Journal of Materials Chemistry B

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/materialsB

Journal of Materials Chemistry B RSCPublishing

COMMUNICATION

A Versatile Stable Platform for Multifunctional Applications: Synthesis of NitroDOPA-PEO-Alkyne Scaffolding for Iron Oxide Nanoparticles

R.C. Stone,[a] B. Qi,[a] D. Trebatoski,[b] R. Jetti,[a] Y.P. Bandera,[a] S.H. Foulger,[a] O.T. Mefford[a]

Cite this: DOI: 10.1039/x0xx00000x

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Contemporary magnetic nanoparticle composites are individually designed for a specific biomedical applications We describe the syntheses and characterization of a heterobifunctional polyethylene oxide (PEO) using nitroDOPA as a robust anchoring group on one end and an alkyne as the reactive surface for additional application specific modification.

Magnetic nanoparticles are an ideal material for bioimaging,[1] therapy, and delivery.[2] These blossoming technologies are not possible without designing and synthesizing specialized materials to stabilize the magnetic nanoparticles in biological environments. For the stabilizing layer, the material must be biologically inert and sufficient steric hindrance to provide a long circulation time in the body.[3] To increase efficiency of materials used in biological imaging, therapy and delivery, the surface must be enhanced with biologically targeting and labeling molecules to both increase the concentration of the particles in the desired location of the body and monitoring the particles' fate.[4]
When designing polymer-particle systems for biomedical applications, it is very important to have a robust anchor to ensure particle stability in protein rich environments. Some traditional anchor groups studied for iron oxide nanoparticles are carboxylic acids,[5] alcohols,[6] amines,[7] silanes,[8] ammonium,[9] and phosphates.[10] Another molecule being heavily researched as a robust anchor for iron oxide nanoparticles are catechols, which were inspired from a protein adhesive featured in mussels.[11] Catechol-derived ligands have been found to displace other binding groups on the surface of iron oxide[12] as well as create stable particles in physiological conditions.[13,14] The catechol groups used for anchoring to iron oxide are generally found in dopamine-derived molecules that can be attached to polymers through the terminal amine using a common crosslinking chemistry that utilizes N-hydroxysuccinimide (NHS) esters to activate carboxylic acid for reactions with amines.[15]

In this letter, we present a heterobifunctional polyethylene oxide (PEO) ligand for the stabilization and functionalization of magnetic nanoparticles. Utilizing ordinary chemistry, we have developed a synthetic route that produces a heterobifunctional PEO ligand with a robust anchoring group and a reactive surface. PEO is ideal for theranostic systems, not only because of how inert the backbone is to protein absorption[16], but this relatively unreactive polymer chain allows for modification of the endgroups with relative ease.[17]
The synthetic design (Figure 1) begins with the anionic ring opening polymerization of ethylene oxide to synthesized a 6000 g mol^{-1} PEO using 2-(tetrahydro-2H-pyran-2-yloxy)ethanol (THP) as the alkoxide initiator (compound 1), based on previous work by Hiki et al.[18] This synthetic approach is ideal for creating narrow molecular weights and is more efficient than partial derivation of diols.[17] Beginning with one protected endgroup allows for precise and efficient modification for desired heterobifunctionality. With one of the endgroups protected, the free alcohol was modified with propargyl bromide via a substitution reaction to produce the alkyne functionality needed (compound 2).[19] After cleaving the tetrahydropyranol protecting group with an acid (compound 3), an alcohol was free to be modified. Through a series of reactions, which included modification with succinic anhydride (SA)[17,20] (compound 4) and the N-hydroxysuccinimide (NHS)[19,21] (compound 5), the polymer was primed to be modified through the amine of NitroDOPA (compound 6).[22,23] NitroDOPA was synthesized using a procedure done by Yang et al[24] and used as our anchoring system based on a study by Amsted et al that compared the stability of a variety of catechol complexes in which they found NitroDOPA having one of the higher binding affinities.[25] Using NMR and FT-IR, we confirmed the nitration of the DOPA by the symmetric and asymmetric stretching peaks at 1330 and 1532 cm^{-1}, respectively.

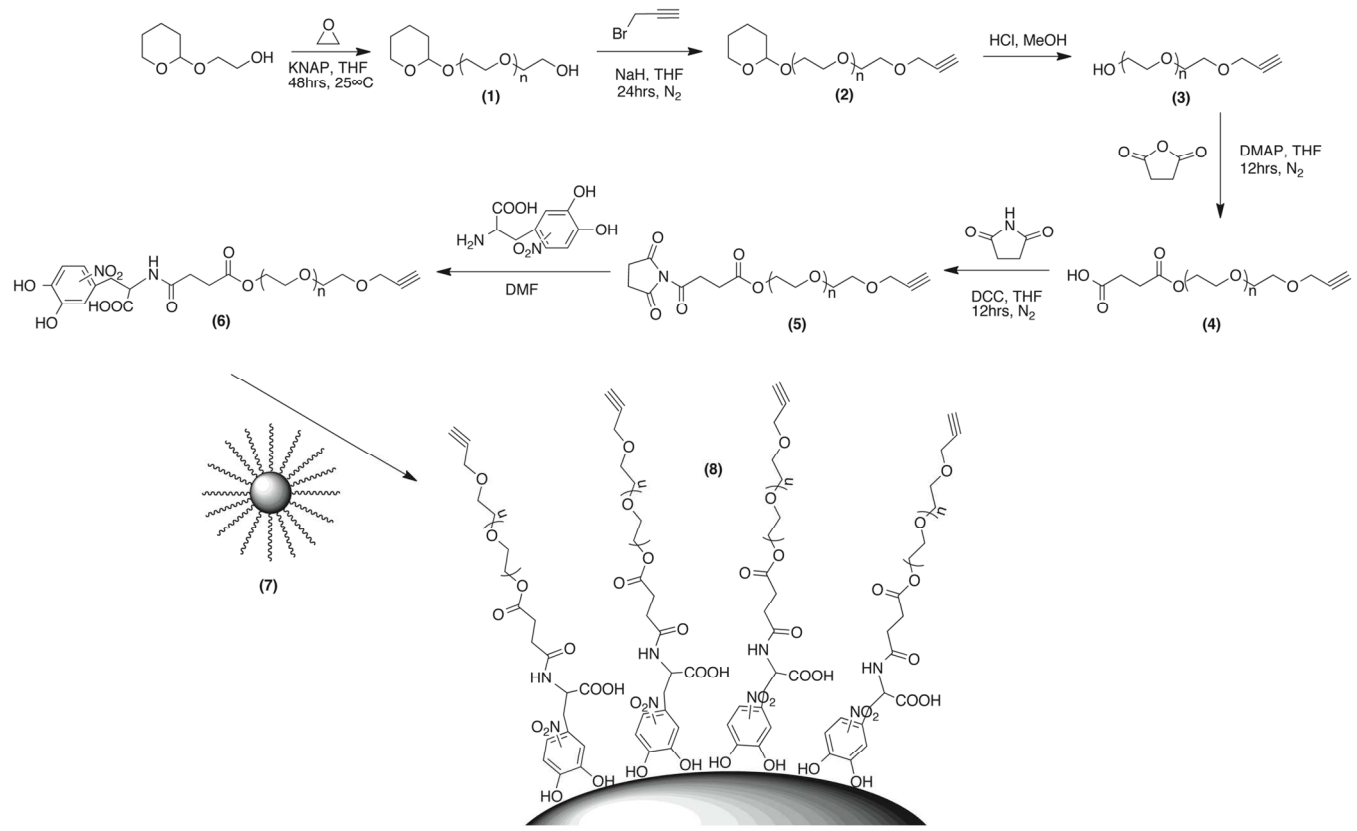


Figure 1. Synthesis of Heterobifuncational PEO with alkyne on one end and NitroDOPA on the other

Monodispersed iron oxide nanoparticles (compound 7) in hexane, were synthesized using a method from Sun et al.[26] The particles core size was found to be 7.2nm ± 0.9nm in diameter by TEM (Figure S1) and the hydrodynamic diameter was found to be 29.2 ± 17.1nm (Table S1). The particles were then modified via ligand exchange using compound 6. Sonication and stirring overnight aided in the replacement of the oleylamine, a hydrophobic ligand, with our polymer ligand in chloroform. The particles were then precipitated with an anti-solvent and finally dispersed into DI water. This aqueous stability was a great indicator that the particles were successfully modified with PEO (compound 8).

When creating nanoparticles for biological applications, analyzing the particle-environment interaction is important.[15,27] Characterizing these particles in ionic and protein rich solutions is a great technique for simulating stability in biological environments. Using dynamic light scattering (DLS), modification of the aforementioned 7.2 nm oleylamine **(7)** coated nanoparticles with the water soluble PEO **(6)** was confirmed. The final hydrodynamic diameter was an average of 77 nm in water, as shown in Figure 1. For drug delivery systems, this size will be able to circulate within the body without getting filtered out by the reticulo-endothelial system (RES).[28] Although we have confirmed their stability in water, this is irrelevant in the complex environment of the human body. The particles were further characterized with thermogravometric analysis (TGA) (Figure S2) to find the surface loading of polymer on the surface of the particle, which was found to be 95% weight loss of polymer. This information was then used in theoretical calculations, based on a density distribution model (described in the ESI), to determine the hydrodynamic diameter to be 56 nm. This indicates that there is a small degree of agglomeration in the system.

Materials used in biological applications can be limited in their effectiveness due to absorption plasma proteins that can inhibit the circulation time in the body.[29] Proteins can attach to the particles and provoke agglomeration rendering the particles useless. To observe how this particles system will behave in a simulated biological environment, phosphate buffer solution (PBS), fetal bovine serum (FBS), and bovine serum albumin (BSA) were added, 10% by volume (0.1 ml), to a dilute solution of the particles (0.9 ml with a concentration of 0.1mg/ml of particles in DI water) and the hydrodynamic diameter was measured.[30] As seen in Figure 2, there was no significant change in hydrodynamic diameter observed when the particles were put in ionic and protein rich environments.

An increase in temperature that is relevant in the body will effect the interactions between the polymer and water molecules, increasing the volume of the PEG in water.[31] This change in size can impact the delivery and cell uptake of these particles if they become too large. Polymer modified magnetic nanoparticles in PBS were then submitted to a biologically relevant temperature of 37°C and compared to the hydrodynamic diameter of particles in DI water and PBS solution, Figure S4. Fortunately, there was no significant change in size observed in the diameter of the nanoparticles after 24 hours and at biologically relevant temperatures.

A long circulation time of these particles is very important for the efficiency of the delivery of the nanoparticles to the infected area. If the size were to increase significantly above approximately 100nm,[28] the RES will recognize the particles as a threat and subsequently remove them. To simulate extreme conditions, the particles were dispersed in a PBS solution and exposed to 70°C for 24 hours. The results in Figure S5 show an increase in the Z-average diameter of about 14nm. This slight increase in hydrodynamic diameter of the polymer modified magnetic nanoparticles keeps them within the acceptable size range for efficient drug delivery.

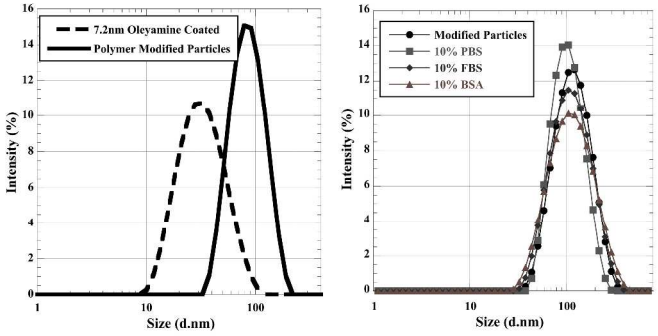


Figure 2. (Left) Hydrodynamic diameter by intensity of magnetic nanoparticles before and after modification with Alkyne-PEO-NitroDOPA(6); size data chart available (Table S1). (Right) Hydrodynamic diameter by intensity of the particles titrated with PBS, FBS, and BSA.

Now that this polymer-particle complex has been successfully synthesized and characterized in simulated biological environments, we now have evidence of a biologically stable scaffolding that we can build upon for biomedical targeting and imaging applications. Fluorescein-azide was then "clicked" in using a Huisgen 1,3-dipolar cycloaddition between the alkyne of the particle surface and the azide of the fluorescent dye molecule, using copper (II) sulfate and sodium ascorbate to drive the reaction.[32] These particles were dialyzed for 3 days in the absence of light to ensure removal of excess dye, catalyst and byproducts. The photoluminescence spectrum (Figure 3) shows a maximum emission at 517nm for the particles modified with fluorescein and no visible emission for the particles before modification. Using solutions of known concentrations of fluorescein azide, the amount of dye on the surface of the nanoparticles was determined to be 1.3 fluorescein molecules per nm^2.

The design and synthesis of this specialty heterobifunctional PEO provides a great platform for biomedical magnetic nanoparticle research. This uncomplicated synthesis of such a specialized material lends itself to the production of biologically stable nanoparticles with an alkyne surface that provides a customizable scaffold for a variety of biomedical applications.

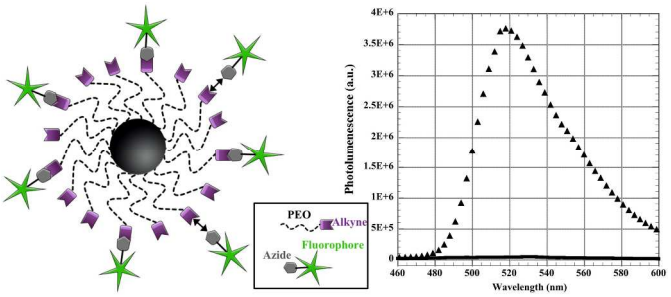


Figure 3. Image on the left represents the final construction of magnetic nanoparticle with functional surface modified with a fluorophore. Image on the right shows a photoluminescence spectrum of particles before and after functionalization with fluorescein.

Synthesis of a unique heterobifunctional PEO was accomplished by ring opening polymerization of EO using a protected initiator and sub-sequential endgroup modifications with relative ease. This polymer is equipped with robust anchoring to magnetite via a catechol, biological stability via PEO, and a functional surface via an alkyne. Confirmed by DLS, the once water-insoluble magnetite nanoparticles, were stabilized in water. These functionalized particles are now capable of carrying a variety targeting and imaging agents, through 'click' chemistry, which is represented in the drawing in Figure 3. The accessibility of the surface functionality was demonstrated by adding an azido-functionalized fluorescein that was detected using photoluminescence, at a wavelength of 517 nm.

To demonstrate that the newly formed complex is magnetic, the particles were subjected to vibrating sample magnetometry (VSM). In Figure S7, when observing the magnetization cure, the particles that were measured exhibit superparamagnetic-like tendencies with a saturation magnetization of about 60 emu g^{-1}. Thus establishing the potential of these complexes to be used in magnetic hyperthermia applications.

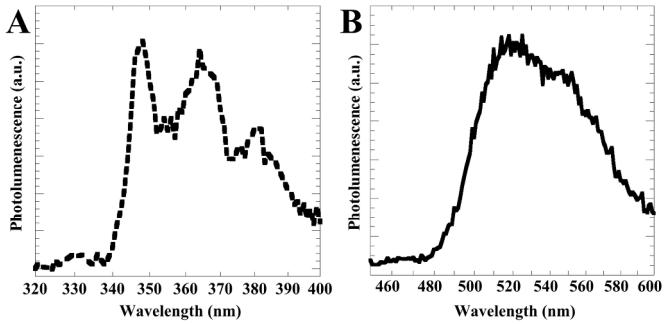


Figure 4. Photoluminescence spectra of magnetic nanoparticles modified with two dyes simultaneously. A) The excitation of carbazole was observed at 348 and 363 nm and B) the excitation of the fluorescein dye was observed at 517 nm.

To demonstrate the ability of these particles to carry multiple functional moieties simultaneously, the particles were modified with two dyes that emit at different wavelengths. Carbazole and fluorescein was added to the surface using a copper (I) catalyzed reaction between the alkyne on the surface of the particle and the azide of the dyes in the same solution. After modification of the particles, they were submitted to dialysis for three days to remove any of the dye not attached and copper (I) catalyst. The particles were

then characterized with photoluminescence, Figure 4, by first exciting at 305 nm and observing an excitation peaks at 348 and 363 nm corresponding to the carbazole dye. They were then excited at 445 nm and a photoluminescent response was observed at 517 nm. Using solutions of known concentrations of fluorescein azide and carbazole azide, the amount of dye on the surface of the nanoparticles was determined to be 1.0 fluorescein molecules per nm^2 and 0.5 carbazole molecules per nm^2.

Conclusions

The work here describes the synthesis of a water-disperable iron oxide nanoparticles that was accomplished by using a simple, but powerful design that can be customized for biomedical imaging, delivery, and/or therapy. This was accomplished by modifying magnetic nanoparticles with PEO that has multiple robust anchors and an alkyne functionality that can be linked with functional moieties that have an azide using 'click' chemistry. The particles' stability was demonstrated by subjecting them to biological conditions and observing no significant difference in hydrodynamic diameter. The particles were then modified with fluorescent dyes, fluorescein and carbazole, to show the flexibility of the surface to be customized for a myriad of theranostic applications.

Acknowledgements

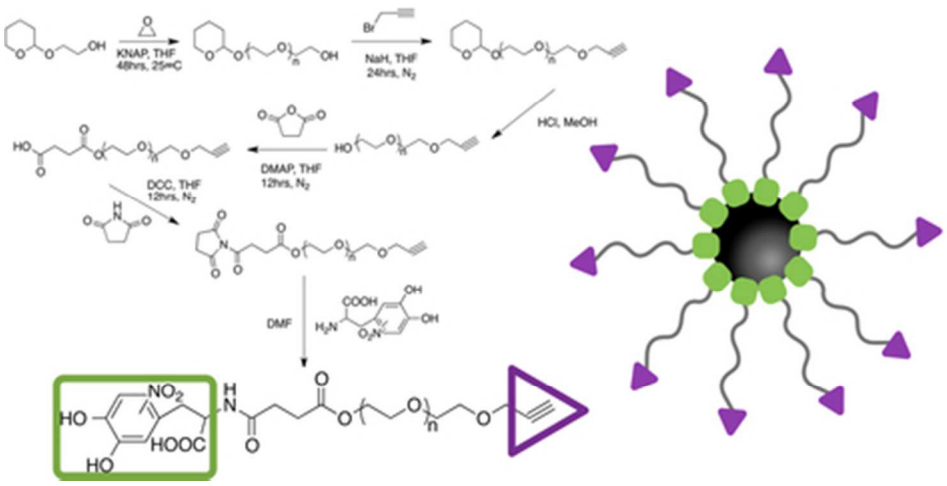
Special thanks to for funding from the National Science Foundation under grant numbers: CMMI-1057633, CMMI-1130819, and ECC1062873. Special thanks to Dr. Thomas Crawford at the University of South Carolina for assistance in acquiring the VSM data.

Notes and references

[a] Clemson University, Materials Science and Engineering, Center for Optical Materials Science and Engineering Technologies, 91 Technology Drive, Anderson, SC 29625, USA.
[b] University of Wisconsin-Madison, Materials Science and Engineering, 1509 University Avenue, Madison WI 53706, USA.

Electronic Supplementary Information (ESI) available: Materials and methods used in the synthesis and characterization of the polymer and particles described in this manuscript See DOI: 10.1039/c000000x/

1. S. K. Yen, D. Janczewski, J. L. Lakshmi, and S. B. Dolmanan, *ACS Nano*, 2013.
2. R. Stone, T. Willi, Y. Rosen, O. T. Mefford, and F. Alexis, *Therapeutic Delivery*, 2011, **2**, 815–838.
3. N. Kohler, G. Fryxell, and M. Zhang, *J. Am. Chem. Soc*, 2004, **126**, 7206–7211.
4. F. Benyettou, I. Chebbi, L. Motte, and O. Seksek, *J. Mater. Chem.*, 2011, **21**, 4813–4820.
5. M. Vadala, M. Thompson, M. Ashworth, Y. Lin, T. Vadala, R. Ragheb, and J. Riffle, *Biomacromolecules*, 2008, **9**, 1035–1043.
6. H. Lin, Y. Watanabe, M. Kimura, K. Hanabusa, and H. Shirai, *Journal of Applied Polymer Science*, 2003, **87**, 1239–1247.
7. M. Aslam, E. Schultz, T. Sun, T. Meade, and V. Dravid, *Crystal Growth & Design*, 2007, **7**, 471–475.
8. M. Ma, Y. Zhang, W. Yu, H. Y. Shen, H. Zhang, and N. Gu, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2003, **212**, 219–226.
9. W. C. Miles, J. D. Goff, P. P. Huffstetler, C. M. Reinholz, N. Pothayee, B. L. Caba, J. S. Boyd, R. M. Davis, and J. Riffle, *Langmuir*, 2008, **25**, 803–813.
10. Y. Sahoo, H. Pizem, T. Fried, D. Golodnitsky, L. Burstein, C. Sukenik, and G. Markovich, *Langmuir*, 2001, **17**, 7907–7911.
11. H. Lee, N. Scherer, and P. Messersmith, *Proceedings of the National Academy of Sciences*, 2006, **103**, 12999.
12. W. Chirdon, W. O'Brien, and R. Robertson, *J. Biomed. Mater. Res.*, 2003, **66B**, 532–538.
13. E. Amstad, S. Zurcher, A. Mashaghi, J. Y. Wong, M. Textor, and E. Reimhult, *Small*, 2009, **5**, 1334–1342.
14. E. Amstad, M. Textor, and E. Reimhult, *Nanoscale*, 2011.
15. S. L. Saville, R. Stone, B. Qi, and O. T. Mefford, *J. Mater. Chem.*, 2012.
16. Y. Zhang, N. Kohler, and M. Zhang, *Biomaterials*, 2002, **23**, 1553–1561.
17. M. Thompson, T. Vadala, M. Vadala, Y. Lin, and J. Riffle, *Polymer*, 2007, **49**, 345–373.
18. S. Hiki and K. Kataoka, *Bioconjugate Chemistry*, 2010, **21**, 248–254.
19. Z. Li and Y. Chau, *Bioconjugate Chemistry*, 2009, **20**, 780–789.
20. T. ISHII, M. YAMADA, and T. HIRASE, *Polymer Journal*, 2005.
21. J. Aswathy, S. Jahnavi, R. Krishna, K. Manzoor, S. Nair, and D. Menon, *J. Nanosci. Nanotech.*, 2011, **11**, 7611–7620.
22. M. Das, D. Bandyopadhyay, D. Mishra, S. Datir, P. Dhak, S. Jain, T. K. Maiti, A. Basak, and P. Pramanik, *Bioconjugate Chemistry*, 2011, **22**, 1181–1193.
23. L. Qu, L. Gu, H. Li, S. Taylor, T. Elkin, P. G. Luo, T.-R. J. Tzeng, X. Jiang, R. A. Latour, F. Stutzenberger, A. Williams, and Y.-P. Sun, *J. Nanosci. Nanotech.*, 2005, **1**, 61–67.
24. X. Yang, H. Hong, J. J. Grailer, I. J. Rowland, A. Javadi, S. A. Hurley, Y. Xiao, Y. Yang, Y. Zhang, R. J. Nickles, W. Cai, D. A. Steeber, and S. Gong, *Biomaterials*, 2011, **32**, 4151–4160.
25. E. Amstad, T. Gillich, I. Bilecka, M. Textor, and E. Reimhult, *Nano Letters*, 2009, **9**, 4042–4048.
26. S. Sun, H. Zeng, D. Robinson, S. Raoux, P. Rice, S. Wang, and G. Li, *J. Am. Chem. Soc*, 2004, **126**, 273–279.
27. J. Goff, P. Huffstetler, W. Miles, N. Pothayee, C. Reinholz, S. Ball, R. Davis, and J. Riffle, *Chemistry of Materials*, 2009, **21**, 4784–4795.
28. A. J. Giustini, R. Ivkov, and P. J. Hoopes, *Nanotechnology*, 2011, **22**, 345101.
29. M. Amiji and K. Park, *Journal of Biomaterials Science, Polymer Edition*, 1993, **4**, 217–234.
30. M. Pavlin and V. B. Bregar, *Dig J Nanomater Bios*, 2012, **7**, 1389–1400.
31. J. S. Pedersen and C. Sommer, *Progress in Colloidal Polymer Science*, 2005, **130**, 70–78.
32. M. A. Daniele, M. L. Shaughnessy, R. Roeder, A. Childress, Y. P. Bandera, and S. Foulger, *ACS Nano*, 2012, **7**, 203–213.



39x19mm (300 x 300 DPI)