Universal Polymer Coatings with Tailorable Bioinert and Biospecific Function

DISSERTATION

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The work presented herein was carried out in the research group of Prof. Dr. Rainer Haag from December 2014 until December 2017 at the Institute of Chemistry and Biochemistry of the Freie Universität Berlin, and in collaboration with Dr. Qiang Wei. in the research group of Prof. Dr. Joachim P. Spatz at the Max-Planck-Institut für medizinische Forschung (MPIMF).

Hereby, I certify that the work presented in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Date and Signature

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1. Introduction

The surface structure and chemistry are essential to solid materials, because they exert a disproportionately large effect on material properties, like surface wettability, adhesiveness, chemical stability, biocompatibility, bioactivity, etc.. Polymer coatings on solid materials play an increasingly important role in modern physical, chemical, and biomedical science.[1] By definition, polymer coating, including the monolayer coating, multilayer coating, polymer brushes, and the surface gel, is a boundary layer between the bulk material and its surrounding phases. All materials interact with the environment through their interfaces (**Figure 1**). Both the kind and the strength of such interactions are largely dependent on the corresponding surface properties.[2] Especially with the fast development and diversification in biomaterial science, there is an increasing utilization of implant devices, blood contacting devices, and biosensors.[3] When a biomaterial contact with a biological environment, the surface chemistry and the surface topography are important parameters that may influence protein adsorption, cell interaction, and ultimately the host response.[4] Biomaterials and medical devices, e.g., artificial organs and biosensors, will quickly induce tissue responses once they are implanted into living tissue or when they get into contact with human blood.[5] Within seconds, nonspecific protein adsorption arises on the implant material surfaces and is quickly followed by cascades of biological response, including foreign body reactions.[6] This biological response may result in the production of a fibrous avascular capsule, which isolates the device from its target tissues, hinders the effectiveness of membranes and biosensors, and prevents drug release from delivery vehicles.[1] Therefore, surface modification of materials is significant and urgent. Under these conditions, surface modification with polymers has drawn much attention over past decades[3,7-10] and has resulted in the developments of polymer chemistry on material surface, coupled with the advanced surface deposition techniques, and is therefore widely employed both in industrial applications and in fundamental research.

Through various fabrication chemistry and engineering approaches, polymer

coatings can be achieved with defined structures associated with designed functionalization.[11,12] Besides the widely used self-assembled monolayer (SAM), Langmuir-Blodgett deposition,[13] layer-by-layer (LbL) assembly,[14] spin coating,[15] chemical vapor deposition (CVD),[16] electrostatic or hydrophobic adsorption[17], surface plasma irradiated polymerization,[18] and polymer grafting[19] are well established. Among all the available surface modification techniques, surface grafting has received great attention over the past few years due to the well-defined and tailorable tethered chain structures with higher grafting density. Benefiting from the advances in anchoring chemistry and the development of polymerization chemistry, especially with the surface-initiation-controlling radical polymerization, e.g., surface-initiated atom transfer radical polymerization (SI-ATPR), surface-initiated reversible addition-fragmentation chain transfer polymerization (SI-RAFT), surface-initiated nitroxide-mediated radical polymerization (SI-NMP), and other techniques, polymer brushes grafted onto various materials surfaces have been greatly developed and broadened their application even in nanotechnologies[20-22] and in the design of bio-interfaces.[23,24] Furthermore, the combination of polymer grafting with lithographic techniques enables the creation of complex surfaces displaying compositionally controlled patterned domains.[19]

However, there are still several problems or limitations of the aforementioned surface modification technologies. Most of them require specific chemical or physical substrate properties and have thus failed to become universal coatings. A universal coating can modify a wide range of material surfaces and is stable under the applied conditions. Ideally, these universal coatings are substrate-independent, regardless of the substrates' chemical composition and physical/structural characteristics. To achieve such coatings, appropriate interactions are required between the coating polymers and the substrate surfaces with intra-coating crosslinking to stabilize them. Moreover, the coatings should present reactive functionalities to be further functionalized.[3] Under these circumstances, mussel inspired catechol/dopamine[25] and polydopamine/polyphenol[26] coating chemistry was developed. Even if this coating

chemistry has several shortcomings, such as not being stable enough on nonpolar surfaces and may dramatically increase the thickness and roughness of the substrates, the mussel inspired coating technology has become the most widely used and new benchmark universal surface modification method in recent decades due to its facile dip-coating approach and nearly substrate independent property.[27,28]

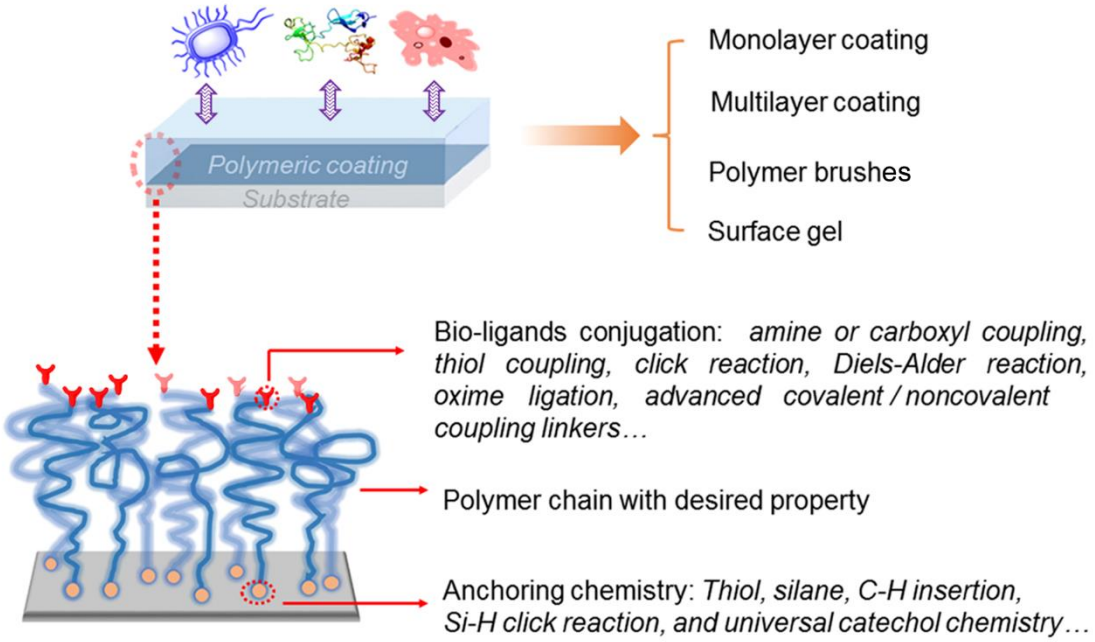


Figure 1. Schematic depiction of the polymer coating, including the monolayer coating, multilayer coating, polymer brushes, and surface gel.

1.1. Chemistry for surface coatings

1.1.1. Anchoring

Functional coatings on solid surfaces are widely utilized to fabricate interfaces with specific properties and characteristics that enable them to interact with their environment in a desired manner,[29] especially the coatings containing reactive functional groups or that can be easily modified under suitable conditions are attractive for various applications that involve immobilization of ligands, peptides and biomolecules.[30] But necessarily and primarily, the functional or reactive coating polymers should be anchored onto the substrate surfaces to form a stable coating layer. With hydrogen bonding, electrostatic, and hydrophobic interactions the surface-

anchoring units can be physically adsorbed onto or be covalently tethered to the functional groups residing on substrate surface (**Figure 2**). However, all the polymeric coatings that are physically anchored onto substrate surface suffer from long-term stability problems when exposed to a liquid medium. This greatly restricts their application in bio- and medical fields. As a result, scientists and engineers have developed several stable and tailorable covalent anchoring strategies. Depending on the interaction mechanism between coating polymers and substrate surfaces, the coating polymers can be strongly and covalently anchored via thiol chemistry (usually for SAMs coating), C-H insertion reactions (suitable for polymeric surface/interfaces modification), silane chemistry (for inorganic substrates modification), Si-H click reaction (PDMS modification method), [2+1] cycloaddition (works well on carbon materials), and the universal catechol chemistry.

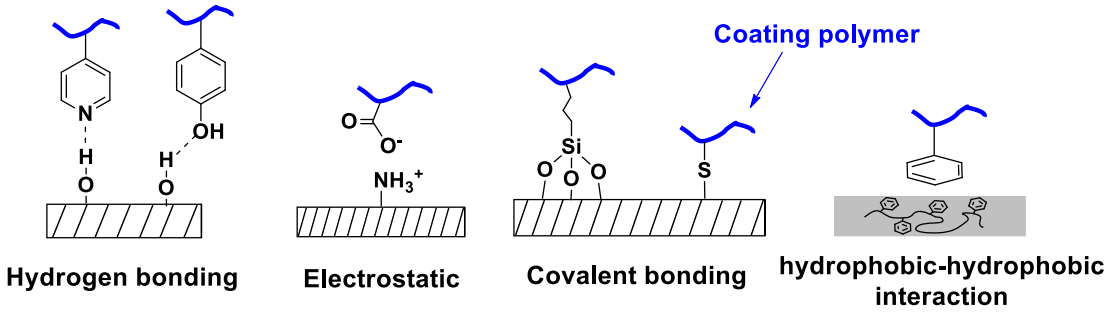


Figure 2. Typical anchoring interaction that immobilize coating polymers with substrate surfaces via hydrogen bonding, electrostatic interaction, covalent bonding, and hydrophobic-hydrophobic interaction.

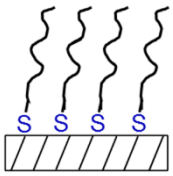
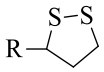
Thiol anchoring groups

Thiols and disulfides can quickly chemisorb onto gold (Au) surface and then self-assemble into a dense monolayer coating, namely SAMs (self-assemble monolayer coatings). This monolayer coating exhibits good molecular order and is relatively stable in ambient conditions. SAMs are key elements in nanoscience and nanotechnology to link inorganic, organic, and biological materials to planar Au surfaces or Au nanoparticles (AuNPs).[31] The initial stage of thiol chemisorption on Au (111) involves the formation of lying-down molecules layer. Upon an increase of surface coverage, a

transition from the lying-down to a standing-up configuration takes place, with the formation of domains of the dense and stable ($\sqrt{3} \times \sqrt{3}$)-R30° and c (4 x 2) thiol lattices, which can coexist on the substrate.[32,33] The chemisorption process induces strong changes in the substrate with the formation of vacancy islands of monatomic depth in the case of aliphatic thiols and of gold islands of monatomic height in most of the aromatic thiols.[34]

A thiol molecule for SAMs usually consists of three parts: i) the sulfur head group, which forms a strong, covalent bond with the metal substrate surface, ii) the hydrocarbon chain (with variable length), which stabilizes the SAM through van der Waals interactions, and iii) the terminal group, which can have different functionalities.[33] The energy related to each part of the molecule has a different order of magnitude: 50 kcal mol^{-1} for the interaction between the S headgroup and the substrate (a thiolate bond), 1-2 kcal mol^{-1} per methylene for the van der Waals interactions between hydrocarbon chains, and only a few kT for energies related to the terminal groups.[35] All three parts of the molecule contribute to the structural, physical, and chemical properties of the SAMs. The commonly used thiols and dithiols for SAMs are alkanethiol (nonanethiol), arenethiol, alkanedithiol, dialkyldisulfide (dinonyl disulfide), and dialkylsulfide (dinonyl sulfide). All these molecules can be self-assembled on different metallic surfaces, e.g. Au (the most important one), Ag, Cu, Pd, Pt, Ni and other semiconductor surfaces (**Table 1**). Since their discovery at the beginning of the 1980s by Nuzzo and Allara,[36] the thiol and dithiol SAMs on metals and particularly on Au, have attracted considerable attention due to their easy preparation, their relatively high stability mediated by the strength of the S-Au bond and by van der Waals interactions, and their easy post modification via the terminal group.

Table 1. The common used thiols and substrates for SAMs coating.

Thiol and dithiol ligands	Substrate	Ref.	Thiol and dithiol ligands	Substrate	Ref
RSH	Ag	37	RSH	Pd	38
	$Ag_{90}Ni_{10}$	39		Pt	40
	AgS	41		Zn	42
	Au	43		ZnSe	44
	AuAg	45		ZnS	46
	CdSe	47	RSAc	Au	48
	CdS	49	RSR’	Au	50
	Cu	51	RSSR’	Ag	52
	GaAs	53		Au	54
	Ge	55		CdS	56
	Hg	57		Pd	38
	Ni	58		Au	59
	PdS	60		Au	61

C-H insertion reactions

With the fast development and diversification in biomaterial science, there is an increasing utilization of polymeric materials, especially for implant devices, blood contacting devices, and biosensors.[3] Polymeric biomaterials used for these purposes are mostly selected on the basis of their bulk mechanical properties, rather than on the suitability of their surface properties. However, the adsorption of blood proteins on the surfaces initiates a cascade of biological responses and also hinders the effectiveness of the internal body-attached sensoric or implant devices.[1,9,62] Therefore, surface modification of polymeric biomaterials has been intense interest recently. Although there is a vast body of literatures regarding strategies for material surface modification,[63-65] it is still difficult to construct dense and stable polymer coatings on nonpolar polymeric surfaces, e.g., polyolefines, such as polystyrene (PS) and extremely unreactive surfaces, i.e., polydimethylsiloxane (PDMS) and polytetrafluoroethylene (PTFE), due to the lack of functional surface groups. The polymerization mainly involves the C-C bond chemistry, i.e., breaking and coupling. Except the stable C-C building blocks, most polymeric materials have abundant C-H bonds on their outermost

surface[66] to provide potential high-density reaction sites. To resolve the chem-inert problem of polymeric material surface and fabricate stable coatings onto it, novel efficient surface C-H activation, especially the saturated sp^3 C-H σ bond, is promising and desirable. In addition, the development of surface C-H activation chemistry can also avoid undesired surface degradation due to C−C or ester cleavage during surface modification, which often induce deterioration of bulk/surface properties such as the surface roughness, chemical stability, and the adhesion strength of coatings.[67,68] However, the reactivity of C-H bond on material surfaces or interfaces is pretty low because the C-H bonds are usually "frozen" in a matrix. It is hard for them to change their steric conformation to adjust the distance with the reactants on the surface and provide sufficient proximity to react. In order to improve the reactivity or produce enough reactive sites on the surface or at interfaces, harsh processes such as ion irradiation,[69] plasma treatment,[70] electrochemistry,[71] as well as enzymatic methods[72] have been adopted. But most of them often give rise to an uncontrolled dissociation of chemical bonds other than C-H and result in unnecessary physical or chemical changes such as the introduction of multiple functional groups, etching, and morphological alterations. Therefore, C-H insertion reaction on the surface / interfaces has been developed as an outstanding method for polymeric materials surface modification. The C-H insertion reactions only need reactants (or anchoring groups) that can form reactive intermediates such as radicals, nitrenes, and carbenes upon excitation without any special substrate pretreatment. The excitation of the anchoring groups usually involves acceptably short UV irradiation and low temperature incubation. Reported groups that can conduct such reactions are benzophenone, sulfonyl azide, phenyl azide, and aryl diazo compound.[73-77] After activation, the reactive intermediates generated from these groups will react with any neighboring C-H groups through a C-H insertion reaction regardless of their chemical nature leading to a crosslinking of the coating polymer (**Figure 3**). Consequently, groups adjacent to the surface react with C-H bonds of the substrate and thus form a network that attaches to the substrate.

Taking benzophenone as an example, upon the irradiation of UV light,

benzophenone groups will undergo an n, π* transition into a biradicaloid triplet state. In this state, the molecule can abstract a hydrogen atom from almost any neighboring aliphatic C-H group. The two resulting carbon-based radicals generated by the hydrogen abstraction process can recombine and form a covalent C-C bond, leading to covalent cross-linking.[78,79] The neighboring aliphatic groups can be part of other polymer chains and the substrate as well. The newly generated C-C bond enables the coating molecules to covalently attach to the substrate (especially for the polymer-based substrate) as an ultrathin film layer, which is desirable to enhance the stability of the coatings against solvents and displacement reagents. Whereas, once the UV radiation is removed, the cross-linking reaction of benzophenone will quickly cease, which minimizes post curing effects.[80] Another merit of benzophenone is its good chemical stability in the absence of light, compatibility with long wavelength UV light (~360 nm), and preferential reactivity toward otherwise unreactive C-H bonds even in the presence of water.[81] Except for benzophenone derivatives, other compounds that generate radicals, nitrenes, and carbenes upon excitation can also be introduced into coating polymers as anchoring groups.

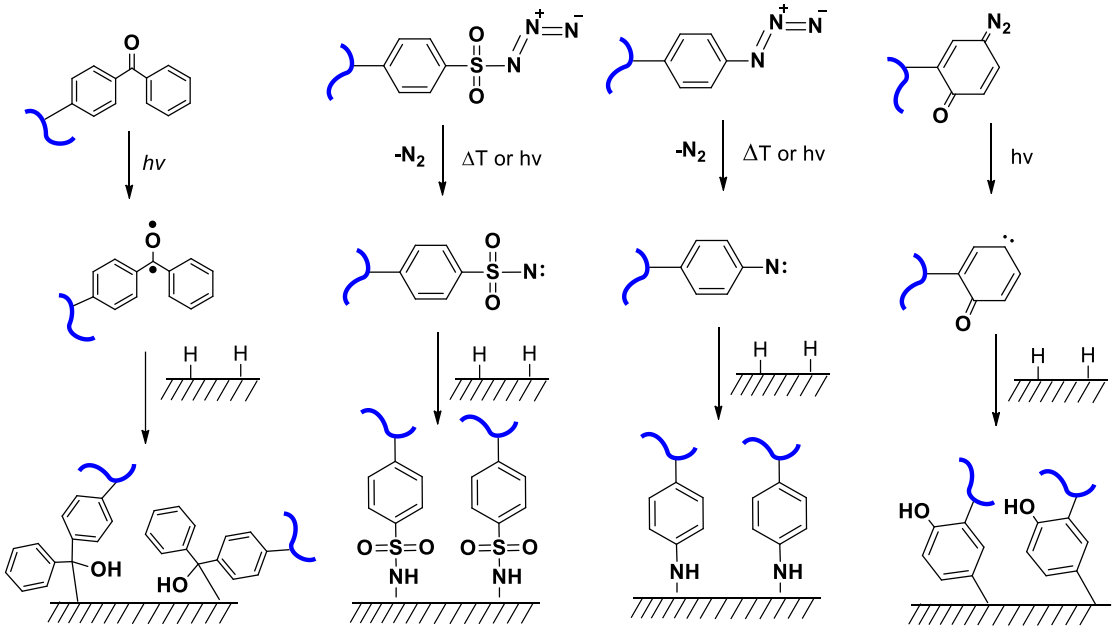


Figure 3. Generation of surface-attached polymer networks via C-H insertion reaction of benzophenone, sulfonyl azide, phenyl azide and aryl diazirine.

Catechol anchoring groups

Marine mussels bind tightly to virtually all types of material surfaces by secreting various types of mussel adhesive proteins (MAPs) such as *Mytilus edulis* foot proteins (Mefp's).[82,83] Among these proteins, Mefp-1 resides in the cuticle of byssal threads and forms a hard outer sheath, which protects the collagenous inner core. Mefp-2 comprises a major component of byssal threads' terminal adhesive plaque, while Mefp-4 located in byssal plaques links the plaques and collagenous threads. Mefp-3, Mefp-5, and Mefp-6 preferentially distribute at the adhesive interface with the substrate surface.[90] Studies discovered that all these proteins are highly rich in lysine and 3,4-dihydroxyphenyl-L-alanine (DOPA),[84,85] which is believed to contribute to the crosslinking of the proteins and form strong covalent and noncovalent interactions with surfaces. Inspired from this mussel adhesion property, catechols, pioneered by Waite[86] and Grätzel[87] and later established by Messersmith,[25] have been recognized as novel and efficient anchoring groups for developing substrate independent coatings. Many different catecholates, which range from synthetic derivatives to natural products in monomeric and/or polymeric forms, have been used to modify various surfaces (**Figure 4**). In a typical coating protocol, the catechol groups in dopamine is first oxidized into quinone in alkaline buffer solution or in the presence of oxidant.[25] The quinones form dopamine dimer via catechol-quinone coupling and dihydroxyindole (DHI) via intramolecular cyclization and then further undergo self-polymerization and aggregate to form a coating layer via hydrogen bonding and π-stacking.[88] Polydopamine and compounds with catechol anchoring groups have been used to coat noble metals (Au, Ag, Pt, Pd) and metal oxides surface (TiO_2, Fe_3O_4, Al_2O_3) via the charge-transfer complex, ceramics (glass, mica, silica, hydroxyapatite) via hydrogen bondings, polymeric materials (PS, PE, PC, PET, PTFE) and carbon materials (graphene, graphene oxide, carbon nanotube) via hydrophobic interaction, π-stacking, and van der Waals' forces. In addition, lateral crosslinking by both covalent and noncovalent bonding could further enhance the stability of polydopamine coatings.[28,89,90] Moreover, under base or oxidizing conditions, the catechols of polydopamine coating can further react with

active nucleophiles (thiols and amines) via Michael addition or Schiff base reactions.[82,84] This enables polydopamine coating to be a versatile platform for further functionalization, thus opening up the possibility of tailoring the coating for various applications.

Tannic acid (TA) is a kind of polyphenol that is rich in tea, red wine, and other plants. This biomolecule is composed by abundant catechol (1,2-dihydroxyphenyl) and gallol (1,2,3-trihydroxyphenyl) functional groups and thus exhibits good affinity to versatile substrates and self-polymerized properties similar to dopamine.[91] Under alkaline condition, they quickly anchor onto the substrate from buffer solution and self-polymerize to form polydopamine-like coating films. Besides TA, other precursors with multi-phenols were pyrogallol, epigallocatechin gallate (EGCG), epigallocatechin (EGC), catechin (Ctn), catechol (Ctl), hydroxyhydroquinone (HHQ), and morin (**Figure 5**), which also have similar anchoring and solid-liquid interfacial properties.[92]

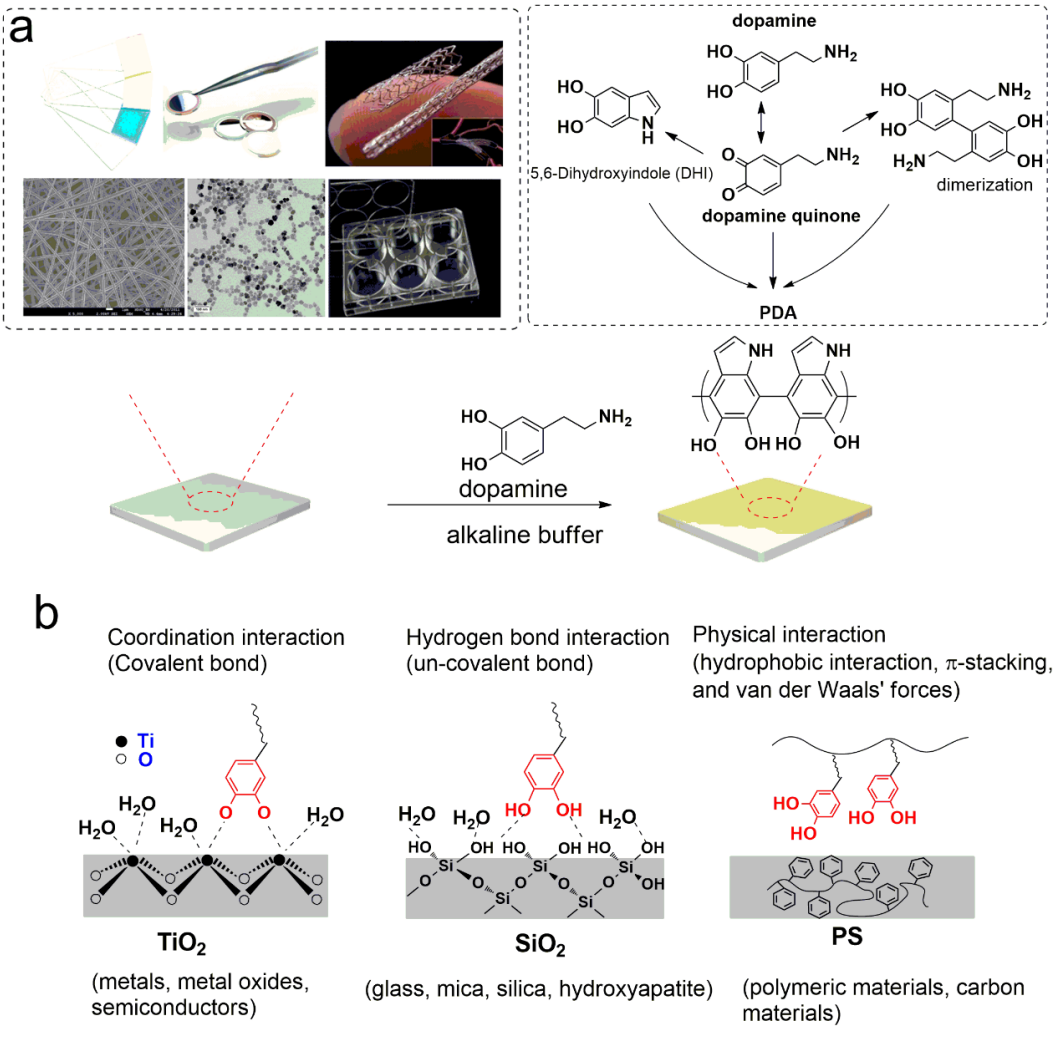


Figure 4. (a) Various substrates can be coated with dopamine and the reactions of dopamine in a weak basic buffer. (b) The interaction mechanisms between catechol groups and different kinds of substrates.

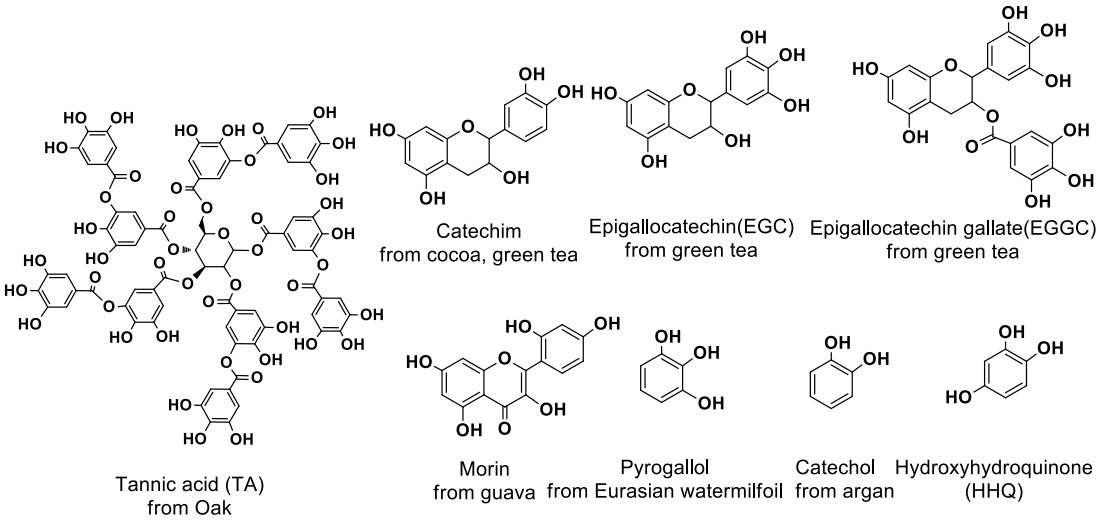
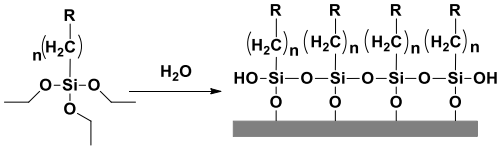
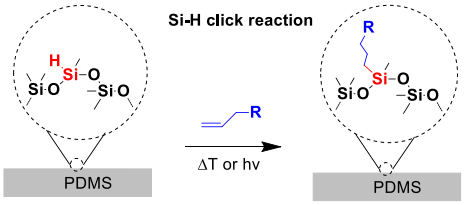
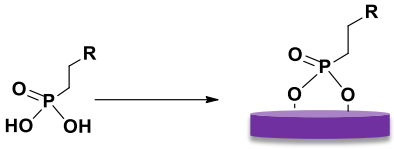
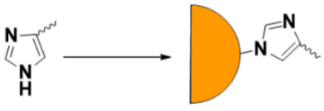


Figure 5. Chemical structures of the natural and synthetic phenols that have been identified to form universal coatings.

Table 2. The other common used anchoring reactions to immobilization polymer coating onto substrate surface.

Anchoring	Substrates	Schematic representation of the interaction
Silane	Inorganic surface bearing OH groups	
Allyl groups (Si-H click reaction)	PDMS with reactive Si-H	
Phosphonates	Hydroxylated metal oxide	
Imidazole	gold nanoparticles (AuNPs), quantum dots (QD)	

Apart from the aforementioned anchoring methods, polymers with silane groups,

allyl groups, phosphonate groups, and histidine groups can also be covalently immobilized onto inorganic surfaces,[93,94] PDMS surface,[95] hydroxylated metal oxide surface,[96] and gold nanoparticles (AuNPs) / quantum dots (QD)[97] (**Table 2**), just to name a few.

1.1.2. Post-modification

Post modification is a powerful technique to introduce functionality into an established polymer coating. The secondary functionalization of the coatings is normally required for specific surface characteristics, e.g., bioinert coatings with antimicrobal agents, functional surfaces with bio-specific ligands for the study of fundamental biological interactions, specific proteins adsorption, rare cell capture and detection, tailorable bio-interface for cell adhesion, migration, differentiation, and bio-sensing platforms. Ligands with specific bio-functionalities are usually small bioactive molecules, peptide sequences, oligonucleotides, and proteins. Therefore, the reaction to conjugate these ligands onto coating surface generally involves an amine or carboxyl coupling, thiol coupling, surface click reaction, and covalent coupling via other active groups. In addition, bifunctional linkers, 4-nitrophenyl chloroformate (NPC), 1,1'-carbonyldiimidazole (CDI), N, N'-disuccinimidyl carbonate (DSC), 3-(maleimido) propionic acid N-hydroxysuccinimide ester (BMPS), 4-(maleinimido) phenyl isocyanate (PMPI), sulfosuccinimidyl 6-(4'-azido-2'-nitrophenylamino) hexanoate (Sulfo-SANPAH), and several bio-linkers are also frequently used.

Covalent coupling reactions

Amines are by far the most common target groups for covalently coupling peptides and proteins. The high abundance of available, surface-exposed amine functional groups means that almost any peptide or protein may be covalently bound to substrates in this way.[98] It is relatively easy to conjugate active amine-bearing bioligands onto a coated surface with carboxyl groups to form an amide bond (typically activated with carbodiimides such as EDC), with hydroxyl to form a carbamate, with aldehyde via Schiff base addition, with isocyanate to form a carbamide, with halogen via

nucleophilic substitution, with epoxide groups, with anhydride, with easy labile pentafluorophenyl and p-nitrophenyl activated ester, and with aryl azide via photocoupling (**Figure 6**). To improve amide formation, coupling agents, DCC (N, N'-dicyclohexyl carbodiimide)/DMAP (4-dimethylakminopyridine), DIC (N, N'-diisopropylcarbodiimide), CDI (1,1'-carbonyldiimidazole), EDCI (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide)/NHS (N-hydroxysuccinimide), are usually used. However, many of these reagents are water sensitive, which can drastically decrease the yield, while evaporating the ligand solution during conjugation can be used to improve yields.[99] Some bioligands, such as biotin, fluorescent dyes, antitumor drugs, antibiotics, are usually activated with NHS. In this case, a coating surface with many active amino groups is a good platform for their immobilization.[100]

Thiols are also commonly used reactive groups with high reactivity for a coating surface post functionalization. Among them, the Michael addition is a frequently used method to graft thiol-ligands onto surfaces with alkene-containing reactive groups such as maleimides, acryloyls, acrylate and vinyl sulfones.[101,102] Maleimides are the most commonly used for immobilizing ligands to surfaces, either directly or to surface amines via an advanced coupling agent, sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (Sulfo-SMCC).[103] Thiol-ene click chemistry has also become popular for spatially controlled immobilization of ligands.[104,105] Another potentially useful chemistry is the use of 2-pyridyldithiol-based crosslinker to form a disulfide bond.[106] Studies shown that haloacetyl group can also bind thiols,[106] but it is rarely used for surface secondary coupling.

The click chemistry concept was introduced by Sharpless and co-workers in 2001.[107] The term "click" refers to a reaction family that display particular properties, including high specificity, stable linkages with orientation control, high yield under mild conditions, and some degree of bio-orthogonality. This highly useful reaction has been widely used in materials design[108,109] and certainly can be used for ligands secondary coupling reactions.[110-112] The copper-mediated azide-alkyne click (CuAAC) reaction is the most well-known one. But the CuAAC requires copper as a catalyst, which might

interact with some proteins in an unwanted fashion during the reaction. The cytotoxic Cu^{2+} (even at low concentrations) is a major issue when this reaction is used for biological applications. Even a highly efficient chelating agent such as ethylenediaminetetra acetic acid (EDTA) can be used to remove the Cu^{2+}, but it is difficult to completely remove Cu^{2+} from the coating surface after reaction. Therefore, there has been significant interest in developing alternative click reactions that do not require any metal catalyst. One elegant approach, involving the reaction of azides with cyclooctyne derivatives, was reported by Bertozzi and co-workers.[113] The high strain in the cyclooctyne greatly promote the azide-alkyne [3+2] cycloaddition reaction and this kind of metal free click reaction is also termed strain-promoted azide-alkyne cycloaddition (SPAAC). The bioligands with difluorinated cyclooctyne (DIFO),[113] bicyclo[6.1.0]nonyne (BCN),[114] and dibenzocyclooctyne (DIBO),[115] biarylazacyclooctynone (BARAC or DBCO)[116] all can be clicked onto coating surface via the SPAAC reaction, whereby the activity of BCN and DBCO is higher than the others.[114,116,117]

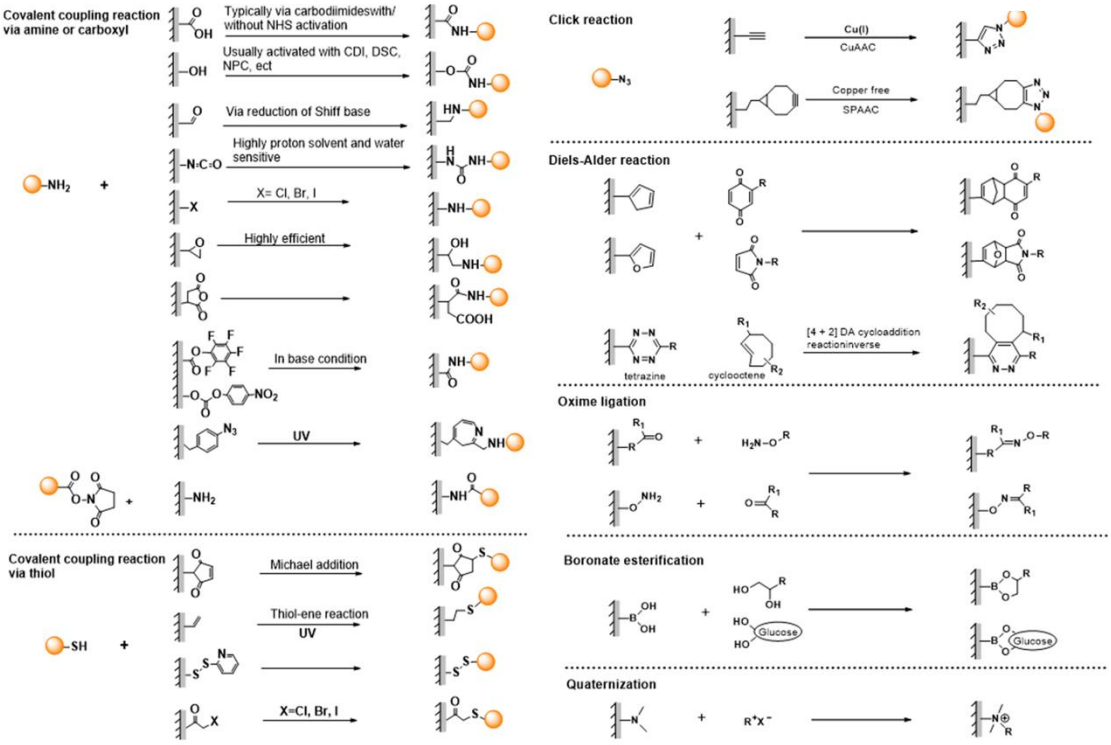


Figure 6. Covalent coupling reactions for ligand post grafting onto a precoated surface, including the coupling reaction via amine or carboxyl, the coupling reaction via thiol, click reaction, Diels-Alder reaction, oxime ligation, boronate esterification, and quaternization.

Diels-Alder (DA)reaction is another kind of click reaction with high bio-orthogonal, which is involved in the conjugation of a diene to a dienophile.[118] Compared with other click reaction, e.g., CuAAC, the DA reaction is a little slower but it does not require any catalyst. The classical diene and dienophile used in DA reactions are furan and maleimide respectively. This reaction is thermally reversible at temperatures higher than 120 °C.[119] Otherwise, this reaction is able to be performed in the presence of many other reactive groups, for example, the DA and the CuAAC reaction can be used synchronously to co-immobilize two different ligands.[120] The cycloaddition of tetrazines and trans-cyclooctene derivatives is based on the inverse electron demand Diels-Alder reaction.[121] The rate of the ligation between trans-cyclooctene and tetrazine is very rapid (k_2 *2000* M^{-1} s^{-1}), the N_2 is the only one byproduct. It tolerates a broad range of functionality and proceeds with high yield in organic solvents, water, cell media, or cell lysate. This fast reactivity enables protein modification at low concentration.[122] The utility of this reaction is also demonstrated by the specific labeling proteins on cell membrane for cell imaging.[122,123]

Oxime ligation was reported to modify quinone activated coating polymers with oxyamine functional ligands in several studies.[124-126] This reaction involves in the aketone/aldehyde and an oxyamine, resulting in a stable oxime bond. The oxime ligation reaction is not sensitive to water and can be conducted in aqueous medium, which is beneficial for the bioligands immobilization. Pauloehrl et al.[127] used it to graft RGD peptide onto an aldehyde patterned surface, revealed by photocleavable protection groups.[127] The boronate esterification and the quaternization are often used to functionalize the side groups of polymer brushes[128,129].

Short peptides and synthetic ligands can generally be designed with almost any

desired reactive groups. However, it is more challenging to modify proteins to introduce some reactive groups onto it. In addition, except for hydroxy or carboxy groups, it is very hard to find any other reactive groups on polymer chains. Moreover, the hydroxy groups in polymers (PHEMA, polyglycerol, PHPMA) are also not reactive enough to do a direct ligand immobilization. In this situation, bifunctional linkers are developed for bioligand coupling. The commonly used bifunctional linkers are 4-nitrophenyl chloroformate (NPC), 1,1'-carbonyldiimidazole (CDI), and N, N'-disuccinimidyl carbonate (DSC), 3-(maleimido) propionic acid N-hydroxysuccinimide ester (BMPS), 4-(maleinimido) phenyl isocyanate (PMPI), sulfosuccinimidyl 6-(4'-azido-2'-nitrophenylamino) hexanoate (sulfo-SANPAH). Among them, the water soluble sulfo-SANPAH is usually used to functionalize polyacrylamide (PA) surface via the photocoupling between the NH_2 groups from PA and the phenyl azide groups from sulfo-SANPAH, and then NH_2-terminated peptide sequence or proteins are immobilized onto material surface via NHS-coupling.[130]

Non-covalent coupling methods

Apart from those chemical linkers, avidin has also been used in many cases as a bio-linker to conjugate proteins (most of them are antibodies) onto a surface based on the biotin-avidin noncovalent interaction.[131-133] Typically, the surfaces are activated with avidin or its derivatives such as streptavidin and NeutrAvidin™ prior to conjugation. The pre-biotinylated ligands are subsequently incubated with the avidin functionalized surface. The yield of the biotin-avidin noncovalent interaction is much higher even when incubation was performed at a low concentration (below 10 μg/mL) with the presence of other proteins in the buffer. The resulting bioactivity is comparable to the EDC/NHS protein conjugation chemistry.[134] In addition, this conjugation method has several advantages for ligand immobilization: i) the interaction between biotin and avidin is the strongest known non-covalent bond (dissociation constant 10^{-15} for avidin), ii) the biotin is a small molecule and considered unlikely to interfere the bioactivity of ligand, iii) the conjugation is very simple and the interaction occurs under mild conditions, iv) consistent orientation of the ligand can be achieved when a single biotin

is added.[135] Another popular coupling method is using metal complexes that can selectively bind oligohistidine residues (His-tag), a tag that is commonly used for the purification of recombinant proteins. Copper and nickel complexes of nitrilotriacetic acid (NTA) have been coupled onto polymer brushes to capture enzymes and His-tagged proteins.[136,137] The polymer brushes functionalized with benzylguanine residues (BG) exhibited similar property, which can specifically couple with alkylguanine-DNA-alkyltransferase (AGT) fusion proteins.[138] This approach offers an excellent control of protein orientation in mild and dilute conditions. Moreover, the human serum albumin/albumin binding domain[139] and barnase/barstar[140] protein pairs also exhibit a high-affinity and noncovalent binding property.

1.2. Polymer brushes

Polymer brushes are ultrathin polymer coatings consisting of polymer chains that are tethered with one chain end to the surface (impenetrable interfaces). At high grafting densities, i.e., when the distance between neighboring grafting points is small, steric repulsion leads to tethered chain stretching and thus forming a brush-type conformation.[141] Surface modifications with polymer brushes play an important role for solid materials in physical, chemical, and biomedical sciences.[142,143] Generally, there are two main methods to fabricate polymer brushes: the physisorption and the covalent attachment (chemisorption). For the physisorption, block copolymers adsorb onto a suitable substrate with one block (typically the hydrophobic block) interacting (noncovalently) strongly with the surface while the other block interacts weakly with the substrate. The covalent attachment can be accomplished by either “grafting to” or “grafting from” approaches. The “grafting to” technique employs a preformed polymer with a reactive end group (anchoring group) to covalently attach the polymer chains onto the substrate. In the “grafting from” strategy, polymer chains are directly synthesized from substrate surface that was previously modified with polymerized initiators or chain transfer agents. It is also called the ‘‘surface-initiated polymerization’’, in which the polymerization occurs exclusively at the surface.

Especially, with the fast development in surface-initiated controlled radical polymerization (SI-CRP), it is easier to synthesize well-defined polymer brushes with precisely controlled polymer architecture, composition, molecular weight, and ultimately brush thickness.

1.2.1. Polymer brushes fabricated via physisorption

The physisorpation involves a process that reversibly tethers polymer chains onto a solid surface without covalent bonding. Usually, the physisorption is achieved by the self-assembly of amphiphilic block copolymers or end-functionalized polymers on a solid surface.[144] In the presence of a selective solvent, the anchoring block, which can strongly and noncovalently interact with the substrate, diffuses from the solution phase and attaches onto the surface of the substrate. While the other block (the "buoy" block), which does not strongly interact with the substrate surface, will be stretched up to form the "brushes" on the surface.[145] In the case of a selective solvent, the ideal solvent is a precipitant for the "anchoring" block to deposit on the surface and a good solvent for the "buoy" block which forms polymer brushes in the solution.[146] The broadly understood process of physisorption consists with (i) an initially fast diffusion regime, during which the polymer chains quickly diffuse from the solution onto substrate surface, (ii) followed by a conformational transition from mushroom to brush and (iii) a slow buildup of dense brushes by the penetration of free chains through the existing brush layer to form a monolayer brush coating with high density.[147-149] The grafting density and other characteristic parameters of brushes are controlled by the thermodynamic equilibrium of block polymers in selective solvent, albeit with possible kinetics.[150] It is very simple to prepare polymer brush coating by using physisorption. However, several problems are inherent with such an approach. The first and the main problem is the stability of the resulting polymer brush. It is easily detached by surfactant, proteins, and even the liquid medium because of the weak, noncovalent interaction with substrate. Since the coating process occurs in a selective solvent, it is not easy to find a suitable solvent. Besides, the concentration of the block copolymer is also very

important. It has been shown that if the concentration of the amphiphilic block copolymer was higher than the critical micelle concentration (CMC), the block copolymer would form micelles in the selective solvent and the adsorbed surface layer itself may have a micellar structure instead of smooth monolayer brushes.[151] A further problem is that the thickness of the monolayer prepared by this technique is very thin with dry film thicknesses typically between 3nm and 5nm. Furthermore, the resulting grafting density is also not very high. The reason for this can be ascribed to the kinetic hindrance for the attachment of polymer chains due to a diffusion barrier created by the already attached molecules.[145]

1.2.2. Polymer brushes fabricated via "grafting to"

The "grafting to" approach refers the interfacial reaction between end-functionalized polymers and a suitable substrate under appropriate conditions to form strongly tethered polymer brushes (**Figure 7**). It is also termed chemisorption. The covalent bond formed between substrate surface and polymer chain makes the polymer brushes robust and resistant to common chemical environmental conditions. This method has been used often in the preparation of polymer brushes. A famous and classical example is the SAM coating. The "grafting to" technique is highly dependent on the anchoring groups in the polymer chain and selective for the substrate, which can react with the anchoring groups. In addition, the "grafting to" technique also suffers from the low grafting density due to steric repulsion from the already grafted chains that in turn preclude the access of new polymer chains to grafting sites on the surface. This effect is more pronounced when dealing with end-functionalized polymers with high molecular weight.[152]

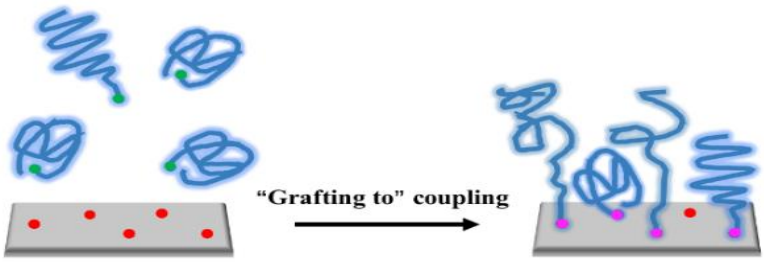


Figure 7. General depiction of the "grafting-to" method to prepare polymer brushes coating.

1.2.3. Polymer brushes fabricated via "grafting from"

To circumvent those problems in physisorption and "grafting to" methods, the "grafting from" approach was introduced and has become more attractive in preparing thick, covalently tethered polymer brushes with a high grafting density.[153-155] In the "grafting from" approach, initiators or chain transfer agents are immobilized onto the substrate surface first and followed by an *in situ* surface polymerization (**Figure 8)**. The initiators and chain transfer agents are typically small molecules. Therefore, the "grafting from" is more promising in the synthesis of polymer brushes with a high grafting density. In addition, this method introduces versatility, reliability, and controllability to the formation of dense polymer brushes and can be implemented with almost all available developing controlled radical polymerization techniques: the surface initiated-atom transfer radical polymerization (SI-ATRP),[155,156] surface initiated-reversible addition fragmentation chain transfer polymerization (SI-RAFT), [19,157] surface initiated-nitroxide mediated polymerization (SI-NMP),[158,159] surface-initiated photo-iniferter mediated polymerization (SI-PIMP), [160,161] and the surface-initiated ring opening polymerization (SI-ROP), [162] just to name some examples.

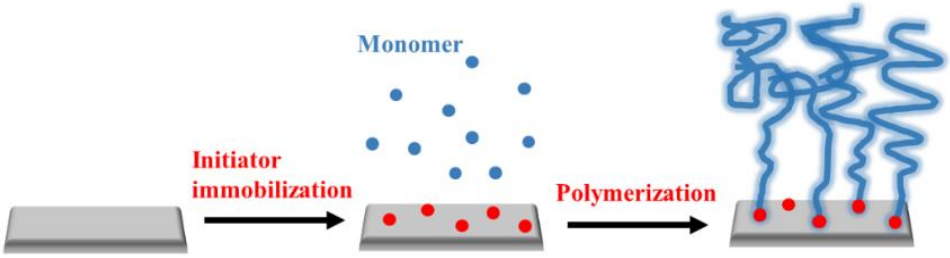


Figure 8. General depiction of the "grafting-from" method to prepare polymer brushes coating.

SI-ROP

With the increasing demand for antifouling surfaces, lots of hydrophilic polymers with high fouling resistance performance are coated onto surfaces. The industry standard in polymeric antifouling coatings are the polyethylene glycol (PEG).[163] Additionally, the polyglycerol and other zwitterionic polymers also have the same antifouling level. The zwitterionic polymers usually introduced onto surfaces via SI-CRP.[164] For PEG and

polyglycerol, the main coating approach is the “grafting to” method. As be discussed afore, the “grafting to” requires the integration of anchoring groups to the polymer chain and the resulting grafting density is also relative lower. To circumvent this challenge, Huck and co-worker activated the silicon wafer with sodium methoxide to deprotonate the silanol groups and then directly polymerized glycidol from the surface (**Figure 9**).[162] A dense hyperbranched polyglycerol brush was formed via the surface-initiated anionic ring opening polymerization (SI-ROP). To avoid bulk polymerization upon addition of the monomer, the sodium methoxide solution should be completely removed with thoroughly rinsed and dried.[165] The SI-ROP has also be used to prepare polymer brushes of poly(N-propionylethyleneimine) (PPEI),[166,167] poly(ε-caprolactone) (PCL),[168] poly (lactic acid) (PLA),[169] poly(L-glutamate),[170] and poly(norbornene)[171] via immobilized initiator on versatile surfaces for special application.

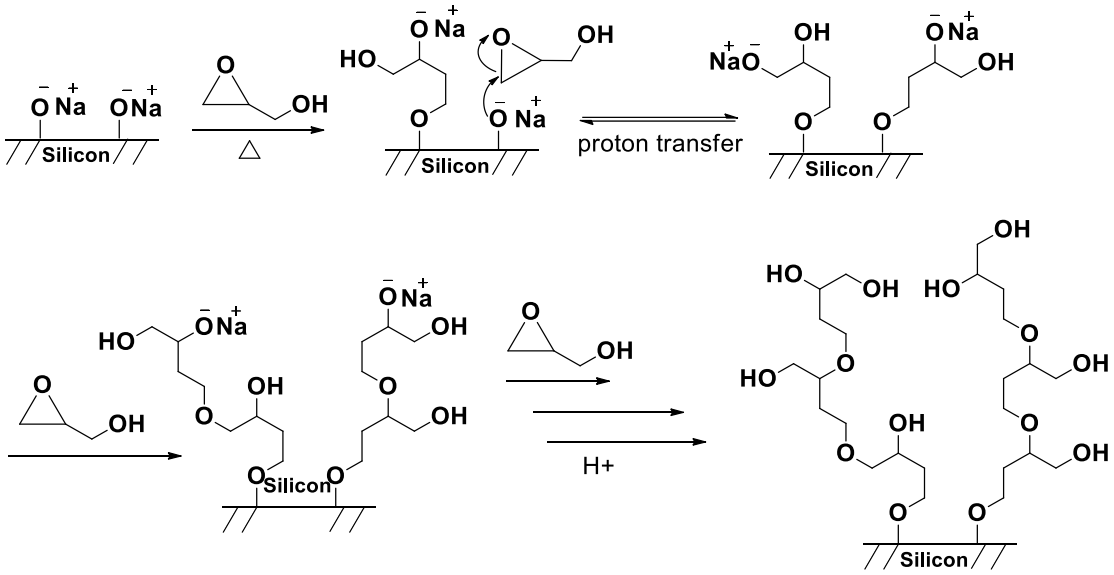


Figure 9. Proposed mechanism for the formation of hyperbranched polyglycidol on a Si wafer surface via anionic ring-opening multibranching polymerization.[162]

1.3. Bioinert polymer coatings

Surface biofouling, which is mainly caused by spontaneous nonspecific protein adsorption, is a ubiquitous and serious problem that may stimulate deleterious biological processes orchestrated by the immune and coagulation systems. It has greatly

limited the application of biomaterials in medical implants, surgical equipment, biosensors, etc.[9] The nonspecific protein adsorption on materials is often associated with surface hydrophobicity.[172] Proteins undergo conformational changes to associate their hydrophobic domains with the material and their hydrophilic domains with the biological environment to create a substantial reduction in surface energy. This reduction outweighs the entropic cost of the conformational change, which also thermodynamically favors protein adsorption onto a hydrophobic material surface.[173] Up to now, modifying material surface with bioinert polymer coating is recognized as a best solution to reduce surface fouling. In designing these bioinert polymer coatings, three main required properties should be kept in mind, i.e., the hydrophilicity, the ability to form hydrogen bonds with water, and the conformational flexibility.[174,175] An additional hydration layer provided by the polymers that prevents protein adhesion is also needed. Based on these principles, many types of bioinert polymers have been developed and employed to effectively decrease the protein adsorption on material surfaces including polypeptides and polypeptoids,[9] oligo-/poly (ethylene glycol) (PEG)s,[176] oligo-/polyglycerols,[177] zwitterionic polymers,[164] polyoxazolines,[178] etc.

To theoretically understand the mechanism of protein resistance, PEG SAMs was selected as the model coating.[174-176,179,180] PEG coatings were recognized as utilizing a passive strategy to increase surface hydrophilicity and resist protein adsorption. PEG polymer chains can hydrogen bond with large amounts of water to form a hydration layer. This layer is sustained by the presence of a hydration pressure that serves as an energetic barrier to non-specific protein adsorption. Additionally, its polyether backbone is inherently flexible. These flexible chains, which are presented as polymer brushes on a surface, would create configurational mobility that blocks the potential sites of protein adsorption due to steric excluded volume effects. In this case, the entropic energy cost to surpass PEG brushes is too high for proteins to favorably adsorb onto a coating surface and hence the protein resistance is achieved. The grafting density of polymers on a given surface and the length of polymers chains are two important parameters in the protein resistant coating. The higher grafting density results in a

higher coverage of the coating polymers. With the increase of chain length of coating polymer, the grafting density should consequently decrease. But the resulting coating thickness correspondingly increases, thereby creating a kinetic barrier that prevents protein adsorption on the surface. Moreover, Grunze and co-workers found that the conformation of the OEG polymer chains on the surface significantly influenced the performance of protein resistance.[181] OEG chains in the SAMs formed on gold surface adopt a helical conformation, which was inert to protein adsorption. In contrast, OEG chains in the SAMs formed on silver surfaces had a trans-conformation, which could not impede the adsorption of proteins.[181] Monte Carlo simulations indicated that the helical conformational is more disordered compared with the trans-conformation. The water molecules can penetrate these coatings more easily, resulting in more water to interact with OEG chains. This result suggests that the interaction with water plays a vital role for protein resistance.[182,183]

Even though PEG is the most commonly used coating polymer to impart protein resistance on a surface, it tends to auto-oxidize and form aldehydes and ethers in the presence of oxygen, which may cause the surfaces to lose their protein resistance ability.[184] Investigation also indicated that the PEG chains undergo degradation in vivo in the presence of enzymes,[185] which gives toxic metabolites.[186] These concerns have prompted research into alternative PEG polymers. One class of polymers is the poly(2-oxazoline) (POx), particularly the two hydrophilic variations, poly(2-methyl-2-oxazoline) (PMeOx) and poly(2-ethyl-2-oxazoline) (PEtOx).[178] Similar to PEG, the POxs can form a hydration layer when tethered onto substrates, and exhibits good antifouling property.[187] In addition, they also have several advantages such as higher stability, lower viscosity, broad variety of oxazoline monomers, as well as functional initiators or terminating agents allowing the synthesis of tailor-made POx.[178] Also, PMeOx has also been found to have good physiological stability.[188]

Another good alternative is the polyglycerol (PG), which is very similar to PEG and exhibits either a linear or a branched structure (**Figure 10**).[189] The polyglycerol combines: 1) a hydrophilic repeating unit, 2) a unit that can hydrogen bond with water

and is hence well hydrated in water. The presence of a hydration pressure serves as an energetic barrier to non-specific protein adsorption. 3) an oligomer/polymer with a very flexibility due to aliphatic ether bonds, which enhances a configurational mobility that blocks potential sites of protein adsorption due to steric excluded volume effects, and 4) bioinert to biomolecules and cells, which enable the PG coating to resist proteins well.[190] The large number of free OH groups on the polymer backbone are also prone to be modified with functional groups or ligands. Besides, PGs exhibit higher thermal and oxidative stabilities[190,191] and less thrombocyte activating[192] compared with PEG. Paired with the multifunctional aspect of its structure, PGs show their versatility and potential to supplant PEG in fouling resistant applications.

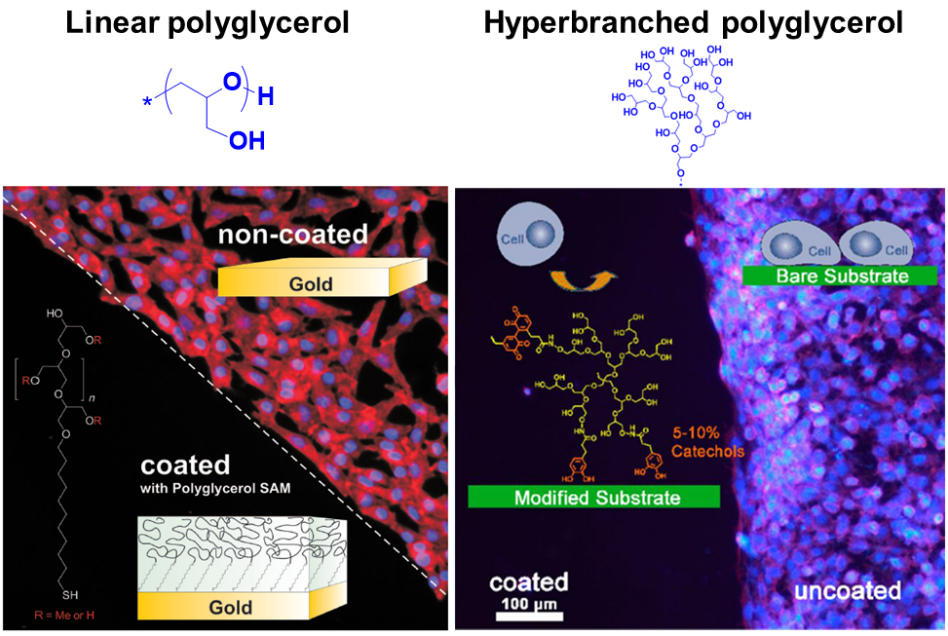


Figure 10. The structure of linear and hyperbranched polyglycerols and their excellent antifouling performance when grafted onto substrates. Reprinted from Ref.[193] and Ref.[194] with kind permission of Wiley-VCH Verlag GmbH & Co. KGaA and The American Chemical Society respectively.

The polymers bear an equimolar number of homogenously distributed anionic and cationic groups along their polymer chains, which are defined as zwitterionic polymers.[195] The combination of oppositely charged moieties grants the polymers ultra-

hydrophilicity and high hydration in aqueous medium. While, at the same time, the polymer chain maintains a neutral overall charge. From an engineering perspective, zwitterionic polymers are generally considered to be a good alternative to the widely used poly (ethylene glycol) (PEG) polymers to prevent nonspecific protein adsorption as well as to minimize bacterial or mammalian cell adhesion.[142,164,196] Their promising antibiofouling characteristics are definitely ascribed to the hydration layer formed around the zwitterionic polymers through electrostatic interactions and hydrogen bonding that are both energetically and kinetically unfavorable for protein disruption. The anionic groups in zwitterionic polymers are commonly the carboxylate, sulfonate, and phosphate groups, while the paired cationic groups are quaternary ammonium, phosphonium, pyridinium, or imidazolium groups. Zwitterionic polymers can be directly synthesized from the polymerization of zwitterionic monomers, i.e., the sulfobetaine methacrylates (SBMA), carboxybetaine methacrylates (CBMA), carboxybetaine acrylamide (CBAA), phosphorylcholine methacrylate (PMC) (**Figure 11a**). Along with the polyOEGMA coatings described previously, polySBMA, polyCBMA, and polyCBAA zwitterionic brushes rank among the most antifouling coatings known against serum and plasma.[9] Many reports[197-199] displayed that the polySBMA and polyCBMA brush coatings exhibited ultra-low fouling adsorption from single-protein solutions or complex media (< 0.3 ng cm^{-2}), undetectable from surface plasmon resonance (SPR) sensor measurements. In addition, the zwitterionic polymer can also prepared with post modification to obtain zwitterionic groups onto the natural polymers. Kellie Seetho et al. used the readily reaction of phosphotriester and tertiary amines to obtain poly(phosphorylcholine) zwitterionic polymer from polyphospholane.[200] This chemistry permits an opportunity to covalently incorporate various functionalities by modification of the R group on the tertiary amine.

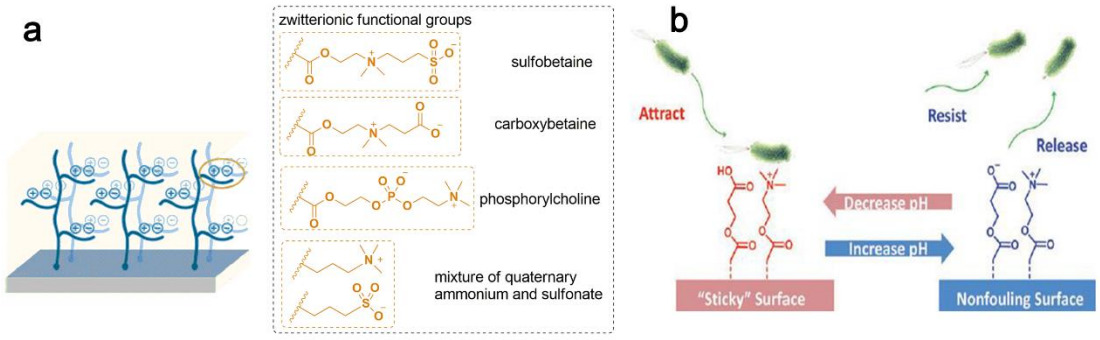


Figure 11. (a) Molecular structures of sulfobetaine, carboxybetaine, phosphorylcholine, and a mixture of trimethylammonium and sulfonate groups (pseudobetaines). (b) A zwitterionic surface that attracts and releases bacterial cells in response to the environmental pH value. Reprinted from Ref.[201] with kind permission of the Elsevier.

Another class of zwitterionic polymers are the "mixed charge" polymers, where the positively and negatively charged groups are completely separately distributed onto different polymer side chains. While this kind of zwitterionic polymer coatings still exhibit a high fouling resistance at an equally low level as the above-mentioned polymers.[201] Furthermore, benefiting from the separated anionic and cationic groups, this "mixed charge" polymers usually display a pH-dependent antifouling property (**Figure 11b**). Under low pH conditions, the coating surface bears a moderately positive charge from protonated carboxylic acid groups that favor the attachment of bacteria cells. In neutral or higher pH solutions, the copolymer has an overall neural charge that gives the copolymer its non-fouling property and releases the bacteria cells.[201] Various derivatives of conventional zwitterionic polymers can either switch between zwitterionic and non-zwitterionic forms[202,203] or carry a charged biologically active molecule as a part of the zwitterionic constituent.[204] This structural diversity brings functional versatility to zwitterionic polymers beyond non-fouling thus distinguishing them from other non-ionic anti-fouling materials.[142]

1.4. Switchable coatings

The ability to reversibly modulate macroscopic surface properties is an important

requirement for numerous biomedical applications, such as cell culture, tissue engineering, biosensors, biofouling, and microfluidics.[205] For instance, dynamic controlled cell adhesion on substrates is a fundamental issue because cell adhesion has profound effects on the cell fate and diverse cellular response behaviors, e.g., migration, differentiation and apoptosis.[206] In order to dynamically control the bio-interfacial interactions between material surface and biomolecule, some stimuli-responsive molecules were introduced as "smart" coatings. The resulting interfacial properties could be tuned by external stimuli.[207-209] The available stimuli includes change of pH values, temperature, ionic strength, magnetic fields, electric fields, light, mechanical forces and chemical interactions.[210] Among various options, light has attracted much attention since it can noninvasively regulate bioligand-material surface interactions with high spatiotemporal precision.[211] The construction and operation principles of photo-responsive polymer coatings are grafting polymers bearing photo sensitive groups onto selected substrate. Upon application of the light stimulus, the photo-responsive groups undergo photo-cleavage or photo-induced conformational changes that result in the responsive change of the coating's interfacial property. The commonly used photo-sensitive functional groups and their primary response mechanisms are summarized in **Figure 12**. Photo-responsive chemistries provide a versatile toolkit that enable spatial and temporal control over stimulus application and therefore stimulus response.

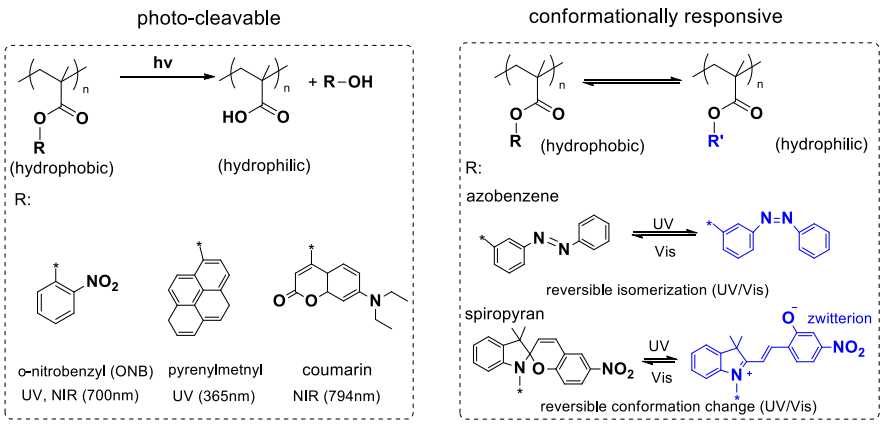


Figure 12. The commonly used photo-senstive functional groups and their primary response mechanism.

In an example of photo-responsive coating surface, Lee et al.[212] used the photolabile 3-(4,5-dimethoxy-2-nitrophenyl)-2-butyl ester (DMNPB) group to cage the cell adhesion ligand, RGD peptide. The photo-sensitive OBN cage can be easily removed with light at prescribed wavelengths to render the RGD peptide fully active. Upon exposure to light (λ~350-365 nm), the caging group was released and resulted in the presentation of the active cyclic RGD peptide onto hydrogel surface (**Figure 13**). Hydrogels with control over the RGD peptide and UV-light-exposed caged RGD peptide supported high levels of adherent cells while the non-exposed hydrogel with caged RGD peptide supported very low numbers of adherent cells with rounded morphology. They establish an outstanding strategy to control the *in vivo* presentation of bioligands temporally and spatially via photo-clearable units. Furthermore, they also realized *in vivo* regulation cell adhesion, inflammation, and vascularization of the material via non-invasive and transdermal activation of RGD peptide on the hydrogel at particular time points after implantation.

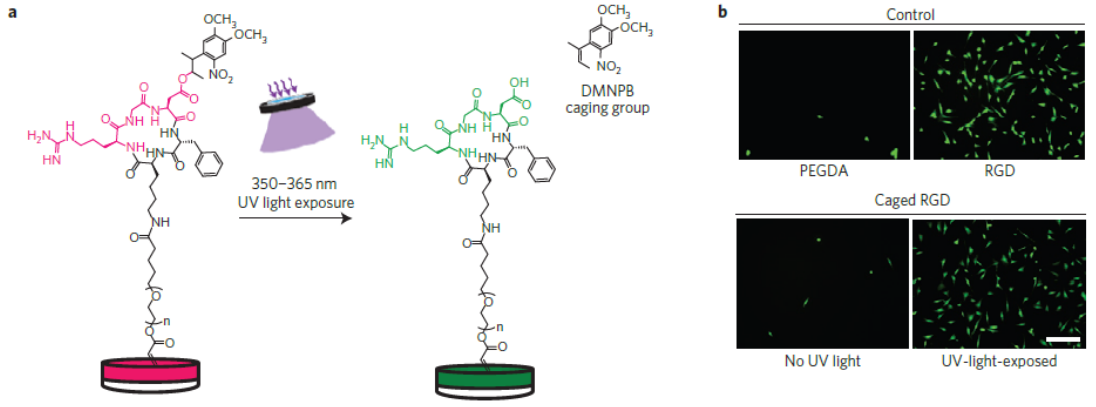


Figure 13. Light-triggered activation of cell adhesion activity of caged RGD peptide on hydrogels. (a) Schematic representation of caged RGD peptide-functionalized PEGDA hydrogels. Light exposure at 365nm cleaves the UV-light-labile caging group to present the active cyclic RGD peptide. (b) Photographs of fluorescently labeled cells cultured on unmodified PEGDA and peptide-modified hydrogels that were either exposed to UV light or not exposed (scale bar, 300 nm). Modified from Ref.[212] with kind permission of Nature Publishing Group.

However, most of the photo-sensitive functional groups respond to ultra-violet (UV) wavelengths (<400 nm), which cannot penetrate deeply into tissue and has a lot of damages to biomolecules and cells. Recent efforts have investigated using near infra-red (NIR) as the trigger light via the assistance of up-conversion nanoparticles (UCNPs).[213] Based on this, Li et al.[214] developed a simple yet versatile strategy to reversibly and noninvasively control cell adhesion/detachment by conjugated spiropyran onto multi-shell UCNPs ($NaYF_4$:Tm/Yb@$NaYF_4$@$NaYF_4$:Er/Yb@$NaYF_4$ core-shell-shell-shell nanoparticles) (**Figure 14**). At a high-power density of NIR, the UCNPs can emit UV photons and activate the isomerization of spiropyran from SP form to the MC form, resulting in the detachment of cell. Conversely, when exposed to NIR with low power density, the same UCNPs can emit visible light to drive the MC form back to the SP form, leading to cell adhesion again. It is a good platform for dynamically regulating interfacial interactions of cell-biomaterials.

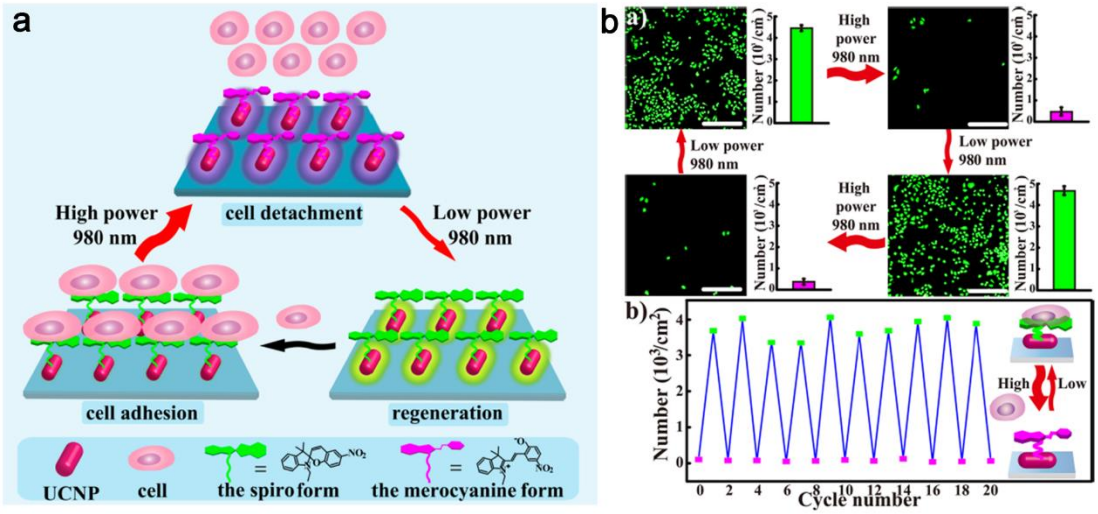


Figure 14. (a) Schematic illustration using SP-UCNP as a NIR-triggered photo-switch for noninvasive and reversible control of cell adhesion/detachment by merely altering the power density of a single-wavelength 980 nm laser. (b) Single-wavelength 980 nm NIR regulates reversible control of cell adhesion and detachment. Scale bars are 100μm. Reprinted from Ref.[214] with kind permission of The American Chemical Society.

1.5. Bio-specific coatings for controlling cell-materials interfaces

The biofunctionalization of implants and devices interfaces is an important element of

design in bioengineering. Cell behaviors, such as the recruitment, adhesion, spreading, motility, matrix deposition, proliferation, and differentiation, are highly related to or controlled by this bio-interface between materials and surrounding cells or tissue. Biologically, the adhesion of cells to culture environment is crucial to their development, including growth and death, cell motility, and differentiation. Such cell-substrate interactions are a complex process that involves protein adsorption to a surface with presentation of specific peptide sequences ("adhesion sequence"). These sequences usually mimic the functions of biological molecules found in the extracellular matrix (ECM).[215] RGD is an ubiquitous cell binding sequence derived from the cell attachment domains of fibronectin. Many integrin receptors recognize this sequence, thereby facilitating adhesion of many cell types to fibronectin in the ECM.[216] Another sequence which can promote cell adhesion is the glycine-phenylalaninehydroxyproline-glycine-glutamate-arginine (GFOGER), which is derived from collagen.[217] Collagen is abundant in mesenchymal tissues and the GFOGER sequence promotes cell adhesion and osteoblast differentiation.[218] An understanding of the ability of these adhesive sequences to exert control over cell adhesion provides an opportunity to integrate them onto a biomaterial surface and thereby mediate the material-cell interfacial interactions and promote better incorporation of the biomedical device into the host. However, further consideration must also be given to the density and spatial arrangement of peptide sequences on the coating surface, which will affect cell adhesion.[219] It has been found that a critical RGD spacing is proposed to be around 70 nm for RGD nanopattern on the material surface to regulate cell functions.[220,221] An RGD spacing below 70 nm favors cell adhesion with a relatively larger spreading area and stronger cytoskeleton. With spacing above 70 nm, the cells poorly adhere and spread on RGD nanopatterns.[220,222] This phenomenon is attributed to the prevention of integrin clustering rather than an insufficient number of adhesive ligands.[259] In addition, one study also showed that cells only develop mature focal adhesions (FAs) on RGD coated substrate with restricted mobility. The fully mobile RGD leads to the formation of podosome-like adhesion in the absence of traction force.[223] To dynamically modulate

the cell-substrate interaction, which is important for fundamental research and practical applications, Wong and co-workers described a simple and easy technique to dynamically and reversibly tune the mobility of tethered RGD peptides by using a magnetic field as graphically illustrated in **Figure 15**.[224] RGD-bearing magnetic nanoparticles (MNPs) were conjugated onto glass substrate via PEG linker with different molecular weights to yield RGD with different tether mobility on the substrate. The nonspecific cell adhesion on the substrate was suppressed by a blocking agent (bovine serum albumin, BSA) before cell seeding. The magnetic field was applied to dynamically tune the mobility of tethered RGD. In their design, the mobility of RGD on the surface was modulated with the applied magnetic field (e.g., strength, frequency, and direction) and the length of a PEG linker. The results indicated that hMSCs cultured on the substrates with restricted RGD mobility exhibit enhanced cell adhesion, spreading, and osteogenic differentiation compared to the hMSCs cultured on substrates with high RGD mobility. This cell response was attributed to the enhanced mechanical feedback via RGD-integrin ligation and activation of intracellular mechano-transduction signaling involving the nuclear translocation of YAP.

Another sequence $FNIII_{7–10}$ was also immobilized onto materials to promote cell adhesion and increase osteoblast differentiation.[225,226] The $FNIII_{7–10}$, a recombinant fragment of FN that presents both the RGD sequence and its PHSRN synergy site.[215] Besides, the CAG (Cys-Ala-Gly) tripeptide, which can significantly enhances endothelial cells adhesion.[227] The REDV (Arg-Glu-Asp-Val) tetrapeptide, which is a fibronectin derived peptide that can specifically bind to the $\alpha_4\beta_1$ integrin and enhance the rapid endothelialization of endothelial cells.[228] The YIGSR (Tyr-Lle-Gly-Ser-Arg) peptide sequence, which is a segment of the basement membrane matrix glycoprotein laminin and mediates the attachment and migration of cells including endothelial cells, fibroblasts and smooth muscle cells.[229] All of them have been immobilized onto the material surface to tune cell-material interactions.[7] The growth factors (bFGF, VEGFs, BMP-2) are another class of bioligands, which are essential components of the cell microenvironment and important cues controlling cell motility, proliferation, and

differentiation. Their role is sometimes associated with matrix adhesion and have has been grafted onto material surfaces or introduced into 3D cell culture microenvironments to study the subsequent cell behavior.[230-232]

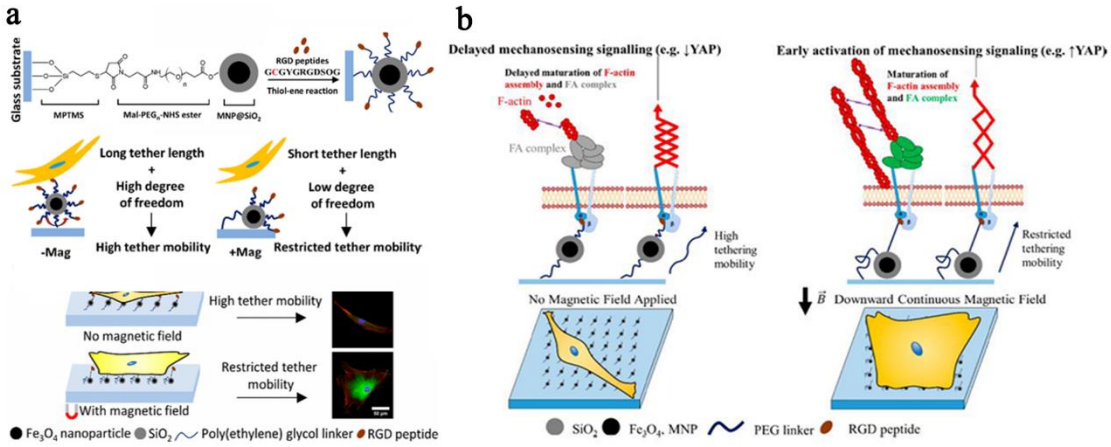


Figure 15. (a) Schematic of fabricating a monolayer of RGD-bearing MNP substrate. Without magnetic field (-Mag), RGD peptides have a very long tether length and higher degree of freedom on the MNP. With the magnetic field (+Mag), RGD peptides have a shorter tether length and a lower degree of freedom on the MNP. (b) Schematic illustration of the potential mechanism underlying the substrate tether mobility to control hMSCs adhesion, spreading, and differentiation, which is governed by mechano-sensing signaling. Modified from Ref.[224] with kind permission of The American Chemical Society.

The specific interaction between an antibody and an antigen has also been broadly used in bio-functional coating design. Antibodies are one of the most important specific molecules that recognize cells with the corresponding surface antigens. For example, anti-EGFR (epidermal growth factor receptor antibody) can be used for specific recognition of lung-cancer cells.[233] Anti-HER2 (human epidermal growth receptor 2 antibody) is capable of recognizing breast cells overexpressing HER2 proteins.[234] EpCAM (epithelial cell adhesion molecule) is a transmembrane glycoprotein mediating Ca^{2+}-independent cell-cell adhesion in epithelia[235] and is found over expressed in a great variety of human adenocarcinoma cells (e.g., circulating tumor cells, CTCs), but it is absent in blood cells.[236] Hence, the overexpressed EpCAM is known as a CTC-

associated biomarker and has been widely used in CTC isolation techniques based on its antibody, the anti-EpCAM.[237] By using this specific cell marker, Liu et al. developed a temperature-responsive polymer coating functionalized with anti-EpCAM to reversibly capture/release CTC cells (**Figure 16**). Therein, nanostructured (nanopillars) substrates were adopted to improve the cell-capture/release performance. Moreover, the cells can stay undamaged during the processes of capture/release in their design, which is significant because it facilitates the subsequent cell culture and single cell analysis.

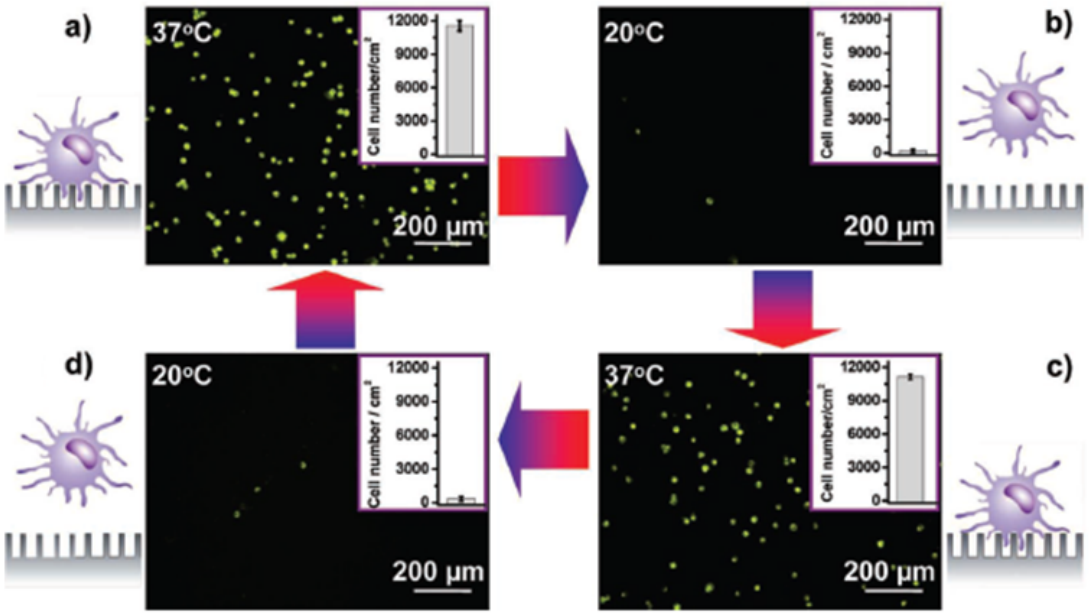


Figure 16. Reversible capture and release of targeted cancer cells on as-prepared surfaces triggered by temperature. The design of thermo-responsive nanostructured surfaces for reversible capture and release of targeted cancer cells is based on hydrophobic interactions and topographic interactions. Hydrophobic interactions mediate capture and release of targeted cancer cells by temperature changes. Topographic interactions between silicon-nanopillars and cell protrusions offer a 3D interfacial contact. Using biotin-BSA as a hydrophobic anchor, targeted MCF-7 cells can be captured onto or released from the PNIPAAm-modified SiNP (PSiNP) reversibly, by changing the temperature between 37 °C and 20 °C. Modified from Ref.[238] with kind permission of Wiley-VCH Verlag GmbH & Co. KGaA.

While the cell-material adhesion is mediated with integrin-ECM proteins, the

bacterial adhesion to their host surfaces is usually dependent on the specific carbohydrates-lectins binding, which is mediated by adhesive organelles of bacteria, called fimbriae.[239] Fimbriae, particularly the type 1 fimbriae which are terminated by an α-D-mannose specific lectin, comprise specialized lectins to recognize carbohydrate ligands.[240] These type 1 fimbriae are expressed in several hundred copies on the bacterial cell surface to achieve tight adhesion through multivalent protein carbohydrate interactions. Therefore, the conjugated mannose ligand onto material surface can efficiently mediate and promote the specific adhesion of mannose-specific (MS) bacteria such as *E. coli*, *Klebsiella pneumoniae*, and *Salmonella spp*[241] and has been the art to design bacteria specific adhesion coatings.[242-244] However, the orientation of the carbohydrate on a glycosylated surface was assumed to be crucial for the adhesion of bacteria. To study this effect, Weber and coworkers [245]immobilized the α-D-mannose onto azobenzene and then prepared a photoswitchable glyco-SAM coating. Strong *E coli* adhesion onto the SAM surface was found when the azobenzene was in its E-configuration and, the mannose was accessible to *E coli*'s fimbriae. Otherwise, only a small amount of *E coli* came from non-specific interactions,[246] which were detected on the carbohydrate surface with azobenzene in Z-configuration. The presence of an azobenzene unit provided a possibility to dynamically control the orientation of surface-bound carbohydrate ligands by an external stimulus (light).

2. Scientific Goals

There is an enormous scientific interest for polymer coating strategies regardless of the chemical composition and physical morphology of material surfaces. However, only a few approaches were developed, and most of them resulted in coatings that were suffering from color, high thickness or high roughness.

Herein, a bio-inspired universal monolayer coating by combining concepts from blood protein adsorption and mussel adhesion is supposed to develop (Figure 17). The resulting monolayer coatings are highly stable, colorless, smooth, only 3-4 nm thick, and can be generated on various planar surfaces and nanosystems.

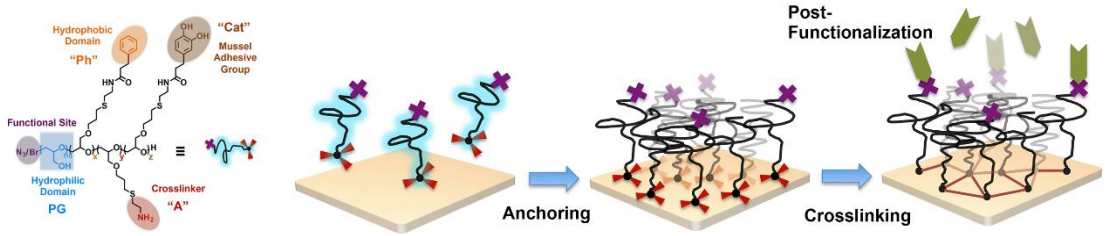


Figure 17. Polyglycerol (PG)-based amphiphilic block copolymer that mimics blood protein adsorption and mussel adhesion. The catechol groups mostly contribute to the coordinative and/or hydrogen bonding on polar surfaces but can also serve as hydrophobic domains together with the phenyl groups for anchoring on non-polar surfaces. The amine groups, on the one hand, increase the crosslinking efficiency and, on the other, displace hydrated cations from the mineral surfaces to stabilize the coatings.

Blood proteins spontaneously adsorb on almost all solid material surfaces by denaturing themselves to expose “anchor domains” to the surfaces. Hydrophobic interactions, hydrogen bond formation, ionic or electrostatic attractions, and coordinative interactions are recognized as the main forces causing and driving this adsorption. Mussels can adhere to a broad range of solid surfaces and are especially stable on metal oxide and mineral surfaces because of mussel foot proteins (mfps). Although the adhesion of mfps is a very complicated process, catecholic anchoring and

subsequent intra-molecular crosslinking are believed to play the most important role. Moreover, the hydrophobic amino acids in mfps, especially in mfp-3 “slow” (mfp-3s), enhance the hydrophobic interaction and shield the catechols from the water phase to provide a microenvironment that retards oxidation. A coating polymer that integrates the above-mentioned amphiphilic interactions and catecholic anchoring, with manifold attachment and chelation as well as a subsequent intralayer crosslinking should be designed to achieve a universal monolayer coating system. The coating ability on various material surfaces (macro-scale) and even the nano-interfaces will be studied. The bioinert performance will be also investigated that benefits from hydrated polyglycerol brushes and the potential post-functionalization via the ω-terminal groups.

Polymer brushes, which are tethered with one chain end to an interface, especially with high surface graft density, are extremely suitable to become protein resistant coatings. Although much research has focused on surface modifications with polymer brush coatings, only a few non-invasive approaches have been developed and utilized on nonpolar substrates due to the lack of reactive surface groups. Even the so-called universal polydopamine/polyphenol or catecholic coatings are not stable enough on nonpolar surfaces compared with polar surfaces and they also dramatically increase the thickness and roughness of the substrates. Moreover, the greatest challenge in constructing polymer brushes is obtaining a high grafting density, which results in lateral steric repulsion to stretch back-folded polymer chains into a brush conformation. This parameter is very important and essential to the antifouling performance of the coatings.

To develop a coating technology that generates a highly dense polymer brush coating on various nonpolar substrates (including the most inert and low-energy surfaces of PDMS and PTFE) with long term stability and high antifouling performance, a new anchoring chemistry and “adsorption - crosslinking” coating concept should be employed. In this part, amphiphilic block copolymers with benzophenone units as the hydrophobic anchor/chemical cross-linker should be synthesized (Figure 18). Further *in situ* tailoring of the established polymer brushes via terminal active groups is

supposed to conduct to construct a bifunctional brush coating that provides a highly stable and robust bioinert background for biospecific adsorption of desired proteins, cells, and bacteria.

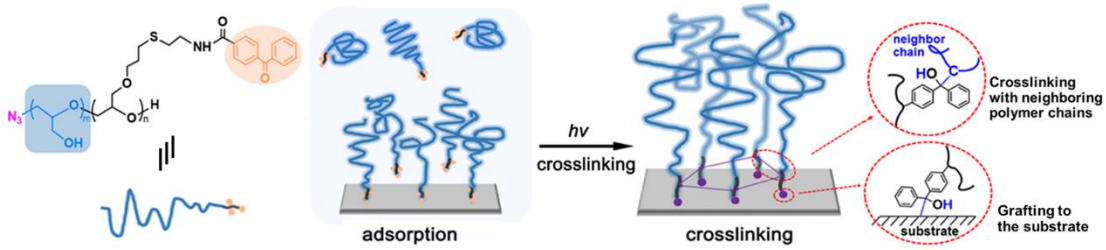


Figure 18. Structure of bifunctional amphiphilic block copolymer PG-BPh and PG-BPh brush coatings fabricated via "adsorption-crosslinking" approach based on a sequence of versatile photo-initiated C-H insertion crosslinking steps.

Furthermore, in order to dynamically regulate the protein adsorption and cell adhesion behavior on the surface, a mussel-inspired polyglycerol coating and the photo-responsive photochrome, i.e., spiropyran, should be integrated to generate a functional coating with an interesting noninvasive light-modulation surface property. On this coating, the light-modulation is based on the light-induced spiropyran-to-merocyanine (SP - MC) isomerization: nonpolar and hydrophobic SP form to the polar, hydrophilic, and zwitterionic form.

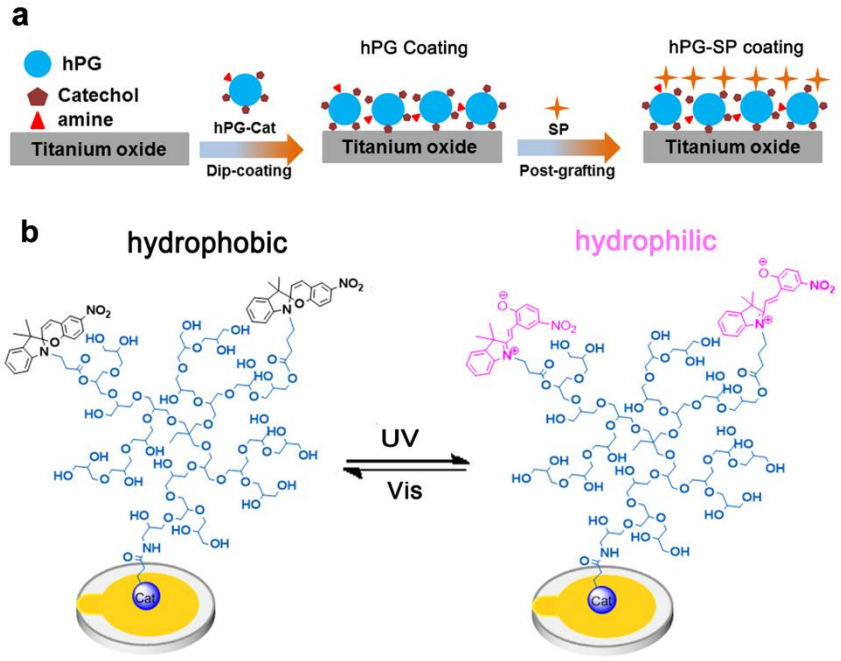
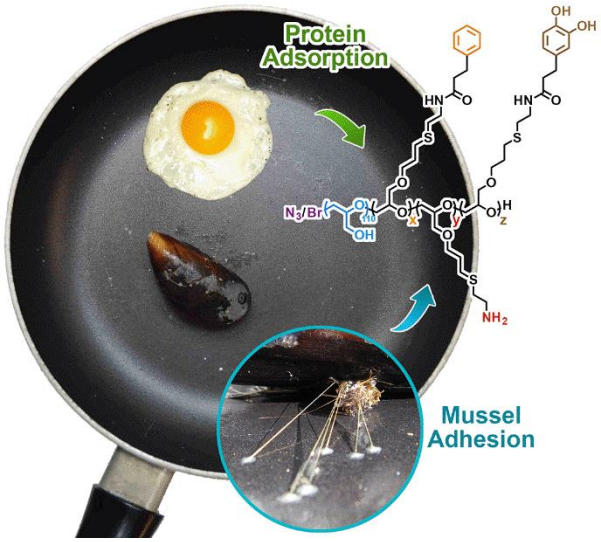


Figure 19. (a) Preparation of hPG-SP coatings on titanium oxide (TiO_2) substrate. (b) The spiropyran moiety on hPG-SP coating can be photochemically converted between the hydrophobic SP form and hydrophilic zwitterionic MC form.

Overall, polyglycerol-based universal polymer coatings on versatile substrate surfaces should be explored with designed anchoring groups. Biofunctional or biospecific ligands are further immobilized onto coating polymers to integrate the bioinert and biospecific to PG coatings simultaneously and broaden their biomedical applications of PG coatings.

3. Publications

3.1. Bioinspired Universal Monolayer Coatings by Combining Concepts from Blood Protein Adsorption and Mussel Adhesion



Leixiao Yu, Chong Cheng, Qidi Ran, Christoph Schlaich, Paul-Ludwig Michael Noeske, Wenzhong Li, Qiang Wei, Rainer Haag

ACS Appl. Mater. Interfaces 2017, 9, 6624−6633

DOI: 10.1021/acsami.6b15834

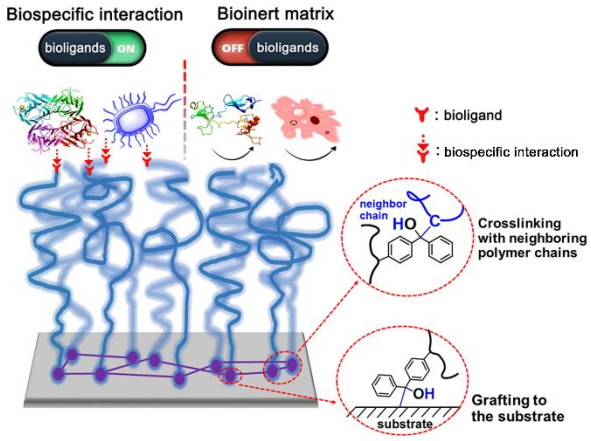
https://doi.org/10.1021/acsami.6b15834

Author contributions:

Leixiao Yu conceived the project, performed the main experiments, and wrote the manuscript.

Chong Cheng performed the experiments with graphene nanosheets and AFM measurements. Qidi Ran performed the experiments with nanoparticles. Christoph Schlaich contributed to the ellipsometry measurement and discussion. Paul-Ludwig Michael Noeske performed and evaluated the XPS measurements on planar surfaces. Wenzhong Li enabled and supported the cell culture experiments. Qiang Wei and Rainer Haag supervised and discussed this project, as well as corrected the manuscript.

3.2. High-antifouling Polymer Brush Coatings on Nonpolar Surfaces via Adsorption-Crosslinking Strategy



Leixiao Yu, Yong Hou, Chong Cheng, Christoph Schlaich, Paul-Ludwig Michael Noeske, Qiang Wei, Rainer Haag

ACS Appl. Mater. Interfaces, 2017, 9, 44281–44292

DOI: 10.1021/acsami.7b13515

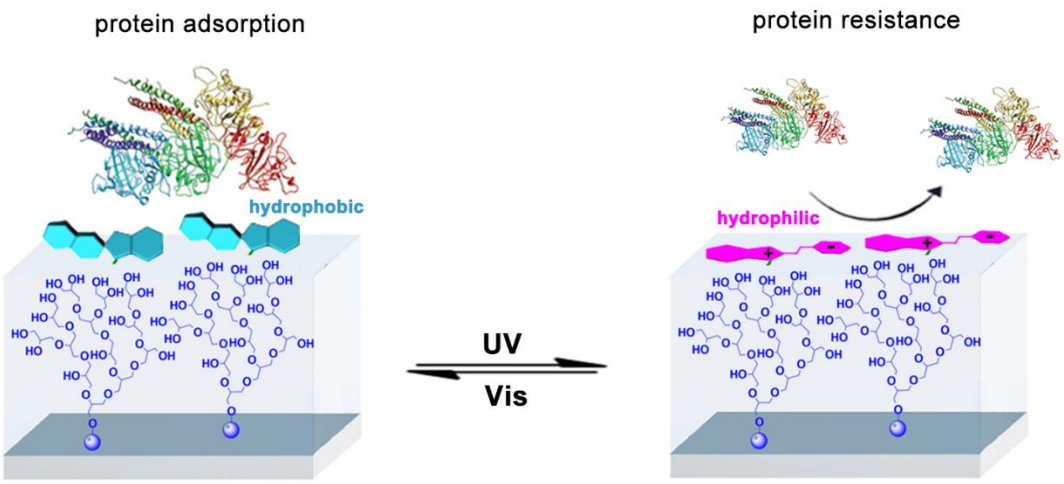
https://doi.org/10.1021/acsami.7b13515

Author contributions:

Leixiao Yu conceived, performed the main experiments, and wrote the manuscript.

Yong Hou synthesized the cyclo-alkyne functionalized mannose. Christoph Schlaich contributed to the ellipsometry measurement and discussion. Dr. Chong Cheng performed and evaluated the AFM measurements. Dr. Paul-Ludwig Michael Noeske performed and evaluated the XPS measurements. Dr. Qiang Wei and Leixiao Yu conceived the idea and designed the research. Prof. Rainer Haag is the principle investigator and supervised the project. Rainer Haag, Qiang Wei, and Leixiao Yu, wrote the manuscript. All authors contributed to discussions and manuscript writing.

3.3. Photo-regulating Antifouling and Bioadhesion Functional Coating Surfaces Based on Spiropyran



Leixiao Yu, Christoph Schlaich, Yong Hou, Jianguang Zhang, Paul-Ludwig Michael Noeske, and Rainer Haag

Chem. Eur. J. 10.1002/chem.201801051

DOI：10.1002/chem.201801051
https://doi.org/10.1002/chem.201801051

Author contributions:

Leixiao Yu conceived the idea and designed the research, performed the main experiments, and wrote the manuscript. Christoph Schlaich contributed to the ellipsometry measurement and discussion. Yong Hou made the cell live/dead staining experiment. Dr. Paul-Ludwig Michael Noeske performed and evaluated the XPS measurements. Jianguang Zhang gave help in the cell culture. Prof. Rainer Haag is the principle investigator and supervised the project. All authors contributed to discussions and manuscript writing.

4. Conclusions

In this thesis, a set of multi-functional polymer coatings with tailorable surface properties was developed via mussel-inspired catecholic chemistry and the "adsorption-crosslinking" technology of benzophenone. The simple dip-coating method was adopted from the point of view of technical applicability. A wide range of material surfaces, including metal oxides, noble metals, ceramics, nonpolar polymeric materials, even the most inert and low-energy surfaces of PDMS and PTFE, and nano-interfaces, e.g., graphene, Fe_3O_4 nano-particles, nano-diamond, were successfully modified by these powerful coating methods to achieve versatile biomedical applications. Especially the "adsorption-crosslinking" coatings technology introduced in the second project, was proven to be efficient for the complex 3D PDMS microfluidic chips, extending its potential application to a lab on chip.

For the bioinspired amphiphilic block copolymer (PG-CatPh) that integrates the concepts from blood protein adsorption and mussel adhesion, the polyglycerol block serves as the hydrophilic domain with excellent bioinert properties, while the anchor domain involves three different functional groups with synergistic effects (see section 3.1). It has been demonstrated that the PG-CatPh polymers are uniformly and robustly coated on both macroscale planar surfaces and nanosystems. The exposed hydrophilic moieties of the coatings hinder the formation of uncontrollable multilayers or agglomerates. Therefore, the universal coating ability of the polymers is suitable for macro/nano-interfaces. With the benefit of a dense assembled monolayer, the coating is thin, ultra-smooth, and colorless. The antifouling performance of the coatings was proven on TiO_2 and PS to prevent unspecific protein adhesion and cell adhesion, respectively. In addition, specific interactions can be generated upon post-functionalization of the terminal groups with cyclic RGD by cellular adhesion and spreading on a Teflon surface. Therefore, this universal monolayer coating provides a new platform for material surface modification and can be used in a wide range of biointerface applications.

However, the aforementioned PG-CatPh coatings on polymeric substrates failed

in the ultra-low fouling resistance from single protein solution and complex serum, which was due to the weak polymer-substrate interaction, low coating density, and possible brush conformation change during the post-crosslinking. Therefore, a dense functional and long-term stable monolayer brush coating for various nonpolar surfaces was further developed by a simple "adsorption-crosslinking" technology based on a multifunctional amphiphilic block copolymer with benzophenone (BPh) as the reactive anchor (see section 3.2). The optimized hydrophobicity of the BPh functional block enabled BPh to be a direct anchor on pristine surfaces, which largely extended the use of BPh for material surface modification. The adsorbed BPhs initiated the unselective chain insertion crosslinking reaction under short UV irradiation to immobilize the polymer chains either on the substrates presented aliphatic C-H groups via covalent bonding or on the other substrates by multivalent adsorption and covalent crosslinking. This process resulted in an ultrathin, smooth, and highly stable monolayer brush coating. Besides the coatings on 2D planar surfaces, the PG-BPh polymers can also be used to coat complex 3D systems, e.g., microfluidics channels. Because of the high graft density, the modified nonpolar surfaces exhibited outstanding antifouling properties and were very stable in a physiological buffer for at least one year. After *in situ* post-modification with biospecific ligands, e.g., mannose, these bioinert surfaces were converted to highly biospecific protein adsorption and bacteria capture coatings via multivalent protein-carbohydrate interactions. Therefore, this highly stable monolayer coating provides a new platform for universal material surface modification and can be used in a wide range of biointerface applications. We believe our work opens up new avenues for the modification of nonpolar material surfaces and *in situ* immobilization of a wide variety of selective biomolecules.

Furthermore, in order to dynamically control the bio-interfacial interactions between material surfaces and biomolecules, we designed and developed an spiropyran (SP)-based, light-responsive functional coating with a good bioinert PG background (see section 3.3). In the normal state, the spiropyran groups on the coating surface were in hydrophobic ring-closed SP form, which could promote the nonspecific protein

adsorption and cell adhesion. After UV light irradiation, the spiro ring of SP opens and converts into a hydrophilic and zwitterionic merocyanine (MC). Both hydrophilicity and zwitterions would contribute to the hydrated layer forming and, therefore, resist the protein adsorption and cell adhesion. Moreover, the controllable adsorption/desorption of proteins, attachment/detachment of cells and even dense cell sheet was also achieved on the SP functionalized coating in a noninvasive mode. This functional coating exhibited a good perspective and potential utilization in bio-responsive surface modification and tissue engineering. The current system may be most appropriate for applications which facilitate sufficient UV illumination and may show limitations when substantial tissue-penetration with light is required.

5. Outlook

This work developed and investigated several technologies to build up universal polymer coatings on versatile material surfaces. These polymer coatings are bioinert and biospecific simultaneously benefiting from advanced polymerization chemistry and post in situ modification. Therefore, these polymer coatings have a broad application potential in biomedicine, especially for implant devices and sensors surface modification, tissue engineering. However, there is only a few anchoring groups that can interact with many substrate types. It is also very hard to fabricate stable and dense polymer coatings on some useful but cheminert material surfaces via present universal anchors. Therefore, it is necessary and urgent to develop new anchoring methods. Learn from nature is a good solution. Besides, more attentions should be paid on the long-term stability of the polymer coatings especially in harsh environments. Future research in this field should address the following points:

- How to control the coating thickness and the coating surface roughness
- Methods to increase grafting density and surface coverage.
- Methods to prepare hierarchic, patterned, and gradient coatings.
- Coatings with more bio-functionality and switchable coatings.
- Advanced chemistry for bioligands conjugation.

6. Kurzzusammenfassung

Ziel dieser Arbeit war die Entwicklung von bifunktionellen Polymerbeschichtungen mit maßgeschneiderten Oberflächeneigenschaften mit Hilfe Muschel-inspirierter Katecholchemie und "Adsorptionsvernetzungs“ von Benzophenon. Dies ermöglichte eine einfache Tauchbeschichtung unter dem Gesichtspunkt der technischen Anwendbarkeit. Mit dem entwickelten Beschichtungsverfahren konnten verschiedenste Materialoberflächen, einschließlich Metalloxide, Edelmetalle, Keramik, unpolare Polymermaterialien, inerte und niederenergetischen PDMS- und PTFE-Oberflächen sowie Nano-Grenzflächen, z.B. Graphen, Fe_3O_4-Nanopartikel und Nano-Diamanten erfolgreich für vielfältige biomedizinische Anwendungen modifiziert werden. Insbesondere die "adsorptionsvernetzende" Beschichtungstechnologie, welche im zweiten Projekt eingeführt wurde, hat bewiesen, dass sie effizient für komplexe 3D-PDMS-Mikrofluidikchips eingesetzt werden kann und deren potentielle Anwendung auf ein *Lab-on-Chip* System erweitert.

Das bioinspirierte amphiphile Blockcopolymer (PG-CatPh) vereint die Konzepte der Blutproteinadsorption und Muscheladhäsion (Siehe Sektion 3.1). Der Polyglycerinblock dient hierbei als hydrophile Domäne mit ausgezeichneten Antifouling- (anwuchsverhindernden) Eigenschaften, während die Ankerdomäne drei verschiedene funktionelle Gruppen mit synergistischer Wirkung umfasst. Es wurde gezeigt, dass die PG-CatPh-Polymere sowohl auf makroskopischen planaren Oberflächen als auch auf Nanosystemen eine gleichmäßige und robuste Beschichtung bilden. Die exponierten hydrophilen Gruppen der Beschichtung behindern die unkontrollierte Bildung von multiplen Schichten und Agglomeraten, so dass diese universelle Polymerbeschichtung für Makro- und Nano-Grenzflächen geeignetist. Die dichte Monoschicht hat den Vorteil, dass sie dünn, sehr glatt und farblos ist. Mittels beschichtetem TiO_2 und PS wurden die Antifouling-Eigenschaften in Proteinadhäsions- und Zelladhäsionsversuchen untersucht. Es konnte gezeigt werden, dass unspezifische Interaktionen verhindert werden. Spezifische Wechselwirkungen mit Zellen konnten durch Postfunktionalisierung der terminalen Gruppen mit cyclischem RGD erreicht

werden. Eine zelluläre Adhäsion und Ausbreitung konnte hierdurch sogar auf Teflonoberflächen erzeugt werden. Die entwickelte universell einsetzbare Monoschicht stellt somit eine neue Plattform für die Modifizierung von Materialoberflächen dar und kann für eine Vielzahl von Biointerface-Anwendungen genutzt werden.

Mit einzelnen Proteinen in Lösung sowie komplexen Medien wie Serum traten jedoch Wechselwirkungen mit der PG-CatPh-Beschichtungen auf. Dies könnte auf die auf die schwache Polymer-Substrat-Wechselwirkung, eine zu geringe Beschichtungsdichte oder eine mögliche Bürstenkonformationsänderung während der Nachvernetzung zurückzuführen sein. Aus diesem Grund wurde eine dichte, bifunktionelle und beständige Monoschicht-Bürstenbeschichtung für verschiedene unpolare Oberflächen entwickelt. Hierfür wurde die "Adsorptions-Vernetzungs"-Technologie unter Verwendung von bifunktionellen amphiphilen Blockcopolymeren mit Benzophenon (BPh) als reaktivem Anker weiterentwickelt (Siehe Sektion 3.2). Die optimierte Hydrophobie des BPh-Funktionsblocks ermöglichte es BPh als direkten Anker für unbehandelten Oberflächen zu verwenden und dadurch die Materialoberfläche zu modifizieren. Die adsorbierten BPhs initiieren eine nicht selektive Ketteninsertions-Vernetzungsreaktion mittels UV-Bestrahlung. Dadurch wird ermöglicht die Polymerketten entweder auf Substraten, welche aliphatische CH-Gruppen über kovalente Bindung präsentierten, oder auf anderen Substraten durch multivalente Adsorption und kovalente Vernetzung zu immobilisieren. Dieses Verfahren führt zu einer ultradünnen, glatten und sehr stabilen Monoschicht-Bürstenbeschichtung. Neben den Beschichtungen auf planaren 2D-Oberflächen können die PG-BPh-Polymere auch zum Beschichten komplexer 3D-Systeme, z. B. Mikrofluidikkanälen, verwendet werden. Aufgrund der hohen Beschichtungsdichte wiesen die modifizierten unpolaren Oberflächen hervorragende Antifouling-Eigenschaften auf und waren mindestens ein Jahr in physiologischem Puffer stabil. Die in-situ-Postmodifizierung mit spezifischen Liganden, z. B. Mannose, ermöglichte hochspezifische Protein- und Bakterienadsorption durch multivalente Protein-Kohlenhydrat-Wechselwirkungen. Daher bietet diese hochstabile Monoschicht-Beschichtung eine neue Technologie für

die universelle Modifikation Materialoberflächen und kann in einer Vielzahl von Biomedizinischen-Anwendungen eingesetzt werden. Die Arbeit eröffnet damit neue Wege für die Modifizierung unpolarer Materialoberflächen durch in-situ-Immobilisierung mit einer Vielzahl von selektiven Biomolekülen.

Um die Wechselwirkungen zwischen Materialoberflächen und Biomolekülen dynamisch zu steuern, wurde außerdem eine Spiropyran (SP)-basierte, lichtempfindliche funktionelle Beschichtung mit einem einer bioinerten PG-Basisbeschichtung entwickelt (Siehe Sektion 3.3). Normalerweise befinden sich die SP-Gruppen auf der Beschichtungsoberfläche in einer hydrophoben, ringgeschlossenen SP-Form, so dass die unspezifische Proteinadsorption und Zelladhäsion gefördert wird. Nach Bestrahlung mit UV-Licht öffnet sich der Spiroring von SP und wandelt sich in ein hydrophileS und zwitterionisch Merocyanin (MC) um. Sowohl Hydrophilie als auch Zwitterionen tragen zur Bildung der hydratisierten Schicht bei und erschweren eine Proteinadsorption und Zelladhäsion. Darüber hinaus wurde die kontrollierte Adsorption und Desorption von Proteinen, das Anheften und Ablösen von Zellen sowie eine dichte Zellschicht durch die SP-funktionalisierten Beschichtung nicht-invasiv erreicht. Die entwickelte funktionelle Beschichtung könnte somit Anwendung für Licht-responsiven Oberflächenmodifizierungen im Tissue Engineering finden. Das gegenwärtige System erfordert jedoch die Anwendung von UV-Licht, so dass z.B. die Lichtdurchlässigkeit von Gewebe ein limitierender Faktor ist.

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8. Appendix

8.1 Publications and Patent Applications

Publications

[1] **Leixiao Yu**, Chong Cheng, Qidi Ran, Christoph Schlaich, Paul-Ludwig Michael Noeske, Wenzhong Li, Qiang Wei, Rainer Haag. Bioinspired Universal Monolayer Coatings by Combining Concepts from Blood Protein Adsorption and Mussel Adhesion. *ACS Appl. Mater. Interfaces* **2017**, 9, 6624−6633.

[2] **Leixiao Yu**, Yong Hou, Chong Cheng, Christoph Schlaich, Paul-Ludwig Michael Noeske, Qiang Wei, Rainer Haag. High-antifouling Polymer Brush Coatings on Nonpolar Surfaces via Adsorption-Crosslinking Strategy. *ACS Appl. Mater. Interfaces*, **2017**, 9, 44281–44292.

[3] **Leixiao Yu**, Christoph Schlaich, Yong Hou, Jianguang Zhang, Paul-Ludwig Michael Noeske, and Rainer Haag. Photo-regulating Antifouling and Bioadhesion Functional Coating Surfaces Based on Spiropyran. *Chem. Eur. J.*, **2018** 10.1002/chem.201801051.

[4] Christoph Schlaich, **Leixiao Yu**, Luis Cuellar Camacho, Qiang Wei, and Rainer Haag. Fluorine-free superwetting systems: construction of environmentally friendly superhydrophilic, superhydrophobic, and slippery surfaces on various substrates. *Polym. Chem.*, **2016**, 7, 7446-7454.

[5] Christoph Schlaich, Luis Cuellar Camacho, **Leixiao Yu**, Katharina Achazi, Qiang Wei, and Rainer Haag. Surface-Independent Hierarchical Coatings with Superamphiphobic Properties. *ACS Appl. Mater. Interfaces*, **2016**, 8, 29117–29127.

[6] Christoph Schlaich, Mingjun Li, Chong Cheng, Ievgen S. Donskyi, **Leixiao Yu**, Geonho Song, Ernesto Osorio, Qiang Wei, and Rainer Haag. Mussel-Inspired Polymer-Based Universal Spray Coating for Surface Modification: Fast Fabrication of Antibacterial and Superhydrophobic Surface Coatings. *Adv. Mater. Interfaces*. **2018**, 1701254.

[7] Jianguang Zhang, Wei Chen, **Leixiao Yu**, Mingjun Li, Falko Neumann, Wenzhong Li, Rainer Haag and Nan Ma, Selective Endothelial Cell Adhesion Via Mussel Inspired

Hybrid Microfibrous Scaffold. *ACS Appl. Nano Mater.,* **2018***,* 1, 1513–1521.

Patents

L. Yu, Q. Wei, R. Haag, M. Weinhart, *Blockcopolymere als bionierte universelle Monoschichtsysteme*, europäische Patentanmeldung 2016, EP 16177279, internationale Patentanmeldung 2017, PCT / EP 2017/066313, WO 2018/002322 A2

8.2 Poster Presentations

[1] **Leixiao Yu**, Rainer Haag

Light Switchable Spiropyran Functionalized hPG as Antifouling Coatings, SFB 658 Kolloquium, Berlin, Germany, 2015

[2] **Leixiao Yu**, Rainer Haag

Bioinspired Universal Monolayer Coatings by Combining Concepts from Blood Protein Adsorption and Mussel Adhesion, International Symposium on "Functional Biointerfaces" and 100th Anniversary of Georg Manecke (1916-1990) Ceremony, Berlin, Germany, 2016

9. Curriculum Vitae

Der Lebenslauf ist aus Gründen des Datenschutzes nicht enthalten