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Norepinephrine: Material-Independent, Multifunctional Surface Modification Reagent

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The performance of most advanced materials is closely connected to their surface chemical characteristics. Examples include biosensors, medical devices, catalysts, nanomaterials, drug delivery carriers, etc.[1−5] Widespread methods for surface modification, such as self-assembled monolayer (SAM) and organosilane chemistry, work well on particular material surfaces compatible with the specific strategy for surface conjugation[6] but lack efficacy on broad ranges of material surfaces. Methods that require the use of organic solvents, in some cases under anhydrous conditions, represent further limitations of existing surface modification strategies. Thus, development of versatile aqueous surface modification chemistry remains an important goal.

Herein, we report a facile surface modification method utilizing norepinephrine, a small catecholamine molecule. Oxidative polymerization of norepinephrine in alkaline aqueous media modified virtually all material surfaces (noble metals, metal oxides, semiconductors, ceramics, and synthetic polymers), and the modified surfaces serve as useful platforms for biomolecule-conjugation and ring-opening polymerization.

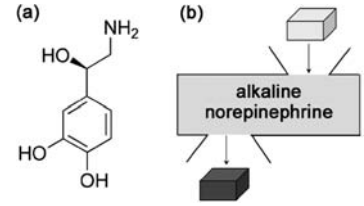
Recently, we reported a material-independent surface functionalization strategy involving self-polymerization of dopamine to form chemically active adherent films on virtually any material surfaces including noble metals, oxides, polymers, semiconductors, and ceramics.[7] The method was inspired by the high content of 3,4-dihydroxy-L-phenylalanine (DOPA) and lysine found in the specialized mussel adhesive protein Mefp-5 (*Mytilus edulis* foot protein-5), which is predominantly located at the interface between the adhesive pad and substrate in byssal attachments.[8] The DOPA-Lys motif consists of ∼40% in total amino acid content of Mefp-5.

Dopamine is considered a small molecule mimic of Mefp-5 in that it contains the catechol and primary amine functional groups found in the side chains of DOPA and Lys residues. Incubation of substrates in an alkaline dopamine solution resulted in oxidative polymerization of dopamine and formation of a heterogeneous polymer coating.[7a] A variety of secondary immobilization reactions using the polydopamine coating as a base or "primer" led to various functional coatings, including grafted polymer coatings, metal films, and self-assembled monolayers.

In an effort to further increase the versatility of this strategy, we now report organic thin film formation by derivatives of dopamine, in particular catecholamines that offer chemical functionalities not present in dopamine. Importantly, we find that norepinephrine shares the material-independent coating-forming properties of dopamine but can also support secondary derivatization of surfaces that is difficult with polydopamine coatings. Unlike polydopamine coatings, coatings derived from norepinephrine have the strong ability to activate surface-initiated, ring-opening polymerization due to the presence of the alkyl hydroxyl group in norepinephrine.

Substrates were modified by simple immersion in a solution of norepinephrine (2 mg of L-(−)-norepinephrine per milliliter of 10 mM Tris, pH 8.5) as described in Scheme 1b. After several hours of incubation in aqueous norepinephrine, substrates exhibited visible changes in color as shown in Figure 1a. The brown color of the coated film is due to catechol oxidation followed by polymerization of norepinephrine at alkaline pH (Figure S1). Analogous to dopamine polymerization,[7a] the product is a surface-bound heterogeneous polymer coating that we refer to as poly(norepinephrine) (PN).

Scheme 1[a]



[a] (a) Chemical structure of L-(−)-norepinephrine; (b) poly(norepinephrine) coating forms by dip-coating of substrates in alkaline norepinephrine.

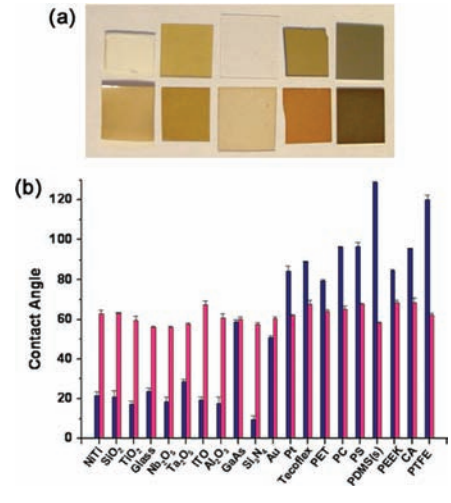


Figure 1. Material-independent surface modification by norepinephrine. (a) Photograph of substrates before (upper row) and after (lower row) immersion in norepinephrine, pH 8.5 for 9 h [left to right: polyurethane (PU), poly(ether ether ketone) (PEEK), glass, gold, and tantalum oxide (Ta_2O_5)]. (b) Static contact angles of unmodified substrates (blue) and PN-coated ones (red).

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A large range of materials, including metal oxides (NiTi, SiO_2, TiO_2, Nb_2O_5, Ta_2O_5, indium−tin oxide (ITO), and Al_2O_3), ceramics (glass), semiconductors (GaAs and Si_3N_4), noble metals (Au and Pt), and synthetic polymers (polyurethane (PU), poly(ethylene terephthalate) (PET), poly(carbonate) (PC), poly(styrene) (PS), poly(dimethylsiloxane) (PDMS), poly(ether ether ketone) (PEEK), cellulose acetate (CA), poly(tetrafluoroethylene) (PTFE)) were successfully coated with poly(norepinephrine). Static water contact angles of PN coated substrates were roughly similar (~62°, red bar) even for substrates with vastly different initial wetting behaviors (Figure 1b, blue bar). PN layers on surfaces were characterized by X-ray photoelectron spectroscopy (XPS) and ellipsometry after overnight coating. The XPS spectrum of PN-coated TiO_2 showed no substrate signal (Ti $2p_{3/2}$, 458.5 eV, $2p_{1/2}$, 464.5 eV). Instead, peaks from the atomic composition of norepinephrine, carbon (C 1s, 285 eV), nitrogen (N 1s, 399.5 eV), and oxygen (O 1s, 523 eV), were detected (Figure 2S). The thickness of PN layer (30−40 nm, 18 h coating) was found to be stable in organic solvents such as ethanol, toluene, and acetone, whereas the layer was partly or completely removed upon treatment of 1 N alkali (decreased to ~0 nm) and 1 N acid (decreased to ~20 nm).

Under alkaline conditions the PN coating is expected to retain residual quinone groups that are reactive toward amine and thiol containing biomolecules.[7−9] Thus, facile conjugation of proteins can be easily achieved due to the presence of N-terminal amine and ϵ-amine of lysine in proteins. To demonstrate this, trypsin was conjugated onto PN-coated Si substrates, and the activity of the surface-tethered enzyme was measured by colorimetric observation of the reaction product of trypsin on its substrate (*N*-α-benzoyl-D,L-arginine *p*-nitroanilide) (Figure 2).

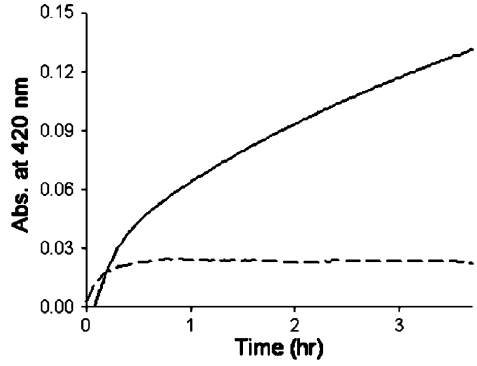

Figure 2. Enzymatic activity of surface-immobilized trypsin on PN-coated Si substrate (solid line) versus unmodified one containing nonspecifically adsorbed trypsin (dashed line).

In addition to biomolecular surface modifications, PN coating allows efficient ring-opening polymerization (ROP) of biodegradable monomers. ROP is a widely used polymerization method for biodegradable polyester coating. Especially, surface-initiated ROP is of interest due to its application in the biomedical areas, such as passivation of biomedical devices.[10] In this case the alkyl hydroxyl group in norepinephrine plays an important role as surface initiator for ROP of lactone monomers. In the presence of tin alkoxide catalyst,[11] biodegradable polymeric thin films can be prepared by the scheme described in Figure 3a.

ROP on the PN layer was investigated using ϵ-caprolactone (ϵ-CL) monomer. After 24-h polymerization of ϵ-CL on the 6-nm-thick PN layer, ellipsometric measurements showed a 34-nm-thick polymeric film of ϵ-CL, and the poly(ϵ-caprolactone) (PCL)-coated substrates became hydrophobic (static water contact angle of 65° → 112°) (Figure 3b,c). Further surface characterization by FT-IR and AFM confirmed successful grafting of PCL to PN. In the FT-IR spectrum, we observed characteristic peaks of polyester (C═O, 1733 cm^{-1}) (Figure S3), and AFM images showed changes in morphology and root-mean-square (rms) roughness. The rms roughness of the substrate was increased after ROP of ϵ-CL: the rms roughness of PCL film grown on the PN was 9.0 nm, whereas the rms roughness of the PN layer was 1.4 nm (Figure S4).

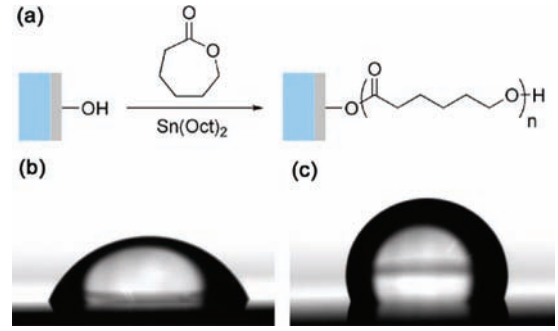

Figure 3. (a) Schematic procedure for ring-opening polymerization of ϵ-caprolactone on the PN layer. Static contact angles of PN-coated gold substrates before (b) and after (c) polymerization of ϵ-caprolactone.

In summary, we developed a facile approach for material-independent surface modification using norepinephrine. Poly(norepinephrine) coatings can serve as a platform for protein bioconjugation as well as ring-opening polymerization of biodegradable polymers. Many types of materials can be modified by this surface modification strategy, which is accomplished under mild aqueous conditions via a dip-coating process.

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Supporting Information Available: Complete list of authors in refs 1b and 4b, experimental details, supporting Figures S1−S4. This material is available free of charge via the Internet at http://pubs.acs.org.

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