# Synthesis and functionalization of monodisperse poly(ethylene glycol) hydrogel microspheres within polyelectrolyte multilayer microcapsules†

**Huiguang Zhu*[a] and Michael J. McShane[ab]**

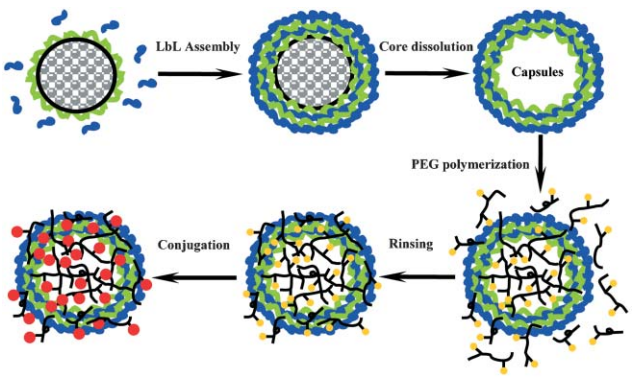
*Received (in Cambridge, UK) 20th September 2005, Accepted 21st October 2005*
*First published as an Advance Article on the web 17th November 2005*
**DOI: 10.1039/b513292d**

**Polyelectrolyte multilayer microcapsules were used as templates to prepare monodisperse poly(ethylene glycol) (PEG) hydrogel microspheres, which can react with amine-bearing molecules.**

Poly(ethylene glycol) (PEG) is a widely used and accepted biomaterial.[1] It is highly soluble in water and soluble in most organic solvents. PEG is nontoxic, and has a rapid clearance from the body. The most important properties of PEG are that it can resist not only cell and protein adhesion but also recognition from the immune system, which allows its use in applications as scaffolds for tissue engineering, as drug delivery carriers, in the prevention of thrombosis, in postoperative tissue repair, in molecular imprinting, and as coatings for biosensors.[2,3] Covalent bonding of PEG to other molecules may enhance the properties of other molecules rendering them nonimmunogenic, water-soluble, and protein-rejecting.[4] These PEGylated molecules not only exhibit many of the properties of PEG, but also retain their biological activity.[5] Because of the high mobility of PEG, molecules that are tethered to it exhibit activity similar to that of a freely soluble molecule. The proteins that are tethered to PEG are not denatured, and because their total size is increased, their rate of clearance through the body is often increased.[6] For practical applications, PEG was usually crosslinked and made into hydrogels because hydrogels are useful in biomedical and pharmaceutical applications.[7] The crosslinking or gelation of PEG can be performed under mild conditions like photopolymerization or enzyme crosslinking, which allows the hydrogel to be generated *in vitro* or *in vivo* from a low viscosity solution of monomer, oligomer, or macromer in a minimally invasive manner.[8] Chemical crosslinking results in hydrogels that possess high water content yet exhibit mechanical properties similar to those of soft tissues. Another advantage of hydrogels is their high permeability for oxygen, nutrients, and other water-soluble metabolites, making them particularly attractive as tissue engineering scaffolds. Bio-active molecules can be immobilized on the surface of the polymer gel or incorporated into the network. The focus of this research was to fabricate monodisperse PEG hydrogel microspheres based on polyelectrolyte multilayer microcapsule templates, which could be potentially used for applications like implantable biosensors, specific drug delivery/targeting, and contrast agents for imaging.

A brief procedure for the synthesis and functionalization of PEG microspheres within polyelectrolyte microcapsules is shown in Scheme 1. Monodisperse $MnCO_3$ microparticles ($\sim$5 μm) were synthesized as described in our previous work,[9] and were coated with eight bilayers of sodium poly(styrene sulfonate)/poly(allylamine hydrochloride) ({PSS/PAH}). The $MnCO_3$ cores were then removed by HCl–EDTA treatment. 25 μL of PEG acrylate monomer (acrl-PEG, Aldrich), 25 μL of photoinitiator (Irgacure 184, 20 mg $mL^{-1}$ in DMF, obtained from Ciba), and 20 μL of the microcapsules were mixed with 430 μL of DI water in a microcentrifuge tube. The mixture was exposed to UV light for 10 min with stirring. The resulting highly viscous product was rinsed with DI water, and then centrifuged three times. For functionalization of PEG microspheres, 20 mg of acrl-PEG-NHS (Nektar), with 10 μL acrl-PEG were used in the photopolymerization.

Since polyelectrolyte multilayer walls contain tiny pores of nanoscale dimension, they allow penetration of molecules with a certain molecular weight. Changing of assembly conditions or post-treatment of the multilayer films, *e.g.* crosslinking, could possibly change the pore size in multilayer films. As a result, the molecular weight of molecules that can pass through the film will change accordingly. This important property of polyelectrolyte multilayer films has been widely studied for the purpose of diffusion control.[10] It is easy for monomers to penetrate into microcapsules, while it is difficult for polymer to diffuse out.[11] The polymerized PEG monomer forms a hydrogel inside




**Scheme 1** Polymerization of PEG monomers in polyelectrolyte microcapsules and PEG-NHS conjugation to amine-bearing molecules.

[a]*Institute for Micromanufacturing, Louisiana Tech University, 911 Hergot Avenue, Ruston, Louisiana, 71272, USA. E-mail: hzhu@latech.edu; Fax: +1-318-2575104; Tel: +1-318-2575127*
[b]*Biomedical Engineering Program, Louisiana Tech University, 911 Hergot Avenue, Ruston, Louisiana, 71272, USA. E-mail: mcshane@latech.edu; Fax: +1-318-2575104; Tel: +1-318-2575100*

† Electronic supplementary information (ESI) available: details of experimental and FTIR, osmotic pressure test results. See DOI: 10.1039/b513292d

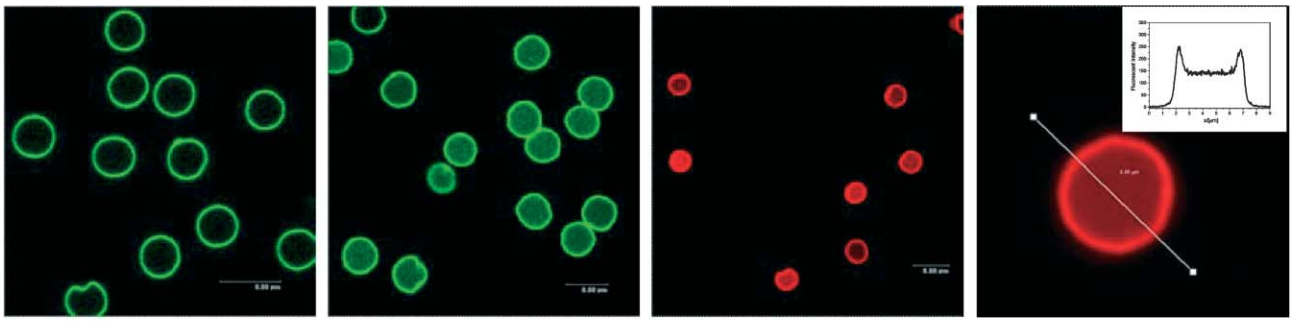



**Fig. 1** Confocal microscopy images of (a) $\{PSS/FITC\text{-}PAH\}_8$ microcapsules, (b) PEG hydrogel microspheres, PEG-NHS conjugated to fluoresceinamine, (c) and (d) PEG hydrogel microspheres, PEG-NHS conjugated to RITC-dextran amine. Inset: intensity line scan of single microsphere.

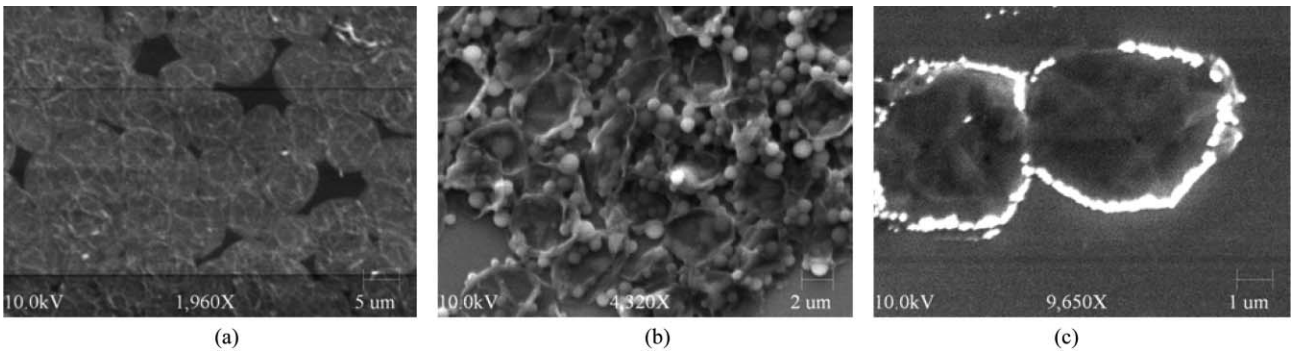



**Fig. 2** SEM images of air-dried (a) $\{PSS/PAH\}_8$ microcapsules, (b) PEG microspheres with $\{PSS/PAH\}_8$ microcapsules, (c) PEG hydrogel microspheres, PEG-NHS conjugated to dextran amine.

microcapsules, and their functionalized end groups could easily react with other amine-bearing molecules.

FTIR analysis was performed to determine the existence of PEG in $\{PAH/PSS\}_8$ microcapsules. The most significant peak in the IR spectrum ($\sim$1100 $cm^{-1}$), attributed to ether bonds, verified the formation of PEG in microcapsules (figure shown in ESI†). Both PEG microspheres and empty microcapsules were placed in 0.25 M NaCl solution to perform osmotic pressure experiments. Under confocal microscopy, it was observed that the empty microcapsule collapsed immediately upon addition of the salt solution, while most of the PEG microspheres remained spherical because of the existence of the hydrogel matrix (figure shown in ESI†).

Confocal microscopy was used to image the $\{PSS/PAH\}_8$ microcapsules and the PEG microspheres functionalized with fluoresceinamine and rhodamine isothiocyanate (RITC, Aldrich)-labeled dextran amine (Fig. 1). The microcapsules fabricated from $MnCO_3$ cores remain spherical and highly monodisperse when wet (Fig. 1a), and are therefore useful as templates to produce monodisperse PEG microspheres. Acrl-PEG-NHS monomer was used for the functionalization of PEG microspheres, where the NHS ester group could easily hydrolyze and react with amine-bearing molecules either before polymerization (fluoresceinamine, Fig. 1b) or after polymerization and rinsing (RITC-dextran amine), Fig. 1c, d). The line-scan of fluorescence intensity in Fig. 1d indicates that the dextran amine was uniformly distributed in the PEG microspheres, though it showed higher concentration in the polyelectrolyte multilayer walls. Most importantly, the polymerized and functionalized PEG microspheres remain monodisperse.

The microcapsules and microspheres were air-dried and investigated with SEM. As shown in Fig. 2a, the $\{PSS/PAH\}_8$ multilayer microcapsules collapsed to thin films when dried. As in our previous work,[9] it was shown that the $MnCO_3$ was completely removed and not detectable by elemental analysis using energy dispersive X-ray spectroscopy after treatment of HCl–EDTA, which is an attractive feature since clean microcapsules are crucial for bio-related applications. It is interesting to note that the polymerized PEG hydrogel apparently comes out of the microcapsules and forms tiny microspheres after the air drying process (Fig. 2b). This may indicate that the molecular weight cut off of the capsule wall is not low enough to hold the PEG hydrogel in the microcapsules. As a result, control of polymerization for the balance between solubility and molecular weight is crucial to this technique. An alternative may be further crosslinking of the hydrogel after polymerization and rinsing. The chemical conjugation of amine-containing material to PEG will increase the crosslinking degree and stability of PEG microspheres, which is verified by SEM investigation. As shown in Fig. 2c, the dextran amine-functionalized PEG was restricted within the multilayer microcapsules. As a result, the functionalized PEG microspheres show great potential for applications such as biosensors and drug delivery/targeting.

In conclusion, monodisperse PEG hydrogel microspheres were synthesized within polyelectrolyte multilayer microcapsules. The PEG microspheres could be functionalized to react with

amine-bearing molecules. The PEG microspheres prepared in this efficient manner should prove to be promising candidates for biomedical and other applications.

This work was supported by the National Institutes of Health (R01 EB00739-01) and the National Science Foundation (NIRT 0210298).

## Notes and references


1 (*a*) J. M. Harris, in *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*, ed. J. M. Harris, Plenum Press, New York, 1992, pp. 1–14; (*b*) K. B. Keys, F. M. Andreopoulos and N. A. Peppas, *Macromolecules*, 1998, **31**, 8149; (*c*) J. A. Hubbell, *J. Controlled Release*, 1996, **39**, 305; (*d*) N. A. Peppas, K. B. Keys, M. Torres-Lugo and A. M. Lowman, *J. Controlled Release*, 1999, **62**, 81.

2 (*a*) J. H. Jeong, S. W. Kim and T. G. Park, *Bioconjugate Chem.*, 2003, **14**, 2, 473; (*b*) Y. Hong, P. Krsko and M. Libera, *Langmuir*, 2004, **20**, 25, 11123; (*c*) K. Itaka, N. Kanayama, N. Nishiyama, W.-D. Jang, Y. Yamasaki, K. Nakamura, H. Kawaguchi and K. Kataoka, *J. Am. Chem. Soc.*, 2004, **126**, 42, 13612; (*d*) D.-A. Wang, C. G. Williams, F. Yang, N. Cher, H. Lee and J. H. Elisseeff, *Tissue Eng.*, 2005, **11**, 1–2, 201; (*e*) R. J. Russell and M. V. Pishko, *Anal. Chem.*, 1999, **71**, 3126.

3 (*a*) K. M. Gattas-Asfura, Y. Zheng, M. Micic, M. J. Snedaker, X. Ji, G. Sui, J. Orbulescu, F. M. Andreopoulos, S. M. Pham, C. Wang and R. M. Leblanc, *J. Phys. Chem. B*, 2003, **107**, 10464; (*b*) A. Revzin, R. J. Russell, V. K. Yadavalli, W.-G. Koh, C. Deister, D. D. Hile, M. B. Mellott and M. V. Pishko, *Langmuir*, 2001, **17**, 5440; (*c*) X. Zhao and J. M. Harris, *J. Pharm. Sci.*, 1998, **87**, 11, 1450; (*d*) K. L. Moffat and K. G. Marra, *J. Biomed. Mater. Res.*, 2004, **71B**, 181; (*e*) W. K. Lee, T. Ichi, T. Ooya, T. Yamamoto, M. Katoh and N. Yui, *J. Biomed. Mater. Res.*, 2003, **67A**, 1087.

4 N. A. Peppas and R. Langer, *Science*, 1994, **263**, 1715.

5 S. Halstenberg, A. Panitch, S. Rizzi, H. Hall and J. A. Hubbell, *Biomacromolecules*, 2002, **3**, 710.

6 K. P. Antonsen and A. S. Hoffman, in *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*, ed. J. M. Harris, Plenum Press, New York, 1992, pp. 15–27.

7 (*a*) Y. Zheng, M. Micic, S. V. Mello, M. Mabrouki, F. M. Andreopoulos, V. Konka, S. M. Pham and R. M. Leblanc, *Macromolecules*, 2002, **35**, 13, 5228; (*b*) Y. Zheng, K. M. Gattas-Asfura, C. Li, F. M. Andreopoulos, S. M. Pham and R. M. Leblanc, *J. Phys. Chem. B*, 2003, **107**, 2, 483; (*c*) J. B. Leach, K. A. Bivens, C. N. Collins and C. E. Schmidt, *J. Biomed. Mater. Res.*, 2004, **70A**, 74; (*d*) J. B. Leacha and C. E. Schmidt, *Biomaterials*, 2005, **26**, 125; (*e*) B. P. Lee, J. L. Dalsin and P. B. Messersmith, *Biomacromolecules*, 2002, **3**, 1038; (*f*) J. J. Sperinde and L. G. Griffith, *Macromolecules*, 2000, **33**, 5476.

8 (*a*) Y. Zheng, F. M. Andreopoulos, M. Micic, Q. Huo, S. M. Pham and R. M. Leblanc, *Adv. Funct. Mater.*, 2001, **11**, 1, 37; (*b*) J. J. Sperinde and L. G. Griffith, *Macromolecules*, 1997, **30**, 5255.

9 H. Zhu, E. W. Stein, Z. Lu, Y. M. Lvov and M. J. McShane, *Chem. Mater.*, 2005, **17**, 9, 2323.

10 A. A. Antipov and G. B. Sukhorukov, *Adv. Colloid Interface Sci.*, 2004, **111**, 49.

11 L. Dähne, S. Leporatti, E. Donath and H. Möhwald, *J. Am. Chem. Soc.*, 2001, **123**, 5431.