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Biomimetic Anchor for Surface-Initiated Polymerization from Metal Substrates

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Abstract: In this paper, we demonstrate the first use of a catecholic initiator for surface-initiated polymerization (SIP) from metal surfaces to create antifouling polymer coatings. A new bifunctional initiator inspired by mussel adhesive proteins was synthesized, which strongly adsorbs to Ti and 316L stainless steel (SS) substrates, providing an anchor for surface immobilization of grafted polymers. Surface-initiated atom transfer radical polymerization (SI-ATRP) was performed through the adsorbed biomimetic initiator to polymerize methyl methacrylate macromonomers with oligo(ethylene glycol) (OEG) side chains. X-ray photoelectron spectroscopy, surface FT-IR, and contact angle analysis confirmed the sequential grafting of initiator and polymer, and ellipsometry indicated the formation of polymer coatings of up to 100 nm thickness. Cell adhesion experiments performed with 3T3-Swiss albino fibroblasts showed substantially reduced cell adhesion onto polymer grafted Ti and 316L SS substrates as compared to the unmodified metals. Moreover, micropatterning of grafted polymer coatings on Ti surfaces was demonstrated by combining SI-ATRP and molecular assembly patterning by lift-off (MAPL), creating cell-adhesive and cell-resistant regions for potential use as cell arrays. Due to the ability of catechols to bind to a large variety of inorganic surfaces, this biomimetic anchoring strategy is expected to be a highly versatile tool for polymer thin film surface modification for biomedical and other applications.

Introduction

Enediol-containing compounds (e.g., catechol, dopamine) are common in nature, playing key roles in many biological reactions and in medical treatment.[1] For example, the catecholic amino acid L-3,4-dihydroxyphenylalanine (L-DOPA) is found in the adhesive pad proteins secreted by marine mussels.[2] Although its function is not fully understood, the presence of L-DOPA at elevated concentrations in proteins found closest to the adhesive−substrate interface[3] suggests a possible role in adhesion, a finding that has stimulated great interest in exploiting catechols for enhancing interfacial adhesion of materials.[4] Catechol derivatives have recently been used to anchor small functional biomolecules onto ferromagnetic nanoparticles (Fe_2O_3) for protein separations,[5] and for linking of DNA and dyes to surfaces of semiconductor nanoparticles (TiO_2).[6]

However, catechols have been underutilized as tools for modification of metal surfaces with synthetic functional polymers. We recently described the use of DOPA-containing peptides for adsorption of the antifouling polymer poly(ethylene glycol) (PEG) onto Ti and Au surfaces.[7,8] Although the resulting surfaces exhibited excellent protein and cell fouling resistance, adsorption of polymer from solution (so-called “graft-to” approach) is generally not capable of achieving high polymer surface densities and thick coatings.[9] In contrast, surface-initiated polymerization (SIP) of monomers from surface bound initiators (“graft-from”) is a more versatile technique capable of producing polymer brush layers of greater density and thickness.[10] Although different types of functional groups have been reported for immobilizing initiators onto metals for SIP, catechols have never been used in this manner.

We describe in this Article a general approach to SIP of antifouling polymers from metal surfaces via a mussel adhesive protein (MAP) mimetic initiator. Our strategy takes advantage of a bifunctional initiator (**2** in Scheme 1) containing a catechol end group for surface anchoring and an alkyl bromine to activate SIP. We demonstrate this approach by grafting PEG-based antifouling coatings on Ti and stainless steel (SS), two important metals commonly used in the fabrication of medical devices

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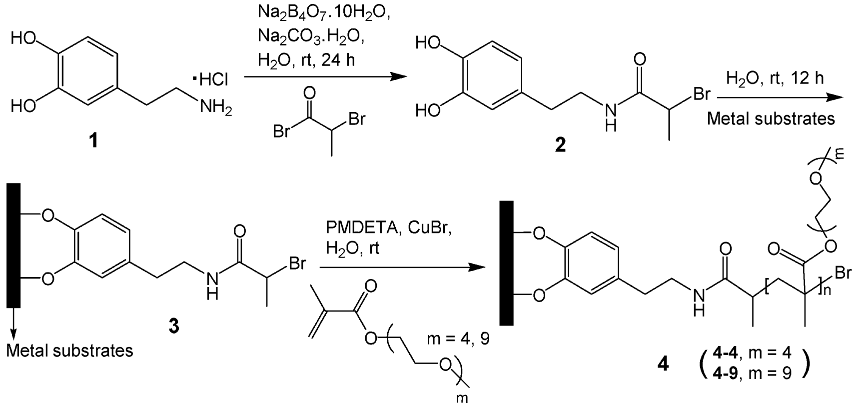
Scheme 1. Synthesis and Anchoring of the DOPA Mimetic Initiator and Subsequent SI-ATRP



due to their high specific strength, corrosion resistance, and biocompatibility.[11] The polymers are grown in situ from the initiator modified surfaces by surface-initiated atom transfer radical polymerization (SI-ATRP), which is a versatile and robust approach widely used in "graft-from" strategies.[12] Moreover, to demonstrate the potential applications of our approach for patterning purposes, we also prepared patterned thin films and subsequent cellular arrays using the combination of SI-ATRP and a simple photolithography method, molecular assembly patterning by lift-off (MAPL).[13]

Results and Discussion

As illustrated in Scheme 1, dopamine was protected in situ as the borate ester using sodium borate decahydrate and then reacted under mildly basic conditions with 2-bromopropionyl bromide to yield initiator **2**. In the design of our biomimetic initiator, rather than using the hydrolytically labile bromoester functionality, which is widely employed in ATRP initiators, we coupled the catechol anchor and Br terminus using an amide linkage that should remain stable under the aqueous conditions of initiator adsorption, SI-ATRP, and in vitro cell adhesion test. Initiator **2** was immobilized on Ti and 316L SS surfaces[14] via adsorption from aqueous solution. Initiator modified substrate **3** was then subjected to SI-ATRP of oligo ethylene glycol methyl ether methacrylate (OEGMEMA) macromonomers with OEG side chains of length 4 or 9 EG repeats (OEGMEMA-4/-9). The grafted poly(OEGMEMA) coatings **4-4** (4 EG repeats) and **4-9** (9 EG repeats) were analyzed by X-ray photoelectron spectroscopy (XPS), ellipsometry, contact angle goniometry, and polarization-modulation infrared reflection-adsorption spectroscopy (PM-IRRAS).

Table 1. Surface Analysis Results of Bare Ti, Initiator Modified Ti, and **4-9** Grafted Ti (3 h SI-ATRP) Samples

	XPS atomic concentration (%)					
sample	[Ti]	[O]	[C]	[N]	[Br]	static water contact angle (deg)
bare Ti	59	30	11	0	0	<10
initiator modified	21.1	41.6	33.4	2.5	1.4	56 ± 2
4-9	0	30.4	69.6	0	0	48 ± 2

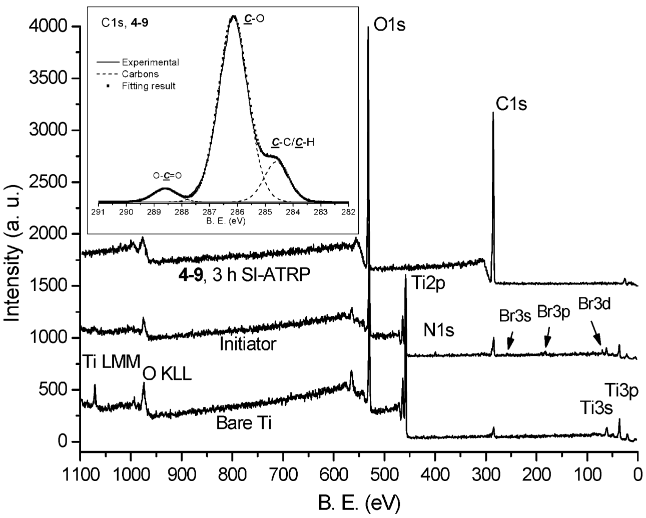


Figure 1. XPS survey spectra of bare, initiator, and **4-9** coated Ti surfaces. A high-resolution spectrum of the C 1s region of **4-9** coated Ti is shown in the inset.

Successful initiator immobilization and polymer grafting were first ascertained by XPS and contact angle measurements. Figure 1 displays XPS survey spectra of unmodified, initiator-treated, and polymer grafted Ti substrates. Surface chemical composition and static water contact angle data of these surfaces are summarized in Table 1. The pristine Ti surface was mainly composed of Ti and O from the native surface oxide as well as a small amount of C resulting from adventitious hydrocarbon contamination. Successful anchoring of initiator **2** was indicated by a significant increase in C concentration (33.4%) and the presence of N and Br signals not detected for the bare Ti substrate. After 3 h SI-ATRP of OEGMEMA-9, polymer grafting was confirmed by a substantial increase in C concentration (69.6%) and the complete disappearance of Ti signal, indicating the thickness of the grafted **4-9** layer (~80 nm, see ellipsometry results below) was greater than the escape depth of the photoelectrons (~10 nm).[9] It should be noted that the [O]/[C] ratio determined by XPS (0.44) was very close to the theoretical value (0.48) of **4-9** polymer. Furthermore, high-resolution XPS spectral analysis demonstrated good agreement with the structure of the grafted polymer coating. As shown in the inset of Figure 1, the C 1s high-resolution scan of **4-9** grafted Ti surface was fitted with aliphatic (*C*−C/*C*−H), ether (*C*−O), and ester (O=*C*−O) carbons corresponding to binding energies at 284.6, 286.1, and 288.6 eV, respectively. The observed area

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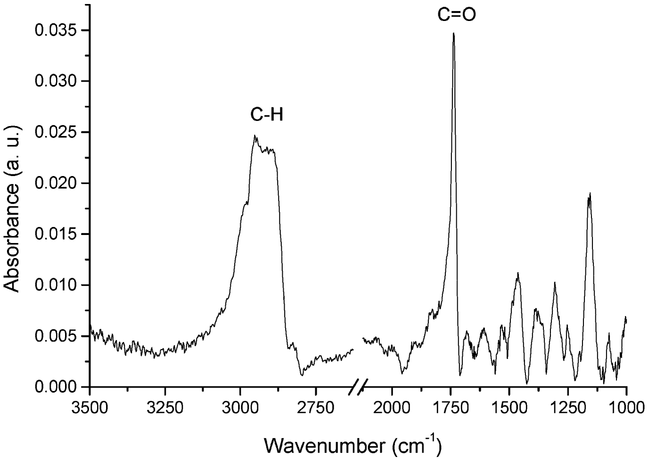


Figure 2. PM-IRRAS spectrum of a **4-4** sample produced by 12 h SI-ATRP of OEGMEMA-4.

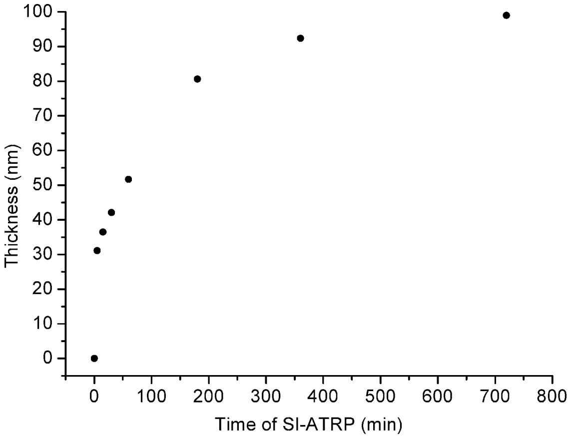


Figure 3. Dry ellipsometric thickness of **4-4** thin films on Ti substrates as a function of polymerization time. Samples were prepared from separate yet identical reactions, and data points represent mean values of at least three different measurements.

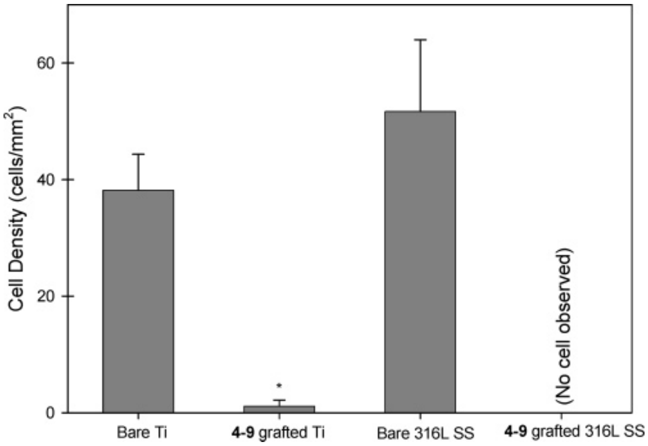
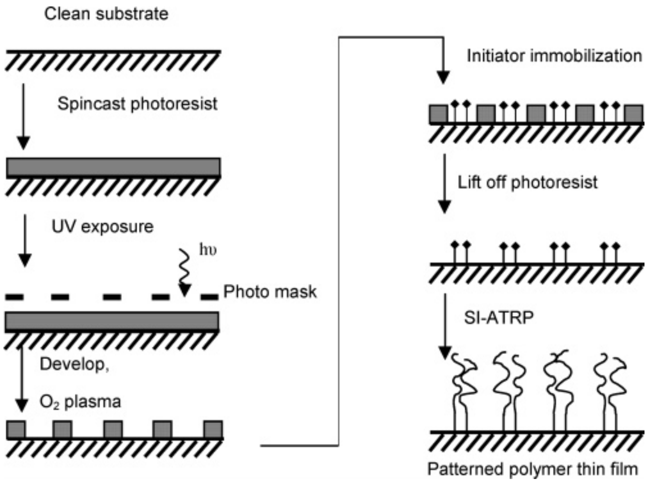


Figure 4. 4 h cell adhesion assay results on bare and **4-9** grafted Ti and 316L SS substrates (*, $p < 0.001$ versus bare Ti).

Scheme 2. MAPL Process for the Preparation of Patterned Polymer Thin Film by SI-ATRP[13]



ratio of these components, 3.1:17.2:1, is consistent with the theoretical ratio 3:19:1 for **4-9** polymer. Similarly consistent XPS results were also obtained from **4-9** coatings grafted on 316L SS and **4-4** coatings grafted on Ti. Contact angle changes also confirmed the sequential formation of the initiator and polymer layers. Prior to initiator adsorption and SI-ATRP, freshly cleaned Ti substrates were hydrophilic with a static water contact angle <10°, which increased to an average value of 56° after immobilization of **2**. After 3 h SI-ATRP of OEGMEMA-9, the contact angle decreased to ~48°, indicating an increase in hydrophilicity due to the grafted polymer. The observed XPS and water contact angle data of grafted polymer surfaces are very consistent with those reported for similar PEG-based polymer coatings grafted from Si substrates.[15,16]

PM-IRRAS was employed to further confirm the presence of grafted coatings by observation of the absorptions of typical functional groups contained in the polymer. Figure 2 demonstrates the PM-IRRAS spectrum of **4-4** after 12 h SI-ATRP of OEGMEMA-4. Typical features of the methyl methacrylate polymer backbone include strong absorptions at 2850−2990 cm^{-1} due to C−H symmetric and asymmetric stretching, a sharp peak at 1738 cm^{-1} from C=O stretch in the ester group, and peaks at 1360−1470 cm^{-1} from CH_2 and CH_3 deformation. Other features associated with the OEG moiety include bands at 1070−1260 cm^{-1} from C−O−C stretches in both ether and ester groups, and a small peak at 2825 cm^{-1} due to C−H stretch in the methyl ether end group.[17] Moreover, the absence of the absorption from the vinyl group (a peak at 1640 cm^{-1} in the spectrum of the monomer, not shown) further excluded the possibility of simple monomer physisorption on the substrate.

Ellipsometry was used to follow the increase in polymer thin film thickness during SI-ATRP. Figure 3 shows the growth of **4-4** as a function of polymerization time. Under the conditions of our experiments, the initial kinetics of aqueous SI-ATRP was very fast, resulting in a polymer thin film of ~30 nm thickness after only 5 min of polymerization, after which the thickness further increased with time to a limiting value of roughly 100 nm for extended reaction times up to 12 h. Growth of **4-9** coatings on Ti showed very similar behavior. The rapid initial rate can be attributed to the solvent (water), which is believed to increase the activity of the catalyst and accelerate ATRP.[18]

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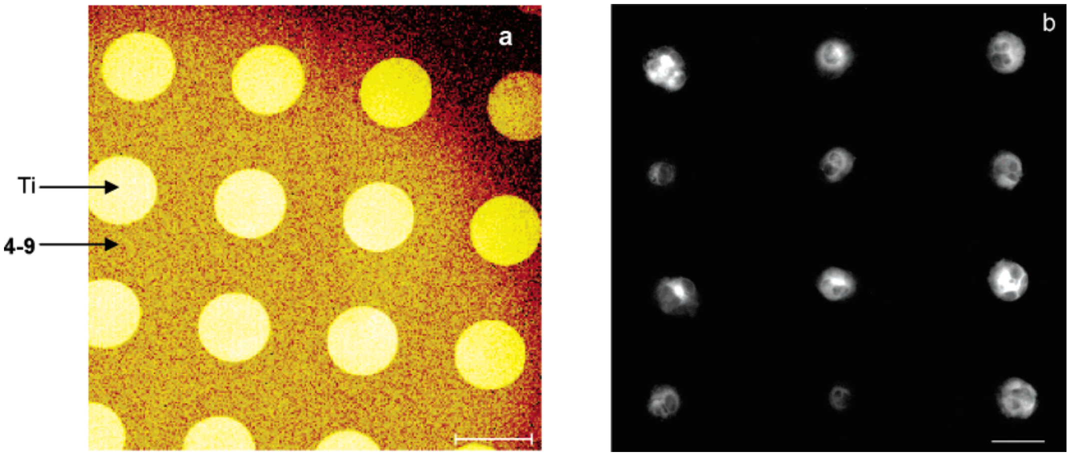

Figure 5. Patterned polymer thin film created by MAPL and SI-ATRP. (a) ToF-SIMS chemical map of Ti^+ ($m/z = 47.89$) signal collected from a patterned **4-9** thin film produced by MAPL and 12 h SI-ATRP. Scale bar = 100 μm. (b) Fluorescence micrograph of fibroblast attachment on a patterned **4-9** surface after 4 h of cell culture. Scale bar = 50 μm. It should be noted that these two images were taken at different locations from samples that contained patterns of variable sizes and shapes.

Similar kinetic results for SI-ATRP in pure aqueous or aqueous alcoholic media have been observed by other investigators.[19,20] Efforts to achieve polymer coatings with better thickness control are underway.

The polymer coatings reported here are significantly thicker than those formed by adsorption of DOPA functionalized polymers from solution ("graft-to" approach) onto similar substrates.[7,8] However, the nature of the chemical interaction between grafted polymer and underlying substrate is expected to be similar for both "graft-from" and "graft-to" approaches. On Ti, the interaction is likely to take the form of a bidentate coordination complex between the catechol oxygens and a Ti atom at the native oxide surface.[21,22] Binding energies between TiO_2 and dopamine have recently been estimated by density functional theory to be on the order of 25−30 kcal/mol,[23] suggesting that grafted polymer chains are strongly bound to the metal surface. Although previous studies demonstrated that similar interactions occur between catechols and Fe_2O_3 surfaces,[22] the situation on 316L SS is potentially much more complicated, as the SS surface is typically composed of mixed Fe and Cr oxides/hydroxides.[24]

The biological response to polymer grafted metal surfaces was assessed by studying resistance to mammalian cell adhesion in the presence of serum. Figure 4 shows the comparative results from cell adhesion assays in which 3T3-Swiss albino fibroblasts were cultured in the presence of unmodified and **4-9** grafted metal substrates after 12 h SI-ATRP. While cells readily attached after 4 h on bare Ti and 316L SS at average densities of approximately 40 and 50 cells/mm^2, respectively, **4-9** grafted surfaces supported attachment of around 1 cell/mm^2 on Ti, and no cells were found on 316L SS. Results from additional experiments in which both thickness and OEG chain length were compared (data not shown) indicated that, for polymer coatings with thickness ∼30 nm or higher, short term cell resistance did not strongly depend on either the thickness or the OEG length of grafted polymer layers.

The short-term antifouling behavior of our polymer grafted metal substrates is similar to that of PEG-based polymer films formed by SI-ATRP on Au[19] and Si[15] surfaces. The observed ∼97% reduction in cell adhesion density on Ti is also comparable to that of our previously reported antifouling PEG-DOPA thin films (∼94% reduction in cell adhesion density) formed by polymer adsorption onto Ti ("graft-to" approach).[7] Although a more detailed study looking at long-term performance of these coatings is in progress, a preliminary long-term cell adhesion experiment with **4-9** showed promising extended antifouling performance, with **4-9** coatings of approximately 100 nm thickness exhibiting roughly 90% less cell attachment than uncoated Ti at 5 weeks of culture (data not shown).

Finally, the patterning of polymer thin films was accomplished by the combination of SI-ATRP and MAPL.[13] A pattern containing circular domains of photoresist on Ti was created using standard photomask lithography, after which initiator was adsorbed to bare Ti regions (Scheme 2). Following removal of the photoresist by rinsing with solvent, 12 h SI-ATRP yielded circular regions of bare Ti within a background of grafted **4-9**. Patterned surfaces were analyzed by time-of-flight secondary ion mass spectroscopy (ToF-SIMS). Figure 5a shows a ToF-SIMS chemical map of a patterned polymer sample where the bright circular regions represent bare Ti substrate that was protected by photoresist during initiator adsorption, whereas the dark matrix is the **4-9** polymer thin film grafted from the initiator layer after photoresist lift-off. Patterned Ti samples were then subjected to cell culture experiments as described above. Figure 5b shows a typical florescence image of fibroblast attachment on a sample patterned with **4-9** polymer. It was observed that a cell pattern was produced in which 1−4 cells were confined within the circular regions of bare Ti, while the grafted polymer region resisted cell attachment in a spatially controlled manner. This result demonstrates the potential of using our biomimetic strategy to generate cellular arrays on metal substrates.

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The vast majority of previous SI-ATRP studies on inorganic materials have utilized silane or thiol initiator designs.[25] Although well-known for strong interactions with inorganic surfaces, catechols have not been used for SIP before. Thus, the catechol-based initiator introduced in this paper adds a new tool that can be used to modify inorganic surfaces with polymers grafted by SI-ATRP. Although we have demonstrated this with OEG-based methacrylate monomers for antifouling coatings, simple extension of this approach to any monomer and solvent system capable of being used in ATRP will expand the practical uses to include polymer functionalization for many other applications and types of materials, including inorganic nanoparticles. It should also be straightforward to generalize the design of the catechol initiator from the 2-bromoamide used here to functional groups suitable for other types of radical polymerization techniques.

Summary

In summary, we have demonstrated a new approach to SIP from metal surfaces that takes advantage of strong interactions between a catechol-based initiator and native metal oxides. The grafted polymer coatings were up to ∼100 nm thick, resistant to fouling by mammalian cells, and capable of being patterned into cell-adhesive and cell-resistant regions. We believe that the antifouling properties of the thick polymer films, combined with the structural and chemical versatility of the SI-ATRP approach, suggest enormous potential in a wide variety of biomedical applications where cell fouling resistance and cell arrays are desired. Recent preliminary results demonstrate successful polymer grafting from several other metal substrates including Ti alloys (Ti-6Al-4V, NiTi) and Au, which suggests this biomimetic strategy represents a general approach to polymer functionalization of many substrate materials. For nonmedical applications, this facile and versatile synthetic strategy should open a new route toward functional thin films and polymer−metal oxide nanocomposites via SIP.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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