Stabilization and Functionalization of Polymer Multilayers and Capsules via Thiol−Ene Click Chemistry

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Polymer-based materials have played a significant role in the design and development of drug delivery systems. Advances in this area impose challenges on the development of new polymer materials with functional and responsive moieties in the polymer chains. Several macromolecular-based delivery systems have been reported, including dendrimers, polymeric micelles, star polymers, and polymer capsules.[1] The layer-by-layer (LbL) assembly technique,[2] the basis of which is the sequential assembly of interacting species (e.g., polymers), has been widely utilized for the preparation of polymer capsules.[3] This method affords capsules with well-defined size, composition, and surface chemistry, as well as tailored physicochemical properties. To prepare polymer capsules that are robust in a range of conditions (e.g., pH, temperature, and salt), covalent stabilization[3c,4] is often employed. Click chemistry, which describes a number of simple, modular, and highly specific chemical reactions featuring high yields under mild conditions, represents a versatile approach to introduce covalent bonds in various materials.[5] Recently, we demonstrated that click chemistry based on the Huisgen 1,3-dipolar cycloaddition of azides and alkynes[5] affords single-component polymer multilayered thin films and capsules.[6] However, this process requires copper to catalyze the reaction, which may be undesirable in certain biological applications. In materials design for biomedical applications, functionalization strategies[7] are also important, as this allows tuning of the polymer capsule properties for areas such as targeted drug delivery. Herein, we report a generalized, dual stabilization and functionalization, metal catalyst-free approach, to prepare stable LbL multilayers and capsules by light initiated thiol−ene click chemistry.

Thiol−ene based chemistry offers significant advantages for the stabilization and functionalization of polymer capsules. This process is photoinitiated, the reaction can be performed at room temperature in the presence of oxygen, and the process is environmentally benign.[8] Importantly, this process does not require any potentially toxic metal catalyst. Furthermore, the vast array of commercially available molecules and biomolecules with either thiol− or ene− functionality makes this a powerful technique to introduce functionality. This chemistry is versatile and has been used for a range of applications, including polymer functionalization,[9] dendrimer synthesis,[10] nanoimprinting,[11] and patterning.[12]
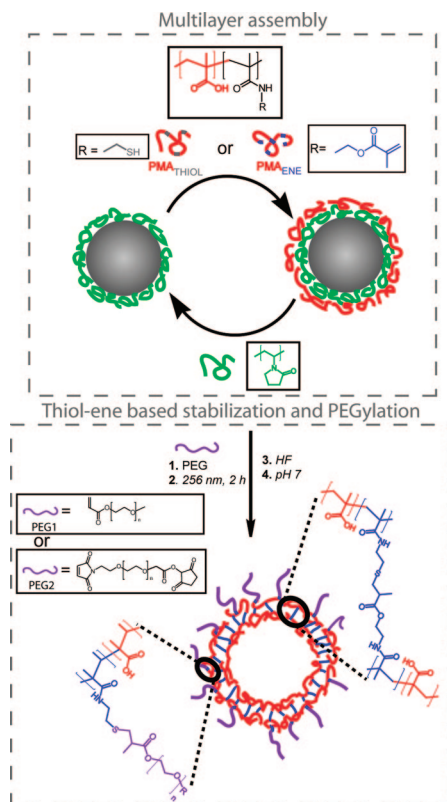
In this communication, we report the LbL assembly of hydrogen bonded multilayers by alternately depositing poly-(methacrylic acid) containing either thiol groups (PMA_{THIOL}) or ene functionality (PMA_{ENE}), with poly(vinylpyrrolidone) (PVP), at pH 4, on silica particles. The nine-layer PVP/PMA-coated particles were subjected to UV light, thus initiating the thiol−ene reaction to induce cross-linking of the PMA layers. We also demonstrate that, upon selective removal of the core and exposure of the particles to pH 7, thiol−ene PMA ($PMA_{T−E}$) capsules are obtained. We further show that the multilayers can be reacted with ene-end functional

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[a] Step (1), PEGylation, is optional in the preparation of the capsules.

Figure 1. Fluorescence intensity of 3 μm-diameter SiO$_2$ particles coated with (PVP/PMA$_{THIOL}$/PVP/PMA$_{ENE}$) multilayers as a function of layer number, as measured by flow cytometry. PVP was labeled with AF488. Measurements were taken after each PVP layer was deposited.

Figure 2. Microscopy images of 3 μm-diameter silica particles coated with (PVP/PMA$_{THIOL}$/PVP/PMA$_{ENE}$)$_2$/PVP and resulting PMA$_{T-E}$ capsules. (a) Phase contrast image of UV-irradiated coated particles at pH 4 and the corresponding fluorescence microscopy image (inset). (b) Fluorescence microscopy image of UV-irradiated PMA$_{T-E}$ capsules at pH 7. (c) Transmission electron and (d) scanning electron microscopy images of PMA$_{T-E}$ capsules at pH 7. PMA was labeled with AF488.

poly(ethylene glycol) (PEG) to create reactive and low fouling polymer multilayer-coated particles.

PMA (15 kDa) was functionalized with thiol or ene moieties using carbodiimide coupling protocols (see Supporting Information). The PMA$_{THIOL}$ contained 12 mol % thiol groups,[4f] and the PMA$_{ENE}$ synthesized contained 13 mol % ene functionality. These polymers were assembled at pH 4 with PVP (Scheme 1).

LbL assembly was performed by alternately exposing the substrates (silica particles or gold electrodes) to solutions of PVP and the functionalized PMA polymers. The interdigitation of polymer multilayers[2b] facilitates reaction between the thiol− and ene− moieties. The growth of the multilayers on the silica particles was monitored by flow cytometry (FC) using fluorescently labeled PVP[13] (Figure 1). The multilayer buildup on the particles was linear, which is accordance with the trend observed for buildup on quartz crystal microbalance electrodes (see Supporting Information).

After multilayer assembly (nine layers), the coated particles were exposed to UV (256 nm) light for 2 h. Under these conditions the thiol self-initiates, forming a radical. The advantages of this approach are that no photoinitiator is required and that the reaction is not sensitive to oxygen. Once the radical is formed, a radical addition reaction can occur
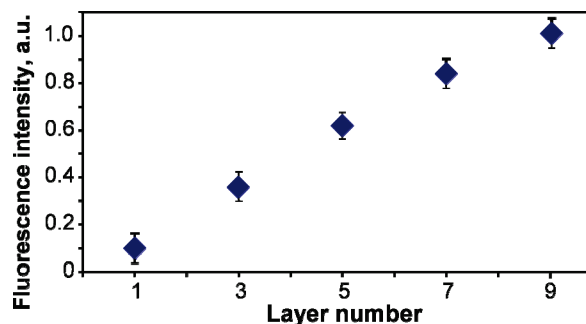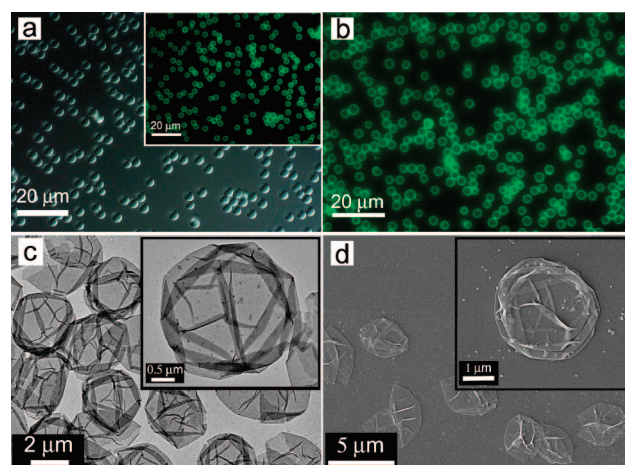
across the double bond of the methacrylate moiety on the PMA$_{ENE}$, stabilizing the multilayer coating. The stability of the multilayers was examined by immersion of the particles into a pH 7 solution, where the methacrylic acid units deprotonate and the H-bonding is disrupted, causing disassembly of the multilayers.[14] Fluorescence microscopy confirmed the formation of PVP/PMA multilayers on the particles after exposure to UV light (Figure 2a, inset), showing uniform ring fluorescence. After hydrogen fluoride etching (to remove the silica template) and incubation at pH 7, PMA$_{T-E}$ capsules were obtained (Figure 2b). The PVP used in the formation of the multilayers is expelled at pH 7 because of the disruption of H-bonding interactions between PMA and PVP.[14] A control experiment was performed whereby no UV irradiation was performed on the coated particles (i.e., no thiol−ene reaction): upon changing the pH to 7, only background fluorescence was recorded, which is attributed to free PMA in solution as a result of disassembly of the capsules. Electron microscopy analysis of the thiol−ene stabilized capsules showed intact polymer capsules with characteristic folding of the capsule walls upon drying[3]

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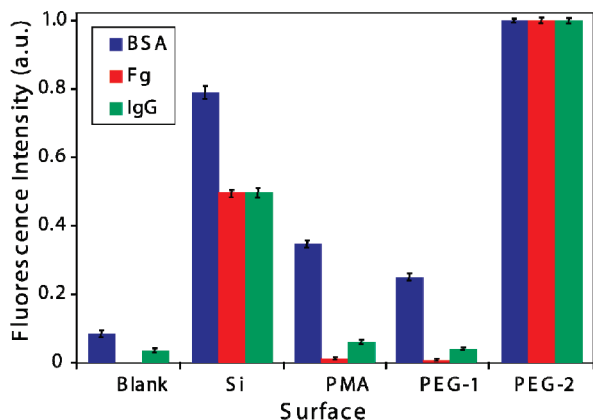(14) Zelikin, A. N.; Li, Q.; Caruso, F. *Chem. Mater.* **2008**, *20*, 2655–2661.

Figure 3. Fluorescence intensity of PVP/PMA-coated particles (of 3 μm-diameter) after incubation with fluorescently labeled proteins, as measured by FC. Blank-background fluorescence of the silica particles before incubation; Si-bare silica particles; and PMA-UV-treated (PVP/PMA$_{THIOL}$/PVP/PMA$_{ENE}$)$_2$-coated particles. PEG-1 and PEG-2: UV-treated particles coated with (PVP/PMA$_{THIOL}$/PVP/PMA$_{ENE}$)$_2$ exposed to the monofunctional PEG-acrylate and maleimide−PEG−succinimidyl ester, respectively.

(Figure 2c,d). PEGylated PMA$_{T-E}$ capsules (data not shown) were similar in morphology to the non-PEGylated capsules.

An advantage of thiol−ene chemistry is the suite of thiol or ene functional molecules available for functionalization of materials. To demonstrate this, we functionalized the outer corona of the PVP/PMA-coated particles with PEG. Two different PEG molecules were employed: a monofunctional PEG−acrylate (PEG 1, $M_w = 526$) and a bifunctional maleimide−PEG−succinimidyl ester (PEG 2, $M_w = 5000$) (Scheme 1). These PEG molecules have one end that can undergo thiol−ene reaction. The PEG-functionalized PMA/PVP-coated particles were incubated with fluorescently labeled proteins (BSA, IgG, Fg) and the fluorescence of the particles was quantified by FC (Figure 3). The results show that the amine-reactive particles (PEG-2) have the highest fluorescence intensity, indicating that the succinimidyl ester end groups react with the amine moieties of the protein.

The other PEGylated particles (PEG-1) exhibit the lowest fluorescence intensity, significantly lower than the bare silica and the PMA-coated particles. These results demonstrate the versatility of this technique, creating two types of PEGylated particles; one with enhanced protein resistance (PEG-1) and the other protein reactive (PEG-2).

In summary, we have presented a robust and general strategy based on thiol−ene click chemistry for the stabilization and functionalization of polymer multilayer-coated particles and capsules. By reacting functionalized PEG through thiol−ene chemistry, the multilayer-coated particles were functionalized, creating protein-resistant or protein (amine)-reactive particles. The versatility and generality of the approach reported is expected to find widespread use in the generation of new particulate delivery systems and microreactors.