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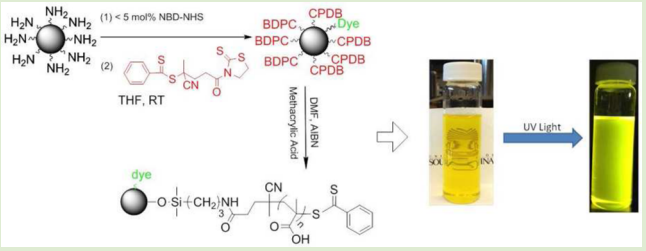
Synthesis and Characterization of Dye-Labeled Poly(methacrylic acid) Grafted Silica Nanoparticles

Lei Wang and Brian C. Benicewicz*

Department of Chemistry and Biochemistry and USC NanoCenter, University of South Carolina, Columbia, South Carolina 29208, United States

Supporting Information

ABSTRACT: The synthesis of dye-labeled poly(methacrylic acid) (PMAA) grafted silica nanoparticles was studied. Surface-initiated reversible addition−fragmentation chain transfer (RAFT) polymerization of *tert*-butylmethacrylate (*t*BuMA) was conducted on dye-labeled CPDB coated silica nanoparticles followed by sequential removal of the thiocarbonylthio end groups and the *tert*-butyl moieties. Additionally, as a more straightforward strategy, direct polymerization of methacrylic acid on silica nanoparticles with a diameter size as small as 15 nm was conducted via the RAFT polymerization technique. A variety of PMAA brushes with different lengths and densities were prepared on nanoparticle surfaces via surface-initiated RAFT polymerization with excellent control and surface grafting densities as high as 0.65 chains/nm^2. The grafted PMAA was methylated by trimethylsilyldiazomethane to conduct organic phase GPC characterization. The dye-labeled PMAA grafted nanoparticles provide a platform to bind biomolecules and to track the movement of the nanoparticles in biological systems.



Reversible addition−fragmentation chain transfer (RAFT) polymerization has been recognized as an important reversible addition radical polymerization (RDRP) technique to prepare polymers with controllable molecular weight and low polydispersities since its invention by Moad and co-workers in 1998.[1] RAFT polymerization has many advantages, such as being adaptable to almost all free radical polymerizable monomers, without participation of inorganic catalysts and mild operation conditions.

Polymer-grafted nanoparticles are of great interest because of their applications in chemosensors, coatings, and organic light-emitting devices (OLEDs).[2] The RAFT polymerization technique has emerged as a powerful tool to modify nanoparticle surfaces with functional polymers containing predetermined molecular weights due to the straightforward attachment chemistry and controllable surface graft density.

Poly(methacrylic acid) (PMAA) and other polymers made from acid-containing monomers represent an important class of stimuli-responsive polymers and have been widely used in membrane transport,[3] biomedical applications,[4] coatings,[5] and sensors.[6] There are few reports about the synthesis of PMAA or other multi-acid-containing polymers on nanoparticle surfaces. For example, Brittain et al.[7] synthesized poly(*tert*-butylacrylate) brushes on silica surface by atom transfer radical polymerization (ATRP), followed by pyrolysis at 200 °C, resulting in PAA-grafted silica substrates. Genzer et al.[8] prepared poly(*tert*-butylacrylate) grafted silicon wafer by ATRP, followed by acid hydrolysis of the polymer to form the immobilized PAA chains. Zhao et al.[9] sequentially prepared poly(*tert*-butylacrylate) brushes by ATRP and polystyrene brushes by nitroxide-mediated radical polymerization (NMRP) on the surface of silica nanoparticles. Subsequent deprotection of the *tert*-butyl moieties with trimethylsilane iodide (TMSI) led to environmentally responsive nanoparticle materials. To avoid the toxicity issue of residual copper from ATRP catalysts in bioapplications, Benicewicz et al.[10] prepared PMAA-grafted silica nanoparticles by surface-initiated RAFT polymerization of *tert*-butyl methacrylate, followed by deprotection of the *tert*-butyl groups by TMSI.

Very few groups have conducted direct surface-initiated RAFT polymerization of methacrylic acid or other acid containing monomers on nanoparticle surfaces. One particular challenge is maintaining good dispersibility of the polymer grafted nanoparticles using small size substrate nanoparticles. Generally, smaller size nanoparticles agglomerate more readily than larger particles. Thus, the size and nature of the substrate nanoparticles are important issues affecting the final dispersibility of polymer grafted nanoparticles. Charpentier et al.[11] used a RAFT agent with a carboxylic acid group to modify TiO_2 nanoparticles and conducted the surface-initiated polymerization of acrylic acid. Yusa et al.[12] synthesized poly(6-(acrylamide)hexanoic acid chains on 11 μm (diameter) size silica particles. The polymer-grafted particles flocculated at low pHs (pH = 3) and dispersed in water at high pHs (pH = 10). However, the large (11 μm diameter) particles are much easier

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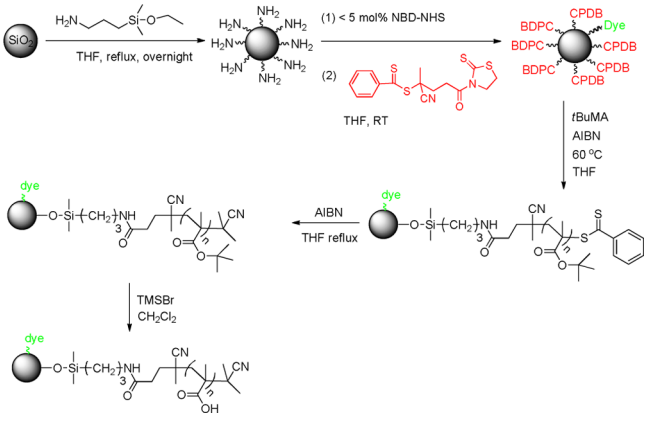
to disperse in solution. In this work, we report the direct polymerization of MAA on small diameter silica nanoparticles (as small as 15 nm) in a controlled manner via surface-initiated RAFT polymerization, which was compared to another procedure for attaching MAA chains to nanoparticles. Cleavage of the methylated chains from the nanoparticle surface enabled accurate measurement of the molecular weights via organic phase GPC and a precise determination of the polymerization kinetics.

Fluorescent nanoparticles have been applied in bioimaging and nanomedicine fields.[13,14] Silica nanoparticles possess a series of properties, such as biocompatibility, controllable particle size, easy fabrication, and powerful surface functionalization chemistry toolbox.[15] Fluorescent silica nanoparticles provide universal imaging probes with other functionalities by powerful surface multifunctionalization with a variety of biomolecules and polymers.[16,17] Additionally, labeling the nanoparticle surface with fluorescent dyes is helpful in monitoring the presence and movement of particles in biological cells or other systems. Nanoparticles with anchored polymer chains containing carboxylic acid moieties have been reported to be useful for fighting bacterial infections and as drug delivery vehicles in the biomedical field.[10,18] Based on the great potential bioapplications, we were motivated to prepare dye-labeled PMAA grafted silica nanoparticles as a powerful platform for such applications.

Initially, the dye-labeled RAFT agent coated silica nanoparticles were synthesized by allowing the amino coated nanoparticles with precisely determined densities to react with a small amount, less than 5 mol % relative to the amines, of activated nitrobenzofurazan derivative, followed by an excess of activated CPDB. This method generated a universal platform for surface-initiated RAFT polymerization of nanoparticles labeled with fluorescent dyes for biomedical tracking. The amount of dye covalently bound to the nanoparticle surface (2.33 μmol/g, 0.01 agents/nm^2) was determined quantitatively by comparing the absorbance for the dye modified particles to a standard UV−vis absorption curve prepared from known amounts of free dye (Supporting Information, Figure S1). The grafted CPDB had a surface density of 54.14 μmol/g (0.19 agents/nm^2) according to the determination of UV−vis absorption at 305 nm.

The initial strategy for preparation of dye-labeled PMAA grafted nanoparticles was demonstrated according to Scheme 1.

Scheme 1. Initial Strategy for Preparation of Dye-Labeled PMAA Grafted Silica Nanoparticles



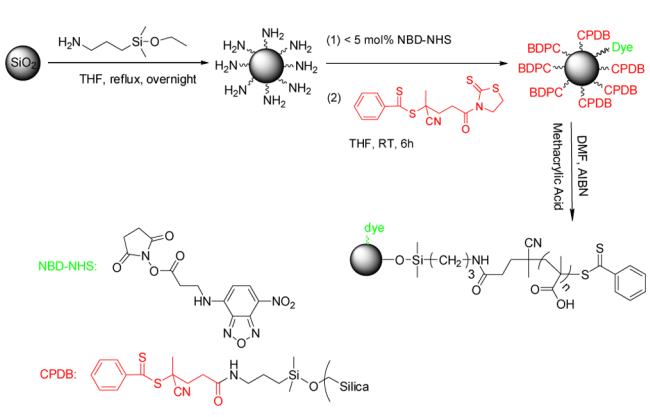
The surface-initiated RAFT polymerization of tBuMA was conducted employing a ratio between species of [tBuMA]/[CPDB]/[AIBN] = 1000:1:0.1 at 60 °C in an appropriate amount of THF. The thermogravimetric analysis (TGA) showed that the grafted poly(*tert*-butylmethacrylate) accounted for 20% by weight (Supporting Information, Figure S2), which was consistent with the UV analysis. After treatment with excess AIBN, the UV−vis spectrum confirmed that the thiocarbonylthio moiety was removed by the absence of the absorption peak at 300 nm (Supporting Information, Figure S3).

Initially, TMSI was used to deprotect the *tert*-butyl groups. However, the fluorescence of the nanoparticles was destroyed after the treatment with TMSI. It was speculated that the attached dye molecules were decomposed during the process. To exclude the possibility of the unstable NBD dye structure, two other fluorescent dyes, an amino coumarin and fluorescein derivative, with completely different molecular structures (Supporting Information, Figure S4) were used to investigate the TMSI process in simple solution experiments. Both of them failed to show fluorescence after the treatment. Thus, the reason may be that the side product iodine attacked these fluorescent dyes with highly conjugated structures which resulted in the fluorescence loss. To test this hypothesis, trimethylsilylbromide (TMSBr) was used as alternative under the same conditions. It was found that the fluorescence of the nanoparticles were maintained after the TMSBr treatment. Thus, TMSBr was used to replace TMSI in the deprotection of the *tert*-butyl groups.

The FTIR analysis of the nanoparticles (Supporting Information, Figure S5) confirmed the absence of the strong absorption peak at ∼2900 cm^{-1} ascribed to the *tert*-butyl moiety after TMSBr treatment. In addition, the presence of a broad peak at ∼3400 cm^{-1} ascribed to the hydroxyl group in −COOH and the shift of the carbonyl stretch peak to 1700 cm^{-1} demonstrated the generation of grafted PMAA chains. The ^{1}H NMR (Supporting Information, Figure S6) reveals the successful formation of the PMAA grafted nanoparticles after the TMSBr treatment. The disappearance of the peak at 1.4 ppm confirmed the complete removal of the *tert*-butyl group.

We recently found that surface-initiated RAFT polymerization of MAA directly on dye-labeled silica nanoparticles can be well controlled in DMF at 60 °C with a ratio between species of [MAA]/[CPDB]/[AIBN] = 1000:1:0.1 (Scheme 2).

Scheme 2. Synthetic Scheme for the Preparation of Dye-Labeled PMAA Grafted Silica Nanoparticles via Direct Polymerization of MAA



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A small amount of 1,3,5-trioxane was added to the solution to monitor the monomer conversion by ^{1}H NMR (Supporting Information, Figure S7). The spectra clearly showed an increase of proton peaks at 1.1 ppm and 1.9 ppm over time assigned to the anchored polymer backbone. The IR analysis of the nanoparticles (Supporting Information, Figure S8) confirmed the presence of the strong absorption peak at ∼2900 cm^{-1} ascribed to the methyl moiety after methylation. In addition, the disappearance of a broad peak at 3500−2500 cm^{-1} ascribed to the hydroxyl group in −COOH and the shift of the carbonyl stretch peak from 1700 to 1725 cm^{-1} demonstrated the methylation of the anchored PMAA chains. The ^{1}H NMR spectra (Supporting Information, Figure S9) shows the PMAA grafted silica nanoparticle before and after methylation by trimethylsilyldiazomethane. The appearance of the peak at ∼3.6 ppm ascribed to the new methyl group further confirms the successful methylation.The TGA demonstrated that the grafted PMAA accounted for 75% by weight (Supporting Information, Figure S10), which was consistent with the UV analysis. The dye-labeled PMAA grafted silica nanoparticle solution was yellow and transparent in dimethylsulfoxide (DMSO; Figure 1). Under UV light with 365 nm wavelength, the nanoparticles

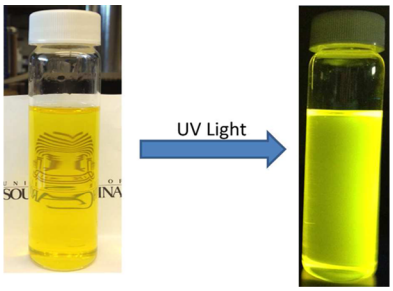

Figure 1. Photograph of dye-labeled PMAA grafted silica nanoparticles in DMSO.

showed very strong fluorescence. The TEM image (Figure 2) illustrates the dye-labeled PMAA grafted silica nanoparticles were well dispersed and shows that the diameter of the individual nanoparticles was around 30 nm.

The kinetic study of surface-initiated RAFT polymerization of MAA on nanoparticles (coated CPDB density: 0.19 agents/nm^2) is shown in Figure 3. A linear relationship between

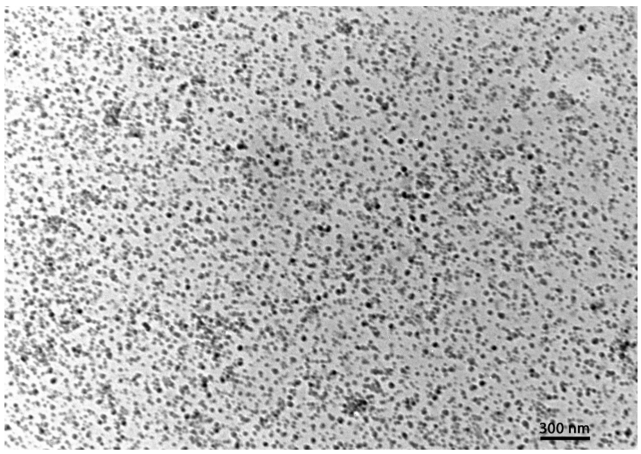

Figure 2. TEM of dye-labeled PMAA grafted silica nanoparticles; size bar = 300 nm.

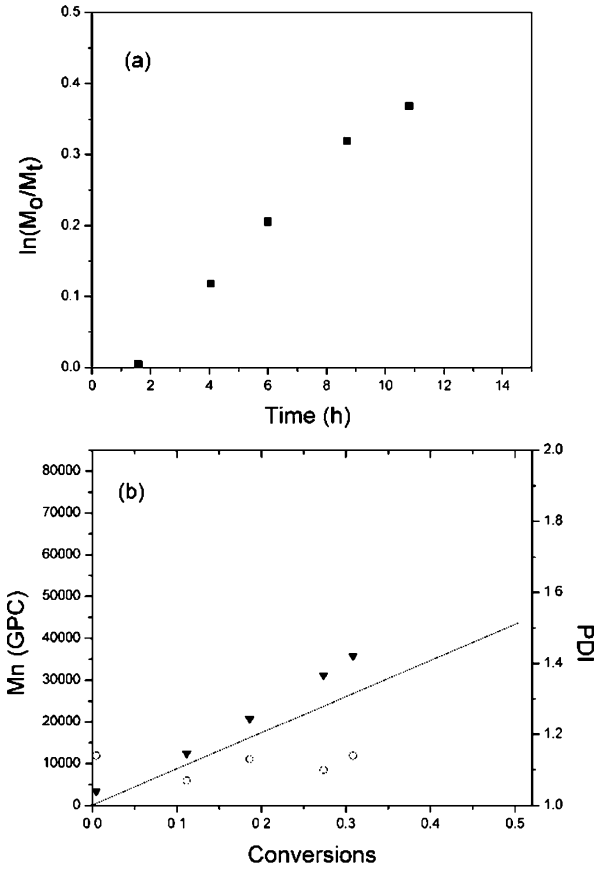

Figure 3. (a) Kinetic plot and (b) dependence of the GPC molecular weight (inverted triangle), theoretical molecular weight (solid line), and polydispersity (unfilled circles) on the conversion for the surface-initiated RAFT polymerization of methacrylic acid ([MAA]/[CPDB]/[AIBN] = 1000:1:0.1) with CPDB anchored silica nanoparticles (RAFT surface density: 54.14 μmol/g) at 60 °C.

$\ln(M_0/M_t)$ (where M_0 is the initial monomer concentration and M_t is the monomer concentration at time t) and polymerization time was observed, which implies a constant radical concentration. Additionally, the Mn determined by GPC of the methylated PMAA chains (calibrated with PMMA standards) increased linearly with monomer conversion and agreed closely with the theoretical molecular weight. The PDIs were approximately 1.1 during the polymerization. These features demonstrated the living/controlled nature of the RAFT polymerization of MAA mediated by CPDB surface anchored nanoparticles. We have previously demonstrated that we can achieve a variety of surface grafting densities of 0.01−0.68 chains/nm^2 using similar surface chemistry to anchor RAFT agents.[19,20] Thus, the surface-initiated RAFT polymerization of MAA was conducted on silica nanoparticles with a high surface density of 0.65 RAFT agents/nm^2, measured by UV−vis spectroscopy.[21] The molecular weight (M_n) of the attached PMAA chains was 41077 g/mol and the PDI was 1.11. Thus, a variety of PMAA brushes with controllable lengths and densities can be synthesized on silica nanoparticles using the direct surface-initiated polymerization approach and with grafting densities as high as 0.65 chains/nm^2.

Compared to our initial synthetic strategy, the direct polymerization of MAA on dye-labeled CPDB coated silica nanoparticles is more straightforward. It also prevents the loss of nanoparticles that occurs in the washing processes after each

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step in the reaction scheme. These concerns are critical for small diameter nanoparticles which are particularly prone to agglomeration. These challenges are largely addressed by the direct polymerization approach described herein. The kinetic study demonstrated the living/controlled nature of the RAFT polymerization of MAA on small diameter nanoparticle surfaces. The dye-labeled PMAA grafted silica nanoparticles generated in this process dispersed well in DMF and DMSO and were stable in these solvents for more than six months.

In conclusion, we demonstrated two methods for the synthesis of dye-labeled PMAA grafted silica nanoparticles. Dye-labeled CPDB coated silica nanoparticles were prepared by treating amino functionalized nanoparticles with activated dyes and followed by activated CPDB. Then surface-initiated RAFT polymerization of *t*BuMA was conducted followed by sequential removal of thiocarbonylthio end groups and *tert*-butyl groups to generate dye-labeled PMAA grafted silica nanoparticles. The second method of direct surface-initiated RAFT polymerization of MAA on small size (15 nm) nanoparticles is more straightforward. A variety of PMAA brushes with different lengths and densities were prepared on nanoparticles with excellent control and surface grafting densities as high as 0.65 chains/nm^2. The synthesis of the dye-labeled PMAA grafted silica nanoparticle was confirmed by FTIR, TGA, ^{1}H NMR analysis, and TEM. The dye-labeled PMAA grafted silica nanoparticles provide a platform to bind biomolecules and to monitor the presence and movement of the nanoparticles for bioapplications.

■ ASSOCIATED CONTENT

Supporting Information

Detailed information about the nanoparticle and polymer grafted nanoparticles. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: benice@sc.edu.

Notes

The authors declare no competing financial interest.

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