59] Z. Liu, W. Cai, L. He, N. Nakayama, K. Chen, X. Sun, X. Chen, H. Dai, In vivo biodistribution and highly efficient tumour targeting of carbon nanotubes in mice, *Nat. Nanotech.* 2 (2006) 47-52.
- [60] X. Zhang, S. Wang, C. Fu, L. Feng, Y. Ji, L. Tao, S. Li, Y. Wei, PolyPEGylated Nanodiamond for Intracellular Delivery of Chemotherapeutic Drug, *Polym. Chem.* 3 (2012) 2716-2719.
- [61] S. Hong, K.Y. Kim, H.J. Wook, S.Y. Park, K.D. Lee, D.Y. Lee, H. Lee, Attenuation of the in vivo toxicity of biomaterials by polydopamine surface modification, *Nanomedicine* 6 (2011) 793-801.

Water dispersible and biocompatible PEGylated carbon nanotubes were prepared via a novel mussel inspired strategy for the first time.