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RAFT Polymerization on Particle Surfaces: Same Goal, Different Strategies

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Reversible addition-fragmentation chain transfer (RAFT) polymerization is an important technique for surface functionalization of nanoparticles. It provides a powerful toolbox to tune the properties of composites. The RAFT agent, 4-cyanopentanoic acid dithiobenzoate (CPDB), was anchored on silica nanoparticles via surface silane chemistry with different graft densities. Methacrylic acid (MAA) and 6-azidohexyl methacrylate (AHMA) were polymerized on nanoparticles in a controlled manner via the RAFT technique. A variety of polyMAA (PMAA) and polyAHMA (PAHMA) brushes with different polymer chain lengths and low PDIs (<1.2) were prepared. The nanoparticles were characterized by FTIR, TGA, ^{1}H NMR and TEM. Postfunctionalization of the surface attached polymers was conducted using bio-functional groups and the resulting polymer nanocomposites may have important biomedical applications.

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Introduction

Polymer grafted nanoparticles are important materials and have found applications in chemosensors, biomedical devices, optoelectronics, coatings and dielectrics.[1-4] Surface functionalization is a key step in the preparation of polymer grafted nanoparticles.[5] The development of nanoparticle surface functionalization strategies has evolved in several stages (Figure 1)[6]: (1) The early stage of simple small molecule modification to alter the hydrophobic/hydrophilic properties of nanoparticles or to further introduce other functional groups; (2) Surface modification with a single population of polymer brushes to introduce new properties to the composites and enhance the matrix compatibility; (3) Functionalization with bimodal polymer brushes to further enhance the compatibility in matrices by independently controlling the entropic/enthalpic interactions; (4) Modification with mixed bimodal brushes to introduce different polymer and property varieties on particles; (5) Modification with multimodal brushes with integrated functionalities on nanoparticles to meet specific applications in complicated systems.[6] This process "from the simple to the advanced" allows researchers to understand the nature of surface functionalization and choose appropriate tools to build a variety of architectures on particle surfaces.

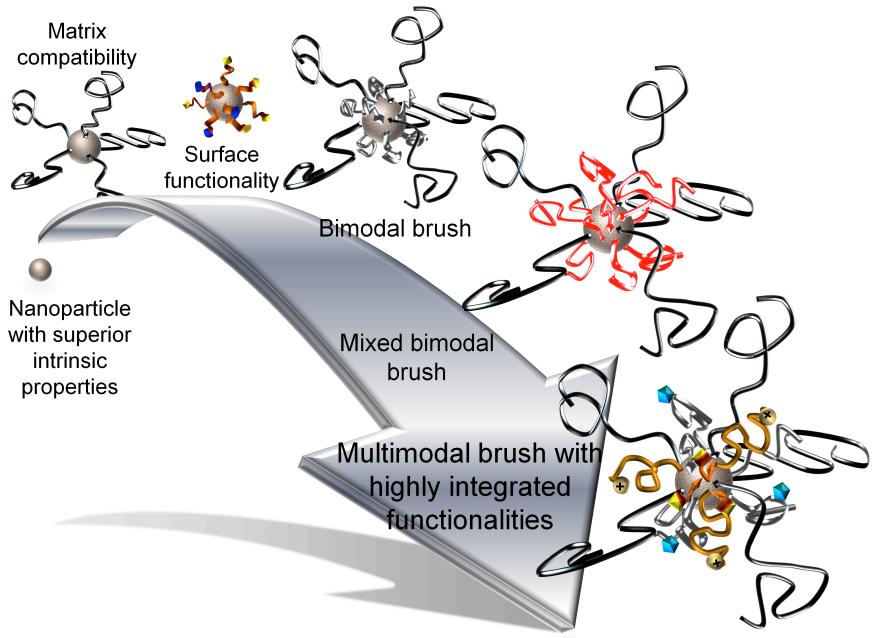


Figure 1. The development of surface functionalization of nanoparticles: from the simple to the advanced. Reproduced with permission from Reference 6. Copyright (2014). American Chemical Society.

The preparation of polymer grafted nanoparticles via surface functionalization is usually conducted using both "grafting to" and "grafting from" strategies. In the "grafting to" approach, free polymers diffuse to the particles and couple with the functional groups on the particle surfaces. It usually provides a relatively low graft density because of the steric hindrance between the previously attached and free polymer brushes during the diffusion process. A variety of polymers, such as polydimethylsiloxane (PDMS) and poly(glycidyl methacrylate) (PGMA) have been coated on nanoparticles.[7-10] In the "grafting from" strategy, polymers are prepared from the surface of nanoparticles. High graft densities can be attained by avoiding the steric limitations of diffusing chains. Controlled radical polymerizations (CRP), including nitroxide-mediated polymerization (NMP),[11] atom transfer radical polymerization (ATRP)[12] and reversible addition-fragmentation chain transfer (RAFT)[13] polymerization, have been widely utilized to prepare polymer grafted nanoparticles via the "grafting from" technique.

The RAFT technique uses a degenerative chain transfer method to control polymerization, rather than employing a persistent radical in the system as in NMP and ATRP.[13] One of unique features of the RAFT technique is its applicability to functional monomers, such as vinyl acetate[14] and N-vinyl pyrrolidone.[15] It has been successfully applied in mediating polymerizations of a variety of monomers under mild conditions with controllable molecular weights, narrow polydispersity and sophisticated architectures. Surface-initiated (SI) RAFT polymerization has been used to grow a variety of polymer shells on different substrate nanoparticles. The properties of the composites can be tailored by choosing different substrate-shell combinations. The SI-RAFT technique can thus be used to affect the dispersion of nanoparticles in small molecule or polymer matrices which would further influence the properties. In this paper, we discuss the RAFT polymerization on particle surfaces to develop polymer-grafted nanoparticles with desired properties.

Poly(carboxylic acids) are significant water soluble polymers with pH responsive properties. They are protonated in low pH environments and deprotonate in high pH systems. Thus, they have been used widely to conjugate other ion moieties to introduce new functionalities. In addition, they have been employed to couple with hydroxyl or amine based functionalities via covalent bonds. Based on these characteristics, carboxylic acid functionalized nanoparticles have been used in the drug delivery fields.[16,17] Thus, we are motivated to develop poly(carboxylic acid) grafted nanoparticles for delivery application. In the first part of this paper, we review our recent work on polyacid grafted particles. In the second part, we report an alternate strategy to achieve the same goal as our previous work. In addition, we report the post-

functionalization of poly(carboxylic acid) grafted nanoparticles for applications in aqueous media.

Cyclodextrin is an effective molecule to capture the signal molecules known as acylated homoserine lactones (AHLs), which are released by bacteria in their quorum sensing (QS) process.[18] QS allows bacteria to communicate with each other and thus makes bacteria much more resistant to antibiotics compared to individual bacteria. Thus, we were motivated to prepare cyclodextrin grafted nanoparticles to bind AHLs, lower their concentration and finally shut down QS. Cyclodextrin grafted nanoparticles could be very important in capturing signal molecules in biofilms, in which bacteria are protected by extracellular polymeric secretions (EPS). Free cyclodextrin will be blocked by the sticky EPS barrier before accessing bacterial cells whereas nanoparticles can penetrate the biofilm's EPS.

Experimental

Materials

All chemicals were purchased from Fisher or Sigma Aldrich and used as-received unless otherwise stated. Trimethylsilyldiazomethane (2.0 M in hexanes) and 4-cyanopentanoic acid dithiobenzoate (CPDB) were purchased from TCI and Strem Chemical Inc., respectively. RAFT agent CPDB coated silica nanoparticles were prepared based on previous literature.[19] 3-Aminopropyldimethylethoxysilane was obtained from Gelest and used as-received. NBD based fluorescent dye was prepared according to the literature.[20] Methacrylic acid (99.5%, Acros) was purified by passing through an activated neutral alumina column. AIBN was purified via recrystallization from methanol before use. The beta-lactam antibiotic penicillin-G was obtained from Sigma-Aldrich Inc. and used for all experiments.

Instrumentation

The ^{1}H NMR characterization was conducted using a Varian Mercury spectrometer 300/400 using CD_3OD or $CDCl_3$ as the solvent. Gel permeation chromatography (GPC) was conducted using a Waters PL-GPC-120 with a 515 HPLC pump, a 2410 refractive index detector, and three Styragel columns (the columns consisted of HR1, HR3 and HR4 with their corresponding effective molecular weight ranges of 100-5000, 500-30000, and 5000-500000, respectively) to characterize the molecular weights and PDI's. THF was employed as the eluent at 30 °C and a flow rate of 1.0 mL/min. Calibration was conducted using poly(methyl methacrylate) or polystyrene standards obtained from Polymer Laboratories. Samples were processed by filtration through microfilters with a pore size of 0.2 μm before analysis. Infrared spectra were

recorded with a PerkinElmer Spectrum 100 spectrometer. TEM imaging was conducted using a Hitachi 8000 transmission electron microscope with an operating voltage of 200 kV. Samples were prepared by dropping sample solutions on the carbon-coated copper grids and subsequent drying in a fume hood before characterization. TGA characterization was conducted using a TA Instruments Q5000 with a heating rate of 10°C/min from 25°C to 800°C~1000°C under nitrogen flow.

Synthesis of 1-Azido-6-hydroxyhexane

1-Chlorohexanol (6.83g, 0.05 mol) and sodium azide (6.50g, 0.10 mol) were dissolved in 50 ml water. The resulting solution was stirred at 80 °C for 12 h. The cooled solution went through extraction with diethyl ether (3×50 mL), drying with anhydrous sodium sulfate and followed by filtration. After removal of the solvent, a colorless liquid was obtained and dried under vacuum to constant weight (yield, 6.00g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.40 (m, 4H, CH_2), 1.51-1.64 (m, 4H, CH_2), 3.24 (t, 2H, CH_2N_3), 3.66 (t, 2H, CH_2O).

Synthesis of 6-Azidohexyl Methacrylate (AHMA)

1-Azido-6-hydroxyhexane (7.34 g, 51 mmol), methacrylic acid (3.87 g, 45 mmol), 4-(dimethylamino)pyridine (DMAP) (1.84 g, 15 mmol) were dissolved in 100 mL methylene chloride and the resulting solution was cooled in an ice bath. A methylene chloride solution (50 mL) of dicyclohexylcarbodiimide (DCC) (10.32 g, 50 mmol) was then added slowly. The resulting solution was then transferred to room temperature and followed by overnight stirring. After removal of the precipitate and solvent, the crude compound was purified via silica gel column chromatography (hexane : ethyl acetate = 10:1). A colorless liquid was obtained and dried under vacuum to constant weight (yield: 5.91 g, 62.2%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.40 (m, 4H, CH_2), 1.55-1.70 (m, 4H, CH_2), 1.95 (s, 3H, CH_3C), 3.24 (t, 2H, CH_2N_3), 4.16 (t, 2H, CH_2O), 5.54 (s, 1H, =CH), 6.12 (s, 1H, =CH). ^{13}C NMR (400 MHz, $CDCl_3$): 18.75, 26.04, 26.86, 28.98, 29.28, 51.6, 64.93, 125.78, 136.60, 167.90.

Surface-initiated RAFT Polymerization of AHMA

AHMA (0.536 g, 2.54 mmol), CPDB immobilized silica nanoparticles (93.05 mg, 0.23 groups/nm^2) and dry THF (2.5 mL) were added to an appropriate size Schlenk tube. The solution was sonicated until all the particles were dissolved in solution. Initiator V-70 (0.102 mL, 5 mM) was then added to the above solution. The resulting solution was degassed by four freeze-pump-thaw cycles followed by back filling with nitrogen. The tube was then placed in an oil bath of 40 °C and quenched in ice water at the desired time.

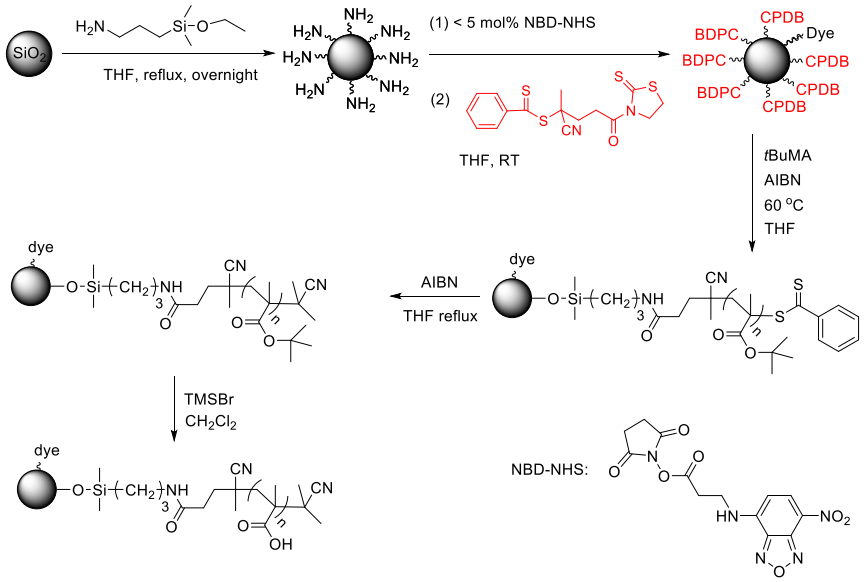
Preparation of Dye-labeled Poly(β-CD) Grafted Silica Nanoparticles

A DMF solution of β-CD (3.711 g, 3.27 mmol), N,N'-dicyclohexylcarbodiimide (DCC, 0.54 g, 2.616 mmol) and 4-dimethylaminopyridine (DMAP, 26.6 mg, 0.218 mmol) were added to a 10 mL dry DMF solution of dye-labeled poly(methacrylic acid) grafted silica nanoparticles (252 mg). The reaction was stirred at room temperature overnight. Then the reaction solution was poured into 200 mL ethyl ether followed by centrifugation at 3000 rpm for 5 min. The recovered particles were then redispersed in 20 mL of ethanol and subjected to a dialysis process to further remove impurities. The isolated dye-labeled poly(β-CD) grafted silica nanoparticles were finally dissolved in water for further use.

Results and Discussion

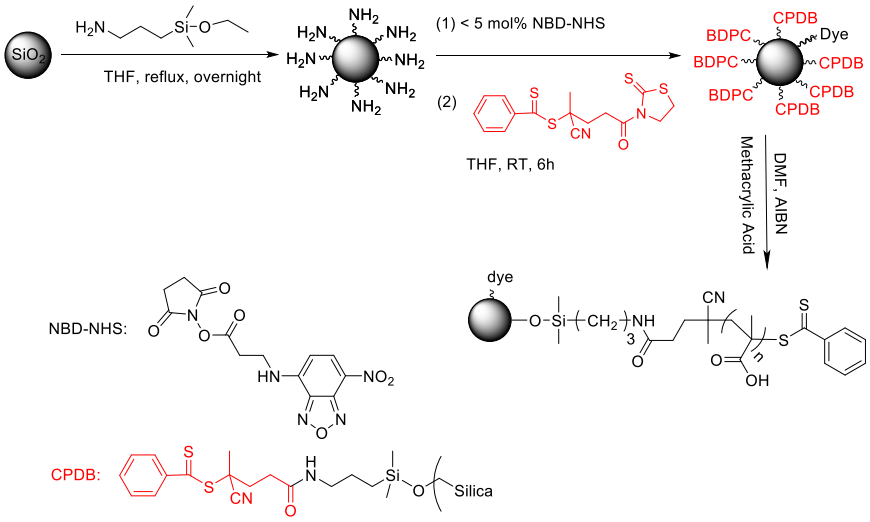
Poly(carboxylic acid) Grafted Nanoparticles

We have developed a series of poly(carboxylic acid) grafted nanoparticles for drug delivery applications via SI-RAFT.[19,21,22] The first synthetic strategy is shown in Scheme 1.[19] Essentially, it contains the preparation of RAFT agent (CPDB) coated nanoparticles with dye labeling and the subsequent SI-RAFT of *tert*-butyl methacrylate followed by deprotection of the *tert*-butyl moieties. The dye labeling on particles was introduced to track the presence and movement of nanoparticles in biological systems. The silane surface chemistry included the treatment of colloidal silica nanoparticles with 3-aminopropyldimethylethoxysilane in an aprotic solvent followed by the reaction between the amino groups and activated CPDB RAFT agent. This allowed us to prepare a variety of CPDB coated silica nanoparticles with a range of controllable graft densities of 0.01-0.7 groups/nm.[2,6,17] Particular attention should be paid on the potential cleavage of RAFT agent by surface-attached amino groups. Thus, in the nanoparticle preparation process, the solution of amino modified particles was added dropwise to an activated CPDB solution. The polymerization kinetic study demonstrated the living/controlled nature of the SI-RAFT of *tert*-butyl methacrylate on CPDB coated silica nanoparticles with different graft densities.[17] The deprotection of *tert*-butyl moieties was conducted by the reaction with trimethylsilyl bromide (TMSBr) and the resulting poly(carboxylic acid) grafted nanoparticles showed excellent dispersion in water.



Scheme 1. The synthesis of dye-labeled poly(carboxylic acid) grafted silica nanoparticles. Reproduced with permission from Reference 19. Copyright (2013). American Chemical Society.

As a more straightforward strategy, direct SI-RAFT of methacrylic acid was developed to prepare poly(carboxylic acid) grafted nanoparticles (Scheme 2).[19] The first step is the same as the method in Scheme 1, which is the preparation of CPDB coated nanoparticles. The second step is the direct polymerization of MAA on particles using DMF as the solvent. The polymerization kinetic study also demonstrated the living/controlled nature of the SI-RAFT of MAA.[19] A varity of polymer brush grafted particles with different chain lengths and densities were synthesized in a controlled manner. Figure 2 shows the ^{1}H NMR results that confirmed the structure of the poly(carboxylic acid) grafted silica nanoparticles.



Scheme 2. The synthesis of dye-labeled poly(carboxylic acid) grafted silica nanoparticles via direct polymerization of MAA. Reproduced with permission from Reference 19. Copyright (2013). American Chemical Society.

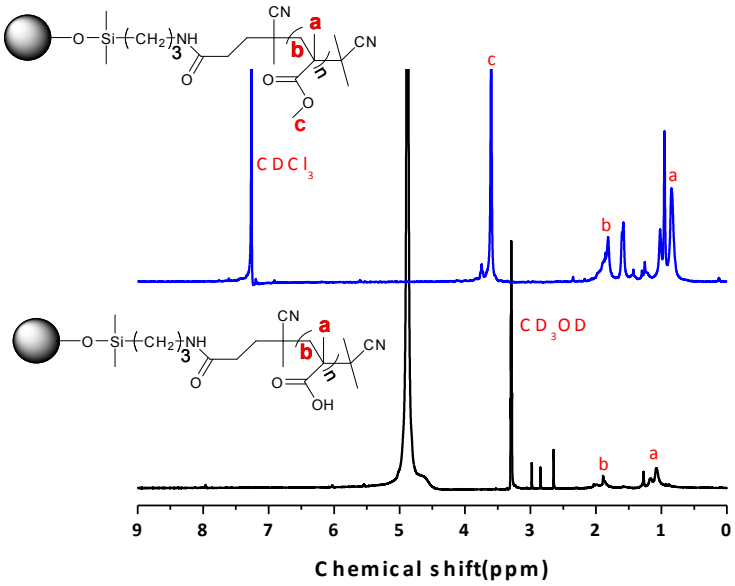


Figure 2. [1]H NMR of poly(carboxylic acid) grafted silica nanoparticles and their methylation by trimethylsilyldiazomethane. Reproduced with permission from Reference 19. Copyright (2013). American Chemical Society.

Trimethylsilyldiazomethane (TMSI) was used to methylate the acid groups on PMAA grafted particles for GPC analysis in an organic solvent. The poly(carboxylic acid) grafted silica nanoparticles with dye-labelling were yellow in DMSO and showed the strong fluorescence under UV radiation (Figure 3).[19] The as-synthesized nanoparticles had a average diameter of 30 nm as shown in the TEM image (Figure 4).[19]

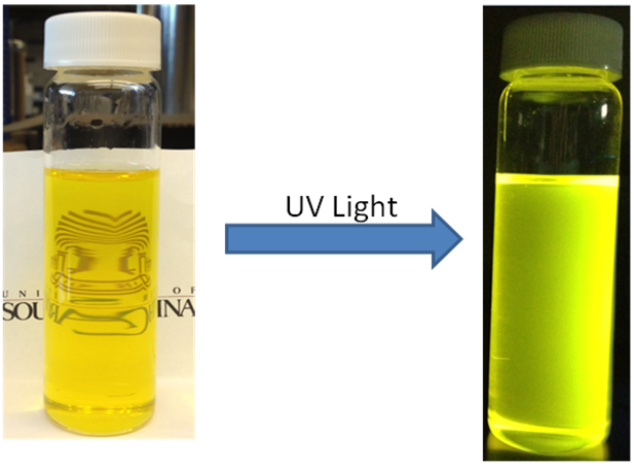


Figure 3. Images of dye-labeled poly(carboxylic acid) grafted silica nanoparticles under UV radiation in DMSO. Reproduced with permission from Reference 19. Copyright (2013). American Chemical Society.

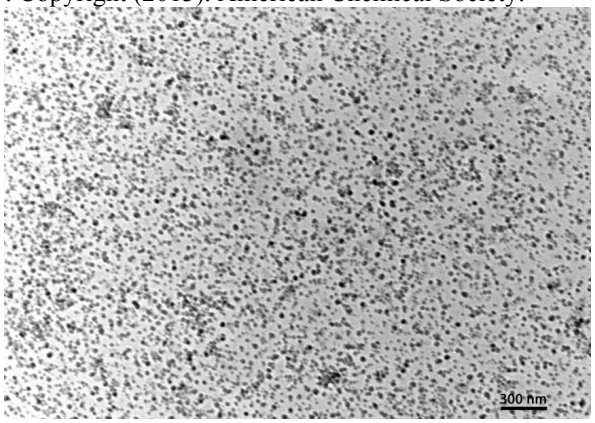
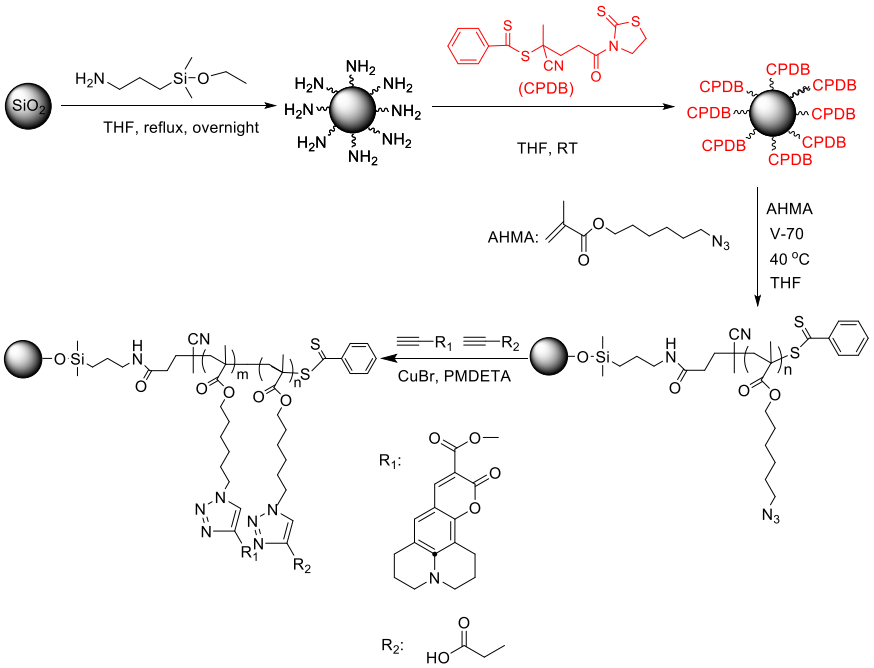


Figure 4. TEM of dye-labeled poly(carboxylic acid) grafted silica nanoparticles. Size bar = 300 nm. Reproduced with permission from Reference 19. Copyright (2013). American Chemical Society.

Alternate Strategy for Dye-labeled Poly(carboxylic acid) Grafted Nanoparticles

An alternate synthetic strategy was developed to prepare the dye-labeled poly(carboxylic acid) grafted nanoparticles, as shown in Scheme 3. Essentially, it was based on the "one-pot" click reactions between the PAHMA grafted silica nanoparticles and alkyne functionalized molecules (alkyne based coumarin 343 fluorescent dye and 4-pentynoic acid). Thus, fluorescent dye molecules and carboxylic acids were incorporated onto the PAHMA grafted nanoparticles sequentially. The PAHMA grafted silica nanoparticles were synthesized by surface-initiated RAFT polymerization of AHMA on CPDB coated silica nanoparticles, which were synthesized by the reaction between activated CPDB and amino-functionalized silica nanoparticles (Scheme 3). The graft density of CPDB coated nanoparticles can be determined by their Uv-vis absorption at 305 nm.



Scheme 3. Synthesis of dye-labeled poly(carboxylic acid) grafted silica nanoparticles.

The surface-initiated RAFT polymerization of AHMA was conducted with a ratio between reactants of ([AHMA]/[CPDB]/[V-70] = 500:1:0.1) at 40 °C in THF. A variety of PAHMA grafted silica nanoparticles with different graft densities and chain lengths were prepared, as shown in Table 1. The molecular weights of these surface attached PAHMA varied from 12,000 to 28,000 g/mol and the PDIs were generally lower than 1.2. The graft densities were 0.23 – 0.42

chains/nm^2. The IR spectra showed that the as-synthesized PAHMA grafted nanoparticles have a peak around 2100 cm^{-1} ascribed to the azide moiety and a peak around 1065 cm^{-1} ascribed to the silica. The loading of the dye molecules and the amount of carboxylic acids can be controlled by using PAHMA grafted nanoparticles with different graft densities and chain lengths, and the feed ratio between the dyes and 4-pentynoic acid.

Table 1 Surface-initiated RAFT polymerization of AHMA on particles

Entry	Mn, GPC (g/mol)	PDI	Graft Density (chains/nm^2)
1	27280	1.37	0.42
2	21310	1.17	0.33
3	28050	1.16	0.24
4	12350	1.12	0.23
5	27060	1.10	0.23

Note: For all the polymerizations, ([AHMA]/[CPDB]/[V-70] = 500:1:0.1) and the reaction temperature was 40 °C. The surface attached polymers were cleaved by hydrofluoric acid (HF) before GPC analysis.

The "click" reaction was conducted between the as-synthesized PAHMA grafted nanoparticles and alkyne functionalized molecules with a ratio of 1: 1.2 between $-N_3$ and the alkyne groups. The amount of alkyne functionalized coumarin 343 accounted for 1 mol % ~ 10 mol % of the alkyne moieties. The CuBr and PMDETA were 0.1 equivalent compared to $-N_3$. After the approximately 10 h reaction between $-N_3$ and alkyne functionalized coumarin 343, 4-pentynoic acid was added to the reaction solution. IR spectroscopy was used to monitor the progress of the "click" reaction. After 24 - 48 hours, the reaction was completed, which was confirmed by the disappearance of the azide peak around 2100 cm^{-1} as shown in Figure 5.

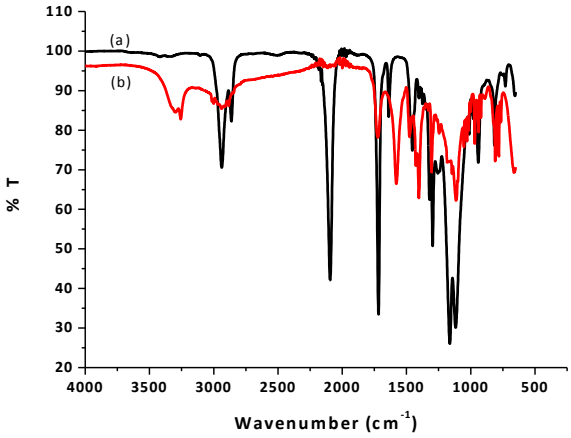


Figure 5. IR spectra of PAHMA grafted nanoparticles (a) before and (b) after "click" reaction.

We recently investigated the antimicrobial application of the poly(carboxylic acid) grafted nanoparticles when conjugated with antibiotics.[21] The nanoparticle-penicillin G (PenG) complex demonstrated much higher activities than free PenG on killing both gram positive and gram negative bacteria. The complexes showed significantly high activities over antibiotic-resistant bacteria such as methicillin-resistant *S. aureus* (MRSA), as shown in Figure 6.

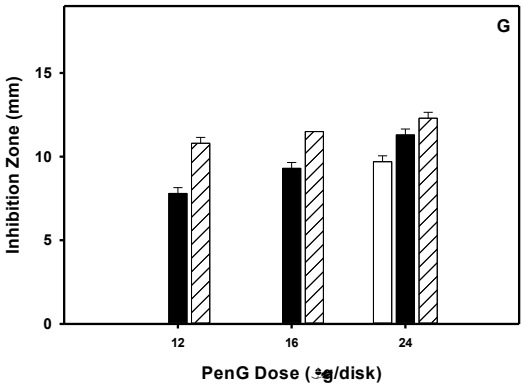


Figure 6. Disk-diffusion assays using community-associated MRSA (CA-MRSA): Antimicrobial activity of free Penicillin G (white), Penicillin G-complexed to the monolayer carboxylic acids coated silica nanoparticles (black), and PenG-complexed to poly(carboxylic acid) grafted silica nanoparticles

(hatched). The same doses of penicillin G were used in the different groups. Please note that the free Penicillin G was tested at all doses, but the inhibition zone at 12 and 16 μg/disk was ~ 0 mm, and thus does not display in the figure. Reproduced with permission from Reference 21. Copyright (2014). Royal Society of Chemistry.

We also have prepared the poly(carboxylic acid) brushes on SiO_2/Fe_3O_4 magnetic nanoparticles via the direct SI-RAFT of methacrylic acid.[22] As shown in Figure 7, the polymer grafted magnetic particles can be collected by a magnet and then redispersed in solution via sonication. The poly(carboxylic acid) grafted magnetic nanoparticles were used recyclably to kill bacteria which can prevent the nano-based pollution to biological environments.[22]

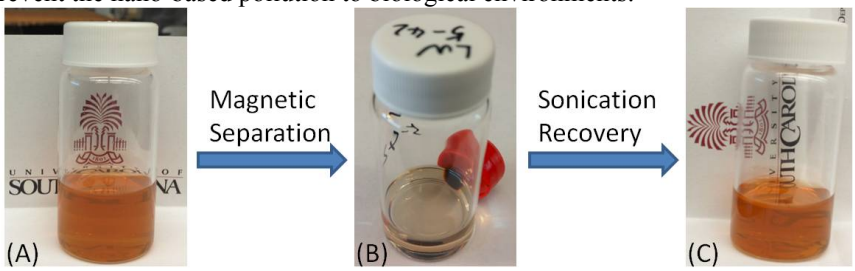


Figure 7. Poly(carboxylic acid) grafted SiO_2/Fe_3O_4 magnetic nanoparticles in dimethylformamide (DMF): (A) Normal state; (B) Under magnetic field; (C) Sonication-recovery and 14 days later. Reproduced with permission from Reference 22. Copyright (2015). Royal Society of Chemistry.

Cyclodextrin (CD) Grafted Nanoparticles

Polymer grafted silica nanoparticles containing β-CD side groups were prepared via the condensation reaction between the grafted poly(carboxylic acid) and the hydroxyl groups on β-CD. The carboxylic acid loading on dye-labeled poly(carboxylic acid) grafted silica nanoparticles can be controlled by tailoring the length of the surface grafted poly(carboxylic acid) brushes as well as the graft densities. The TGA data showed that the surface polymer supported chains with multiple β-CD accounted for 61.7% by weight for particles having a poly(carboxylic acid) brush density of 0.18 chains/nm^2 and molecular weight of 54,900 mol/g (Figure 8). The β-CD side chain based polymer grafted nanoparticles showed strong fluorescence under UV light even after multiple-step surface chemical modifications (Figure 9). The polycyclodextrin grafted nanoparticles can be used to trap the signal molecule AHLs in the bacterial quorum sensing (QS) process.

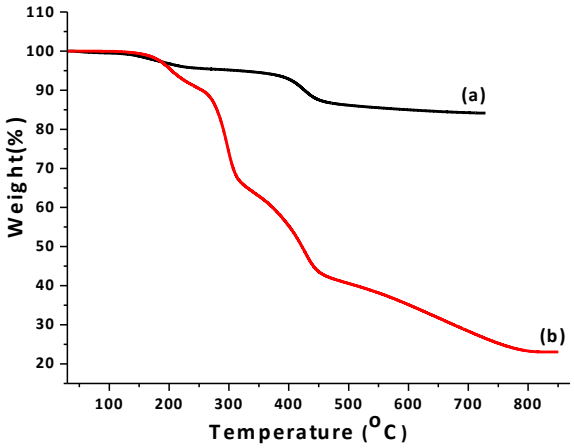


Figure 8. TGA of (a) dye-labeled poly(methacrylic acid) grafted silica nanoparticles; (b) dye-labeled poly(β-CD) grafted silica nanoparticles.

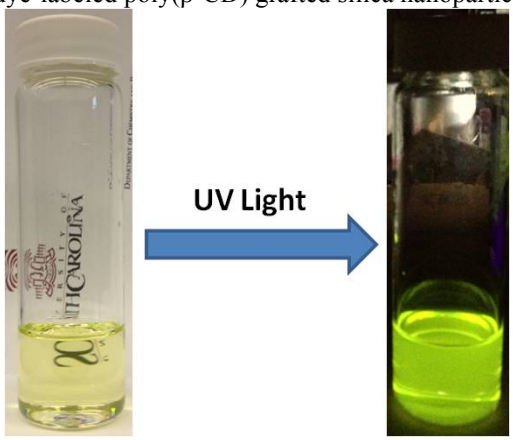


Figure 9. Photograph of dye-labeled poly(β-CD) grafted silica nanoparticles in DMSO.

Conclusions

Surface functionalization is critical in the preparation of polymer grafted nanoparticles. RAFT polymerization is a significant technique in the surface functionalization of nanoparticles with polymers. A RAFT agent was anchored on nanoparticles through surface silane chemistry and the graft densities were

controllably adjusted by the reaction conditions. A variety of poly(carboxylic acid) and poly(AHMA) grafted nanoparticles were prepared with different chain lengths and graft densities in a controlled manner, using several different synthetic strategies. Poly(carboxylic acid) grafted particles had excellent dispersion in water and are important platforms for further bio-molecule conjugation/attachment via covalent or non-covalent linkages. Poly(AHMA) grafted particles are also convenient platforms for postfunctionalization via the alkyne-azide click reaction. Cyclodextrin grafted nanoparticles were prepared based on the synthetic strategy of postfunctionalization. This strategy may find broad relevance in biomedical applications.

Acknowledgments

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References

1. Zou, H.; Wu, S.; Shen, J. *Chem. Rev*. **2008**, *108*, 3893-3957.
2. Pothayee, N.; Pothayee, N.; Jain, N.; Hu, N.; Balasubramaniam, S.; Johnson, L.; Davis, R.; Sriranganathan, N.; Riffle, J. *Chem. Mater.* **2012**, *24*, 2056-2063.
3. Qiao, Y.; Islam, M. S.; Wang, L.; Yan, Y.; Zhang, J.; Benicewicz, B. C.; Ploehn, H.; Tang. C. *Chem. Mater.* **2014**, 26, 5319-5326.
4. Li, Y.; Wang, L.; Natarajan, B.; Tao, P.; Benicewicz, B. C.; Ullal, C; Schadler, L. S. *RSC Adv.* **2015**, 5, 14788-14795.
5. Li, J.; Wang, L.; Benicewicz, B. C. *Langmuir* **2013**, *29*, 11547-11553.
6. Li, Y.; Krentz, T. M.; Wang, L.; Benicewicz, B. C.; Schadler, L. S. *ACS Appl. Mater. Interfaces*. **2014**, *6*, 6005-6021.
7. Tao, P. ;L., Y.; Siegel, R. W.; Schadler, L. S. *J. Mater. Chem. C*. **2013**, *1*, 86-94.
8. Li, Y. ;Tao, P.; Viswanath, A.; Benicewicz, B. C.; Schadler, L. S. *Langmuir*. **2013**, *29*, 1211-1220.
9. Tao, P. ;Li, Y.; Rungta, A.; Viswanath, A.; Gao, J.; Benicewicz, B.; Siegel, R.; Schadler, L. *J. Mater. Chem.* **2011**, *21*, 18623-18629.
10. Tao, P. ;Viswanath, A.; Schadler, L.; Benicewicz, B.; Siegel, R. *ACS Appl. Mater. Interfaces*. **2011**, *3*, 3638-3645.
11. Hawker, C. J.; Bosman, A. W.; Harth, E. *Chem. Rev.* **2001**, *101*, 3661-3688.
12. Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921-2990.
13. Barner-Kowollik, C. In *Handbook of RAFT Polymerization*; Barner-Kowollik, C., Ed.; Wiley-VCH: Weinheim: Germany, **2008**, p 1-4.

14. Schmitt, J.; Blanchard, N.; Poly, J. *Polym. Chem.* **2011**,*2*, 2231-2238.
15. Guinaudeau, A.; Mazières, S.; Wilsonb, D. J.; Destarac, M. *Polym. Chem.* **2012**, *3*, 81-84.
16. Fuqua C.; Parsek M. R.; Greenberg, E. P. *Annu Rev Genet* **2003**, *35*, 439-468.
17. Cash, B. M.; Wang, L.; Benicewicz, B. C. *J. Polym. Sci., Part A: Polym. Chem*. **2012**, *50*, 2533-2540.
18. Pothayee, N.; Pothayee, N.; Jain, N.; Hu, N.; Balasubramaniam, S.; Johnson, L.; Davis, R.; Sriranganathan, N.; Riffle, J. Chem. Mater. 2012, 24, 2056-2063.
19. Wang, L.; Benicewicz., B. *ACS Macro Lett.* **2013**, *2*, 173-176.
20. Mayer, T. G.;Weingart, R.;Münstermann, F.;Kawada, T.;Kurzchalia, T.;Schmidt R. R. *Eur. J. Org. Chem.* **1999**, *10*, 2563-2571.
21. Wang, L.; Chen, Y. P.; Miller, K. P.; Cash, B. M.; Jones, S.; Glenn, S.; Benicewicz, B. C.; Decho, A. W. *Chem. Commun.* **2014**, *50*, 12030-12033.
22. Wang, L.; Cole, M.; Li, J.; Zheng, Y.; Chen, Y. P.; Miller, K. P.; Decho, A. W.; Benicewicz, B. C. *Polym. Chem.* **2015**, *6*, 248-255.