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Multilayered polymer capsules with switchable permeability

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ABSTRACT

The preparation of layer-by-layer (LbL) polymer capsules stabilized by a combination of copper-catalyzed azide-alkyne cycloaddition (CuAAC, "click chemistry") and disulfide cross-linking is reported. The capsules obtained possess gated permeability due to the reversible nature of the disulfide cross-linking. Poly(methacrylic acid) (PMA) was modified with different ratios of alkyne and protected thiol functionalities ($PMA_{Alk,SH}$). Exploiting hydrogen bonding interactions, multilayered films were prepared by the alternate deposition of $PMA_{Alk,SH}$ (hydrogen bonding donor) and poly(*N*-vinylpyrrolidone) (PVPON; hydrogen bonding acceptor), yielding stable $PMA_{Alk,SH}$ capsules. Capsule pH responsive behavior and permeability, using a series of different size and labeled dextrans, were investigated in both the "closed" state (disulfide cross-linking intact) and "open" state (free thiol groups). To demonstrate the potential of these gateable systems, post-loading studies with fluorescent 45-base pair (bp) linear double-stranded (ds) DNA were performed. Fluorescence studies revealed the potential of these capsules to reversibly encapsulate cargo: cleavage and reformation of the disulfide groups resulted in reversible permeability to the DNA.

1. Introduction

The design of "smart" nanostructured materials that respond to changes in their environment is of high interest for applications ranging from drug delivery to microreactors [1–3]. The layer-by-layer (LbL) technique is a robust method for the fabrication of multilayer thin films and the nanoscale engineered assembly of materials. In particular, the versatility of this process is demonstrated by its application to a variety of templates, such as planar surfaces [4,5], particles [6], red blood cells [7], and emulsions [8]. To date, different LbL assembly strategies have been applied [9], including the deposition of polymer layers through electrostatic [10], hydrogen bonding [11], and covalent interactions [12]. A distinct advantage of LbL assembly is the precise control over film properties, such as thickness and morphology. In the past years, the development of LbL capsules that respond to specific stimuli for therapeutic delivery applications has gained significant interest [13]. Pre- and post-loading approaches allow the encapsulation of a range of therapeutic or sensing cargo [2,14,15]. However, systems which exhibit switchable layer/wall permeability are of particular interest for both loading/release and microreactor applications. The permeability of LbL capsules can be controlled by a number of factors, such as the choice of polymer, number of polymer layers, and surface modification. Depending on the material, the capsules can be assembled to be responsive to external stimuli such as redox reactions [16], salt [17], temperature [18,19] and pH [20–22]. For example, Tong et al. synthesized poly(acrylic acid)/branched poly(ethyleneimine) capsules that were cross-linked using glutaraldehyde [23]. These capsules displayed pH-responsive permeability; below pH 6, 2000 g mol^{-1} dextran could readily diffuse into the interior of the capsules, while above pH 6 it was excluded. Despite such studies, to the best of our knowledge, there are no reports on the design and assembly of LbL capsules that exhibit gated permeability based on reversible covalent linkages.

In the present study, a biologically relevant mechanism for reversible capsule permeability, based on a combination of non-reducible copper-catalyzed azide-alkyne cycloaddition (CuAAC) and reversible disulfide bond formation cross-linking, is presented. Disulfides are of interest as a triggered release system, as they are stable *in vivo* but can be degraded to their thiol components intracellularly [24]. Disulfide cross-linking has been used to stabilize poly(methacrylic acid) (PMA) capsules, which were formed by the alternate assembly of a thiol-modified PMA (PMA_{SH}) with poly(*N*-vinylpyrrolidone) (PVPON), followed by oxidation of the thiol groups in the film and removal of PVPON at physiological pH [25]. Such disulfide cross-linked PMA capsules have been used to release peptide cargo both *in vitro* [26] and *in vivo* [27] upon disulfide cleavage. However, as the disulfide bonds provide the sole source of stabilization for the multilayers, the capsules disassemble

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upon disulfide cleavage [28]. To control the permeability of the capsule systems while retaining their integrity, another stabilization factor needs to be incorporated into the multilayers. Recently, reactions summarized under the term "click chemistry" have generated interest for the synthesis of tailor-made materials due to the highly selective, mild and quantitative nature of these reactions [29–31]. To date, the "click" approach has been exploited for the synthesis of various architectures [32]. In particular, the classical "click reaction" (CuAAC) [33], has been used to assemble a range of LbL films, and to functionalize the resulting films to tune their surface properties [34]. In contrast to films constructed using disulfide chemistry, films with CuAAC linkages are stable under different redox environments. Thus, using reversible disulfide cross-linkers in combination with other non-degradable cross-linkers (e.g., *via* "click chemistry") would enable the design of reversibly permeable, gated capsule systems. Such capsule systems are expected to be switchable between a "closed" state (disulfide bonds) and an "open" state (free thiol groups).

Herein, the synthesis of bifunctional PMA and its application for the assembly of LbL capsules with both disulfide and CuAAC cross-links is reported. The combination of these two chemistries offers new possibilities for responsive, nanoengineered capsules. To this end, the preparation and characterization of respective polymers are described in detail. Polymers with different degrees of functionalization were synthesized and examined for their potential to form stable capsules. Permeability studies with dextrans of different molar mass provide insights into the switchable character of the systems. Furthermore, the ability of these capsule systems to load and release DNA is demonstrated. This control of permeability, through disulfide exchange chemistry, could be useful for the application of LbL capsules in drug delivery, as it allows post-loading of sensitive cargo and offers the potential to release the cargo specifically within the reducing intracellular environment of cells.

2. Experimental section

2.1. Materials and instrumentation

Silica particles of 1.16 μm diameter were purchased from Micro-Particles GmbH (Germany) as a 5 wt% suspension and were used as received. Poly(methacrylic acid, sodium salt) (PMA, 30 wt.%), M_w 15 kDa, was purchased from Polysciences (USA). Poly(*N*-vinylpyrrolidone) (PVPON), M_w 10 kDa, fluorescein isothiocyanate-dextran, M_W 10, 59–77, 250, 500, and 2000 kDa, dithiothreitol (DTT), 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM), propargylamine hydrochloride, *N*-chloro-*p*-toluenesulfonamide sodium salt hydrate (chloramine-T hydrate), 2,2′-dithiodipyridine (DTDP), phosphate buffered saline (PBS) tablets, and buffering salts were purchased from Sigma–Aldrich and used as received. 2-(pyridylthio)-ethylamine hydrochloride was obtained from Speed Chemical (China), and azido-dPEG$_3$-amine was obtained from Quanta BioDesign (USA). Alexa Fluor 647 azide, and triethylammonium salt (AF647$_{Az}$) were obtained from Invitrogen. DNA sequences were obtained from GeneWorks (Adelaide, Australia). High-purity water with a resistivity greater than 18 MΩ cm was obtained from an in-line Millipore RiOs/Origin water purification system (Milli-Q water). A stock solution of saline sodium citrate (SSC buffer) was prepared as outlined previously [35].

Fluorescence microscopy images of the capsules were performed with an inverted Olympus IX71 microscope equipped with a DIC slider (U-DICT, Olympus) and a 60 × objective lens (Olympus UPLFL20/0.5 N.A., W.D. 1.6) was used to image the capsules. A CCD camera (Cool SNAP *fx*, Photometrics, Tucson, AZ) was mounted on the left hand port of the microscope. Fluorescence images were illuminated with an Hg arc lamp using a UF1032 filter cube. Confocal laser scanning microscopy (CLSM) images were taken using a Leica TCS-SP2 confocal laser scanning microscope using a Picoquant 405-nm pulsed diode laser as the excitation source. Fluorescent click capsules were imaged in *x*–*y* mode with a 63x planapochromatic oil immersion objective using a PMT gain of 550 V, a digital zoom of 2 × , a line frequency of 400 Hz, and 4x line averaging in 12-bit acquisition mode. Images were analyzed using Image J (1.47v). AFM measurements were carried out with a JPK NanoWizard II BioAFM (JPK Instruments AG, Germany). Glass slides (Livingstone, Australia) were cleaned with piranha solution (*Caution! Piranha solution reacts violently with organic compounds.* Extreme care should be taken when handling piranha solution, and only small quantities should be prepared.) The sample solution (4 μL) was placed onto the glass slide and air-dried. The images were obtained in intermittent contact mode with a scanning rate of 0.5 Hz. Tapping mode cantilevers (BudgetSensors, Tap300Al-G, resonance frequency; ~300 kHz, force constant; ~40 N m^{-1}) with a digital resolution of 512 × 512 pixels were used.

2.2. Polymer post-modifications

PMA$_{Alk,SH}$ was synthesized by either first introducing the alkyne or the protected thiol (PDA) functionality. The functionalization with propargylamine hydrochloride was performed in Milli-Q water, whereas PBS was used as a medium for PDA functionalization. To an aqueous PMA solution (30 wt%), DMTMM (20 g L^{-1}, 2-fold excess) was added and stirred for 15 min at room temperature. Subsequently, propargylamine hydrochloride or PDA (20 g L^{-1}, 1.5-fold excess) was added and stirred overnight at room temperature to obtain PMA$_{Alk}$ and PMA$_{PDA}$, respectively. The polymer was purified by extensive dialysis against Milli-Q water (3–4 days, with at least two water exchanges per day) and was recovered by freeze-drying. ^{1}H NMR: δ_H (400 MHz; D_2O): PMA$_{Alk}$: 0.5–1.25 (C*H*$_3$), 1.25–2.2 (C*H*$_2$ backbone), 2.3–2.54 (C*H* alkyne), 3.6–3.85 (NH–C*H*$_2$). PMA$_{PDA}$: 0.5–1.25 (C*H*$_3$), 1.25–2.2 (C*H*$_2$ backbone), 2.65–3 (S–C*H*$_2$), 3.2–3.5 (NH–C*H*$_2$), 7.0–7.3, 7.5–7.9, 8.25–8.4 (C*H* pyridine).

The second functionalization was introduced accordingly. The mono-functional PMA was dissolved in Milli-Q water (20 mg mL^{-1}) and DMTMM (20 mg mL^{-1}, 2-fold excess) and the second reagent (20 mg mL^{-1}, 1.5-fold excess) were added and the solution was kept overnight at room temperature. The resulting polymer was purified by passing it through a NAP-25 column, and it was recovered by freeze-drying. ^{1}H NMR: δ_H (400 MHz; D_2O): PMA$_{Alk,PDA}$: 0.5–1.25 (C*H*$_3$), 1.25–2.2 (C*H*$_2$ backbone), 2.34–2.54 (C*H* alkyne), 2.65–3 (S–C*H*$_2$), 3.2–3.5 (NH–C*H*$_2$), 3.6–3.85 (NH–C*H*$_2$ alkyne), 7.0–7.3, 7.5–7.9, 8.25–8.4 (C*H* pyridine). Degree of functionalization (DF) was determined by ^{1}H NMR spectroscopy. A detailed overview of the amounts used and the DF values are provided in Table 1.

2.3. LbL assembly on silica particles

Prior to use, PMA$_{Alk,PDA}$ was dissolved at a concentration of 100 g L^{-1} with 0.5 M of DTT solution in MOPS buffer (20 mM, pH

Table 1
Sample characteristics of bifunctional polymers and nomenclature for the capsules (DF = degree of functionalization).

	DF$_{Alk}$[a] [%]	DF$_{SH}$[a] [%]	DF$_{total}$[a] [%]	M_n[b] [g mol^{-1}]	PDI[b]	Capsule description
PMA$_{Alk,PDA_1}$	7	5	12	35,600	1.9	C0
PMA$_{Alk,PDA_2}$	14	5	19	38,100	2.1	C1
PMA$_{Alk,PDA_3}$	12	11	23	36,000	1.9	C2
PMA$_{Alk,PDA_4}$	9	16	25	40,400	1.8	C3

[a] determined by ^{1}H NMR (D_2O, 400 MHz).
[b] determined by SEC (eluent: water).