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Clickable Poly(2-oxazoline) Architectures for the Fabrication of Low-Fouling Polymer Capsules

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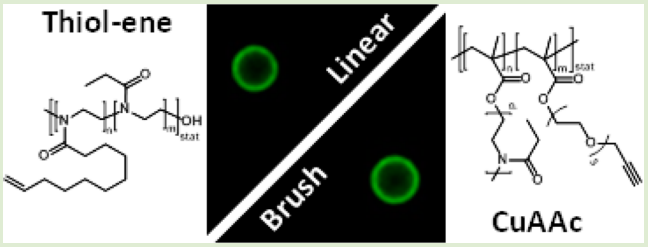
Supporting Information

ABSTRACT: Hollow polymer capsules were prepared from linear as well as brushlike poly(2-oxazoline)s (POxs). Linear POxs containing alkene functionalities were obtained by cationic ring-opening polymerization (CROP), whereas the brush POxs bearing alkyne moieties were synthesized by a combination of CROP and reversible addition−fragmentation chain transfer (RAFT) polymerization. Multilayers consisting of POx/poly(methacrylic acid) (PMA) were sequentially deposited onto silica particle templates, and the films were stabilized either by thiol−ene (TE) chemistry or copper-catalyzed azide−alkyne cycloaddition (CuAAc). Stable, monodisperse capsules were formed after removal of the silica particles with hydrofluoric acid and were observed using fluorescence and atomic force microscopy (AFM). Both architectures exhibited low-fouling behavior, an essential criteria for therapeutic carriers to be utilized in bioapplications. In particular, the brush-like POx capsules show potential as a viable alternative material for the fabrication of low-fouling capsules.



The control of interactions between biological materials such as cells, proteins, and bacteria and synthetic carriers is of significant interest in drug delivery research. To permit efficient delivery to the site of action, therapeutic carriers need to have a low level of nonspecific binding to biological materials, allowing for long circulation in blood. To avoid their recognition and clearance from the body, low-fouling and "stealth" behavior are of major importance for smart carrier systems. A polymer that is conventionally used to impart this property is poly(ethylene glycol) (PEG).[1] However, several limitations associated with PEG,[2] such as degradation by (auto)oxidation, hypersensitivity caused by the polymer, as well as synthesis side products, high viscosity of concentrated solutions which leads to a poor bioavailability, and non-biodegradability, have led to recent research on the development of new alternative materials.[3,4] Among them, poly(2-oxazoline)s (POxs) have emerged as interesting candidates,[5] which combine excellent biocompatibility,[5,6] "stealth" behavior, and protein repellence,[7,8] with the ease of synthesizing highly functional systems.[9−11] To date, POx-based particulate systems have been obtained by self-assembly of amphiphilic block copolymers, nanoprecipitation,[12] or surface modification of existing particles.[13] However, in recent years, a highly versatile technique for the fabrication of multilayer thin films and nanoscale engineered assembly of materials, known as the layer-by-layer (LbL) technique,[14−16] has gained significant interest. Recently, hydrogen-bonded LbL assembly has been used to prepare POx/tannic acid multilayers.[17,18] The LbL technique has also been exploited for the fabrication of functional drug carriers[19] with crucial properties that include "stealth" behavior, targeting groups, and release mechanisms.[20] Typically, "stealth" is achieved by decorating the capsule corona with PEG moieties and layers, respectively. Furthermore, LbL has previously been used to prepare single-component cross-linked PEG[21] and poly(vinylpyrrolidone) (PVPON)[22,23] films and capsules, which exhibit low-fouling properties. These systems have recently been used for specific targeting of cancer cells.[24−26] Despite these studies, there still exists a need for exploring simple and modular chemistries to assemble low-fouling materials with readily tunable properties. The modularity of POx allows for the straightforward incorporation of side and end group functionalities.[11,27] Fine-tuning of the material properties is achieved by copolymerization of different substituted 2-oxazolines[28] and, as recently reported, by the combination with other living/controlled polymerization techniques.[29−31] This versatility provides access to a vast variety of polymer architectures, such as linear, brush, and star-shaped polymers with readily tunable properties. Herein, we report the utilization of linear as well as brushlike POx for the fabrication of multilayer films on both planar and particle supports. Film stabilization was achieved using two different efficient coupling reactions: thiol−ene (TE) chemistry and copper-catalyzed azide−alkyne cycloaddition (CuAAc), respectively. The influence of the POx architecture on the protein fouling behavior of the respective capsules was also investigated.

Two different POx architectures, namely, linear and brushlike POx, which bear functionalities that are suitable to undergo efficient postpolymerization modification reactions, were

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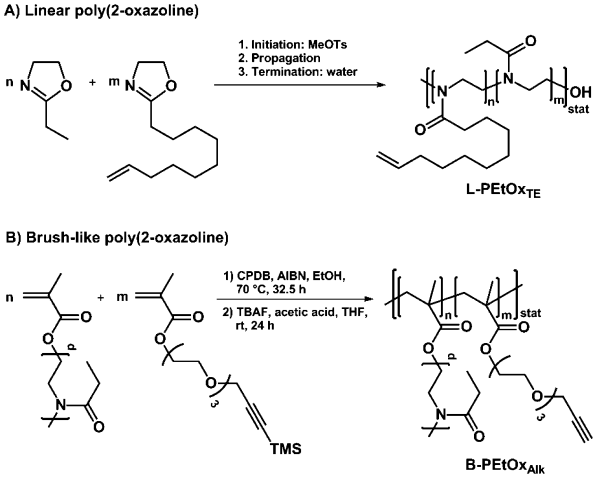
prepared. Functional poly(2-ethyl-2-oxazoline) (PEtOx)-based copolymers were obtained by copolymerization with alkene and alkyne-containing comonomers that are able to react in TE and CuAAc reactions, respectively. The linear POx, referred to as **L-PEtOx$_{TE}$**, was obtained by CROP of EtOx and 2-dec-(9-enyl)-2-oxazoline (DecEnOx; Scheme 1A), a comonomer employed for functionalization and cross-linking via TE reactions.[27] To prepare brushlike POx, referred to as **B-PEtOx$_{Alk}$**, an oligo(2-ethyl-2-oxazoline)methacrylate (OEtOxMA) macromonomer[32] was copolymerized with a trimethylsilyl (TMS)-protected alkyne monomer by reversible addition–fragmentation chain transfer (RAFT) polymerization (Scheme 1B).

Scheme 1. Schematic Representation of the Synthesis of Linear (L-PEtOx$_{TE}$, A) and Brushlike (B-PEtOx$_{Alk}$, B) Poly(2-oxazoline)s (POxs)



This study, to our knowledge, represents the first report of a POx brush with clickable moieties in the side chain obtained by RAFT. The amount of clickable monomer units was determined by ^{1}H NMR to be 7% and 14% for **L-PEtOx$_{TE}$** and **B-PEtOx$_{Alk}$**, respectively (Figures S1 and S2, Supporting Information). It is noteworthy that the latter system required a higher percentage of functional groups to efficiently stabilize the capsule walls. **B-PEtOx$_{Alk}$** with 8% functionalization resulted in the formation of ill-defined capsules (data not shown). Size exclusion chromatography (SEC) measurements revealed the synthesis of well-defined copolymers with polydispersity index values <1.3. For a summary of the characterization data refer to Table S1 (Supporting Information).

The assembly of POx and PMA multilayer films via hydrogen bonding was monitored on planar supports using a quartz crystal microbalance (QCM). To investigate the influence of pH on the film assembly, the buffer pH was adjusted to 2.5, 4, and 5. **L-PEtOx$_{TE}$** (Figure 1A) and **B-PEtOx$_{Alk}$** (Figure 1B) showed the same trend for film buildup, where the frequency decreased with the number of layers deposited. Thus, the polymers examined were able to undergo hydrogen bonding and form multilayer films at all pH values used (2.5, 4, and 5). As reported for PEtOx/PMA systems, hydrogen bonding is observed up to pH 5.[33] The copolymers used in this study showed a similar behavior. Multilayer buildup at pH 5 differed from buildup at lower pH values, where significantly lower frequency changes were observed. The changes in QCM frequency of the films per bilayer were in a similar range for pH

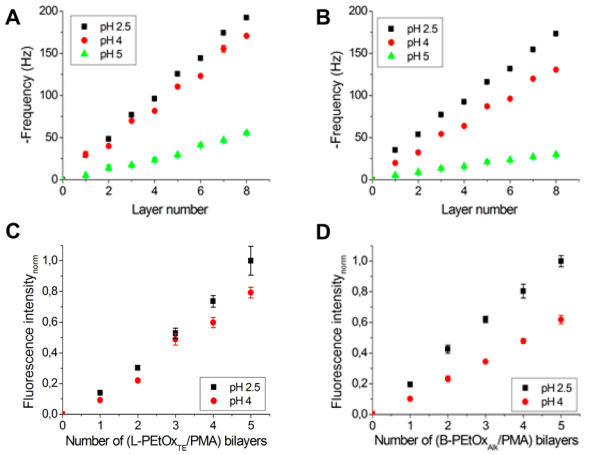


Figure 1. LbL assembly of **L-PEtOx$_{TE}$**/PMA (left) and **B-PEtOx$_{Alk}$**/PMA (right) bilayers (23 °C, 20 mM NaOAc) at different pH. (A, B) Film assembly on planar supports, as monitored by QCM. Layer 0 corresponds to the PMA layer deposited via electrostatic interactions onto a PEI-coated surface. Odd and even numbers correspond to POx and PMA layers, respectively. (C, D) Film assembly on particle supports (SiO_2, 2.59 μm), as monitored by flow cytometry. PMA was prelabeled with AF488-cadaverine. Measurements were taken after each PMA layer was deposited.

2.5 and 4 (40–50 Hz per bilayer). These values are similar to other hydrogen-bonded systems, such as PMA/PVPON,[34] suggesting the formation of thin POx/PMA films. However, due to the higher protonation of the PMA at low pH, hydrogen bond interactions are more pronounced, resulting in a slightly larger frequency change per deposited layer.

The results obtained from the planar support studies were applied for the buildup of films on particle templates. The formation of POx/PMA multilayers was examined on monodisperse SiO_2 particles (2.59 μm in diameter) at pH 2.5 and 4 (20 mM NaOAc). To quantify the buildup, PMA was prelabeled with AF488-cadaverine, and the particle fluorescence was measured by flow cytometry after deposition of each bilayer. Linear and regular buildup was observed in each case, independent of the architecture of the POx (Figure 1C, D). However, the difference in the deposition at pH 2.5 and 4 was more pronounced for **B-PEtOx$_{Alk}$**. These results are in agreement with the QCM data for the buildup on planar substrates. However, the film buildup is ddifferent compared to brushlike PEG, which showed an exponential trend.[21] This PEG growth behavior was attributed to polymer migration within the multilayers during the assembly. We note that polymer capsules based on linear PEG have not yet been reported, to our knowledge.

To study the cross-linking conditions for the preparation of stable hollow POx capsules (i.e., capsules that retain their structural integrity at 37 °C and pH 7.4), five bilayers of **L-PEtOx$_{TE}$** or **B-PEtOx$_{Alk}$** and PMA were deposited onto silica particle templates. In addition, two nonfunctionalized sacrificial PVPON/PMA bilayers were added as capping layers to minimize interparticle cross-linking (Scheme 2). From the studies on both the planar and particle supports, we chose to conduct the multilayer assembly at pH 4, as this pH ensures film buildup.

The cross-linking of **B-PEtOx$_{Alk}$** layers, to yield the respective capsules (Figure 2B), was performed in a similar manner to other reported CuAAc "click" capsules,[35] whereas the TE cross-linking, presented here, required establishing

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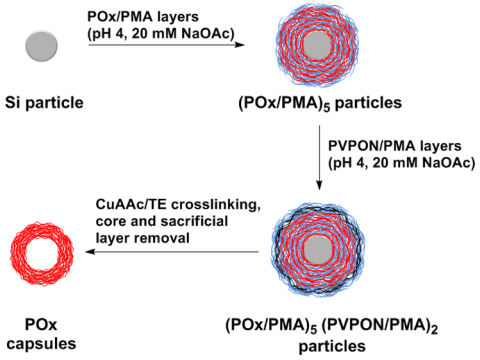
Scheme 2. Preparation of "Click"-Stabilized POx Capsules via Hydrogen-Bonded LbL Assembly of POx and PMA



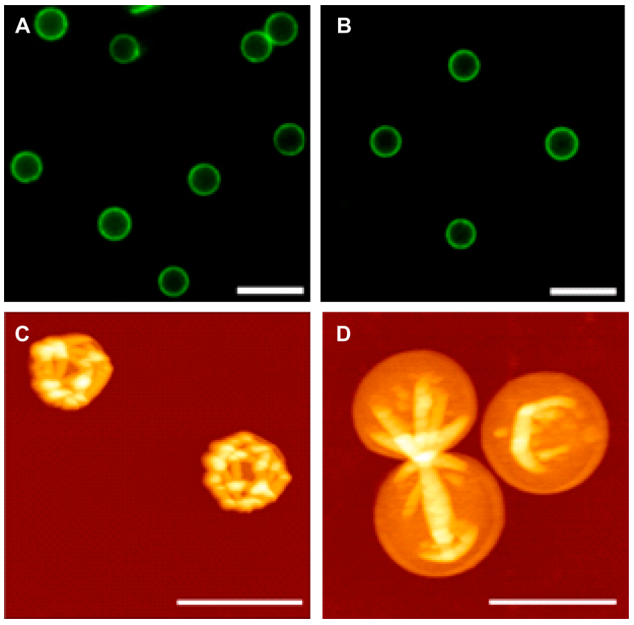


Figure 2. Fluorescence microscopy (top) and atomic force microscopy (bottom) images of **L-PEtOx$_{TE}$** (A, C) and **B-PEtOx$_{Alk}$** (B, D) capsules assembled on a sacrificial core of 2.59 μm diameter SiO_2 particles at pH 4 (NaOAc, 20 mM). Scale bars = 5 μm.

appropriate conditions for stabilization of the **L-PEtOx$_{TE}$**-based systems. The efficient cross-linking of multilayers exploiting polar TE reactions, referred to as Michael-addition, has been previously reported.[36] In contrast, nonactivated alkene groups, such as the terminal double bond present in the DecEnOx monomer, are susceptible to radical TE reactions.[37,38] More recently, functional hydrogels were prepared from DecEnOx and difunctional thiols.[39] In general, the radical TE reaction can be initiated by either UV irradiation or thermal heating.[40] Here, water-soluble UV as well as thermal radical initiators and two different dithiols, namely, dithiothreitol (DTT) and 2′,2′-(ethylenedioxy)-diethanethiol (EDDT), were tested for cross-linking of the multilayer films. UV-initiated thiol−ene reactions were not successful. It was assumed that at the pH conditions chosen the initiator became inactive. In contrast, thermal cross-linking yielded stable capsules after removal of the particle template and the sacrificial PMA by washing the capsules into PBS (Figure 2A, C). However, it was observed that the cross-linker influenced the capsule stability. Using the shorter cross-linker (DTT), the multilayers could be readily stabilized, whereas the longer cross-linker (EDDT) yielded only a marginal number of capsules, which appeared less robust. This observation might be attributed to denser cross-linking using the shorter cross-linker. **L-PEtOx$_{TE}$**, with a rather low molar mass (5 kDa), yielded stable capsules, as observed under the fluorescence microscope (Figure 2A). In addition, the capsules were analyzed using atomic force microscopy (AFM). Analysis of the air-dried POx capsules revealed spherical structures with folds and creases, which are typical for polymer capsules prepared by the LbL technique (Figure 2C, D).

Extended blood circulation times are crucial for systems applied in biology since it increases the bioavailability for delivery to the site of action. As human serum contains numerous different proteins, the ability of the POx capsules to resist protein adsorption was investigated using two representative proteins, namely, bovine serum albumin (BSA) and lysozyme. In particular, the influence of the architecture of the POx systems used, linear (**L-PEtOx$_{TE}$**) and brushlike (**B-PEtOx$_{Alk}$**), on the fouling behavior was considered for this study. The capsules were incubated with FITC-labeled proteins at 37 °C for 1 h. After incubation, the capsules were washed with PBS, and the relative amount of adsorbed proteins on the particles was determined using flow cytometry. As a comparison, PVPON capsules stabilized by CuAAc were synthesized and treated in the same way, as PVPON capsules have been reported to exhibit low fouling behavior compared to higher fouling systems such as PSS, PAH, and PDPA.[22] These highly charged systems show protein adsorption of about 6 times higher than PVPON, highlighting the low-fouling character of the PVPON system. The fouling experiments revealed that POx capsules were similarly capable of resisting proteins as PVPON capsules (Figure 3). Specifically, **B-PEtOx$_{Alk}$** capsules showed an improvement in fouling behavior, with ∼40% lower associated protein than the **L-PEtOx$_{TE}$** capsules, in both protein solutions.

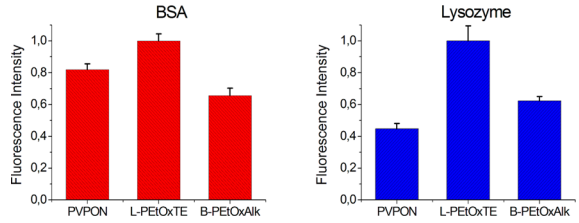


Figure 3. Flow cytometry analysis of the fouling behavior of PVPON, **L-PEtOx$_{TE}$**, and **B-PEtOx$_{Alk}$** capsules with FITC-BSA (left) and FITC-lysozyme (right). Unlabeled capsules were incubated with 1 mg mL^{-1} of protein (BSA or lysozyme) in pH 7.4 PBS for 1 h, before being measured for protein association on the capsule surfaces. Each measurement was performed in triplicate. Error bars show the average deviation from the mean.

It is known that a low-fouling system typically requires a well-hydrated polymer to be capable of repelling proteins.[41] The statistical distribution of the alkene groups within the **L-PEtOx$_{TE}$** copolymer[12] causes a relatively flat arrangement of the POx chains on the surface after cross-linking, preventing the exposure of entangled chains from the surface, limiting effective hydration. This is supported by the low swelling of hydrogels prepared from P(EtOx-*co*-DecEnOx) systems.[39] Furthermore, the hydrophobicity of DecEnOx units, and free thiol groups, might increase the interactions of the capsules with proteins. In contrast, the **B-PEtOx$_{Alk}$** capsules display PEtOx brushes, which might be better hydrated, thus having potentially

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increased protein resistance properties.[41] However, even though **B-PEtOx$_{Alk}$** contains only a minor amount of comonomer, we cannot rule out some influence of the OPEG chains.

In conclusion, the fabrication of POx-based polymer capsules is reported. To this end, two different polymers with linear and brushlike architecture were prepared. Their deposition on planar or particle supports revealed regular and linear film buildup at pH 2.5 and 4. CuAAc and TE chemistries were employed for the cross-linking of the films, yielding in both cases stable thin-film polymer capsules. Both POx architectures rendered the corresponding capsules low-fouling, with brush-like POx capsules showing lower protein adsorption. The interaction of POx capsules with biological material and their response to external stimuli for triggered release of cargo are currently under investigation in our laboratory.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental data and supporting spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

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