JOURNAL OF Applied Polymer SCIENCE

Special Issue: Bioactive Surface Functionalization

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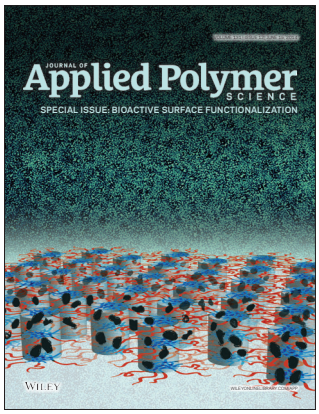

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REVIEW

Orthogonal Surface Functionalization Through Bioactive Vapor-Based Polymer Coatings

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ABSTRACT: Reactive chemical vapor deposition (CVD) polymerization provides a substrate-independent platform for effective functionalization of virtually any solid substrates, flat, or curved, even with complex geometries. This article reviews bioactive surface functionalization strategies based on CVD polymerization and highlights commonly used surface chemistries. These reactions include alkyne–azide "click" chemistry, reactions of active esters with amine, aldehydes/ketones with hydrazides and alkoxyamines, thiols with alkenes and alkynes and surface-initiated atom transfer radical polymerization. The resulting biofunctional surface coatings can facilitate orthogonal immobilization of more than one type of ligand on a substrate. CVD polymer coatings with nanoscale thicknesses are widely applicable in biomedical applications and can be easily integrated into micro- and nanodevice fabrication. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40315.

KEYWORDS: biomedical applications; coatings; surfaces and interfaces; biomaterials; functionalization of polymers

Received 21 October 2013; accepted 12 December 2013

DOI: 10.1002/app.40315

INTRODUCTION

Bioactive surface functionalization is of vital importance to many biomedical applications such as drug delivery, biosensors, medical implants, and tissue engineering, just to name a few.[1–3] Commonly used surface functionalization methods include fatty acids on metal oxide surfaces, silane chemistry for silica or silica-like substrates, organosulfur compounds for coinage metals, especially gold, and photochemical and plasma treatments, etc.[3–9] These methods have contributed significantly to the development of bioactive surface modifications. For instance, fatty acids, silanes, siloxanes and alkanethiols are commonly used in the form of self-assembled monolayers (SAMs), but they are highly substrate specific and feature variable degrees of stability. Moreover, there are other barriers to achieving repeatable surface functionalization results. For example, silane chemistry is frequently used for functionalizing transparent substrates such as glass and silica. However, silane molecules are highly moisture sensitive and can self-polymerize, which leads to surface contamination and difficulties in preparing reproducible, high-quality SAMs.[9] Other techniques like plasma treatment have lower substrate dependence, but show limitations with respect to the choice of specific functional groups that can be introduced into a surface.[10] Photochemical methods have similar shortcomings and not every photochemical reaction has the high yields ideally expected from a surface functionalization method.[11,12]

For biomaterials design, selecting the bulk materials is an important effort, which needs to take into consideration a number of factors, including mechanical properties, ease of processing, pore-forming properties, etc.[2] However, the best selection of the bulk material would not necessarily be the easiest for surface functionalization, which is essential for controlling the biological response to a particular biomaterial.[2] Therefore, an ideal technique for bioactive surface functionalization should be able to retain the beneficial bulk material properties of the substrate, while modifying the surface with high chemical specificity. This allows for accurately controlling the immobilization reactions and a diverse range of different specifications can be accommodated.

Reactive polymer coatings prepared through chemical vapor deposition (CVD) polymerization of functionalized [2.2]paracyclophanes (Scheme 1) can combine a number of these specifications: (1) CVD polymerization is substrate-independent: Virtually any solid substrates, no matter flat or with complex three-dimensional geometries, can be conformally coated and functionalized as long as they are stable in vacuum at or below room temperature.[10,13] (2) An unprecedented library of reactive chemical groups has been established for the reactive CVD coatings (Scheme 1), which provide a wide selection of functional groups for precisely immobilizing biomolecules according to specific application requirements. (3) Multiple functional groups can be applied to the surfaces for orthogonal

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Joerg Lahann is a Professor of Chemical Engineering and the inaugural Director of the Biointerfaces Institute at the University of Michigan, and the co-director of the Institute of Functional Interfaces at the Karlsruhe Institute of Technology (KIT). He is a fellow of the American Institute of Medical and Biological Engineers, the author of more than 140 publications, and the inventor or co-inventor of more than 25 patents or patent applications.

immobilization of multiple biomolecules.[14–16] As shown in Scheme 2, the spatial distributions of multiple functional groups can be accurately controlled as functional group gradients, as random distributions with variable ratios, or as defined patterns.[10,15,17,18] These spatial arrangements feature different biological functions. A gradient surface provides gradual and continuous change of two different functional groups and the immobilized molecules on the surface, which opens interesting possibilities, such as directed molecule transportation or cell migration. The random distribution is preferred for applications requiring an even distribution of the different immobilized molecules in close proximity. In addition, more than two functional groups can be applied and their ratios are adjustable.[19] A patterned surface allows control of feature sizes ranging from nanometers to millimeters, as they are widely used in cell fate studies or in high-throughput bioMEMs devices.[2,20] (4) The nanoscale CVD polymer coatings can be easily incorporated into micro-/nanofabrication processes to cater to the miniaturization trend of biomedical devices.[2,21] In addition to surface functionalization, the CVD coatings can also serve other functions such as polymer matrix in polymer composites or an insulation layer for microelectrodes.[21,22] The synthesis mechanism of [2.2]paracyclophanes, the CVD polymerization process and vapor-assisted surface patterning techniques have been elaborated in previous review articles.[23–25] This article will focus on the recent progress of bioactive surface functionalization through reactive CVD polymer coatings with a profound emphasis on the surface chemistries.

When applying the reactive CVD polymer coatings for surface functionalization, a wide range of bioconjugation chemistries can be selected for immobilization of biomolecules.[11] Because of the delicate nature of biomolecules (protein, peptide, sugar, DNA, etc.), the surface chemistries need to be highly efficient, proceed under mild conditions, and be compatible with the physiological conditions conducive to native biomolecules. When immobilizing multiple biomolecules, orthogonal sets of highly efficient bioconjugation reactions need to be selected to prevent cross-reactions and ensure precise control of the biomolecule immobilization.[14,15,26] Herein, we review the recent progress of applying some of the bioconjugation chemistries for surface functionalization based on the reactive CVD polymer coatings.

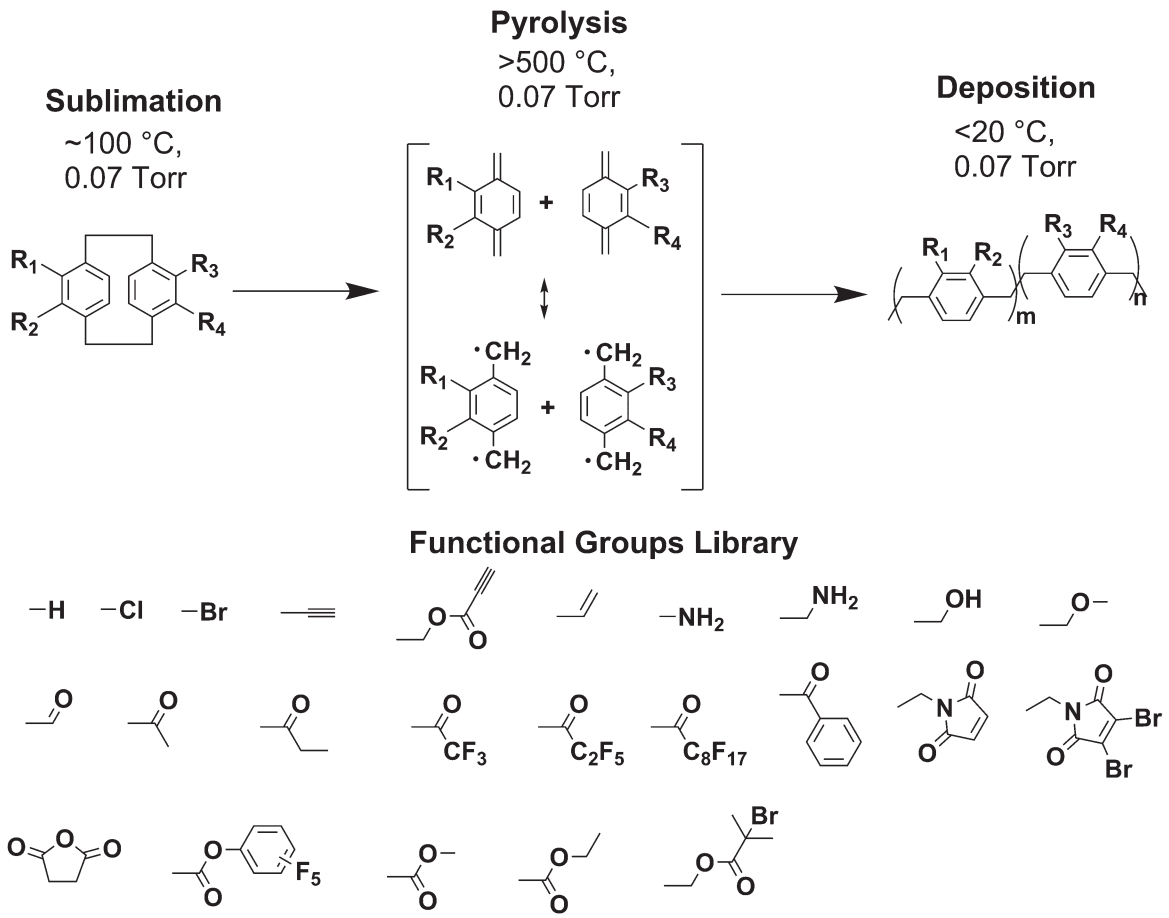
COMMON BIOACTIVE SURFACE FUNCTIONALIZATION REACTIONS

Alkyne–Azide "Click" Reactions

Copper-catalyzed azide–alkyne Huisgen cycloaddition (CuAAC), an archetype example of "click chemistry", has been widely used since its introduction by the Sharpless and Meldal research groups in the early 2000s.[26–29] CuAAC is highly efficient under mild conditions, forming stable triazole linkages that are resistant to hydrolysis, reduction, or oxidation.[30] More recent efforts have been focused on reducing or even eliminating the use of the cytotoxic copper catalyst by activating the alkyne through incorporating an electron-withdrawing group, or taking advantage of ring-strained molecular structures.[31–34]

The first example of CVD polymer coatings that can be functionalized with alkyne groups took advantage of a alkyne-modified poly(*p*-xylylene).[35] Similarly, Gleason and co-workers[36] reported poly(propargyl methacrylate) (PPMA) films prepared by initiated CVD (iCVD) from commercially available monomer. The PPMA films are directly e-beam patternable and click-active with the CuAAC chemistry.[36] More recently, Lahann and co-workers[15] reported a "double-click" cascade using the different reactivity of activated and nonactivated alkyne with azide groups on the surface coated with differently functionalized polymers in selected separate areas (Figure 1). The activated and nonactivated alkyne groups were applied in predefined separated pattern regions on the surface through CVD polymerization. Under the copper free condition, only the areas with activated alkyne (activated by the electron withdrawing ester group) could react with azide-functionalized molecules. For the subsequent step, now using the copper catalyst, the areas with nonactivated alkyne could then react with another molecule with azide group.

The most commonly seen copper-free click regents are cyclooctyne species with both ring strain and electron-withdrawing substituents.[37] They have been gradually commercialized so that

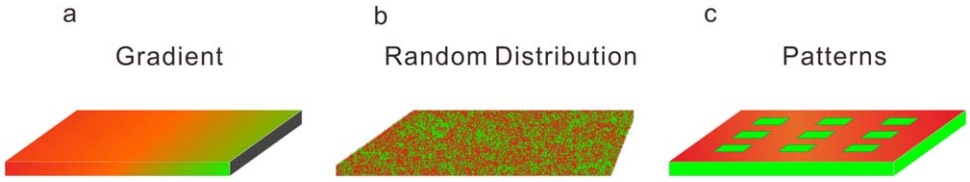


Scheme 1. Scheme for the CVD polymerization process of different functionalized [2.2]paracyclophanes and the established and constantly updating functional group library. The functionalized [2.2]paracyclophanes (the precursors or dimers) sublimate around 100°C in vacuum, flow with the argon carrier gas to the pyrolysis zone (>500°C) and break into radicals. The radicals adsorb on the substrates placed on the cooled deposition stage (<20°C) and form the functionalized polymer coatings.

the challenging synthesis is less of a barrier to their applications. Under physiological conditions, the reactant pair of the alkyne–azide click reaction is not reactive with any other functional groups in biological systems, which gives it the advantage of naturally orthogonal to other bioconjugation reactions.[11] The copper-free alkyne–azide "click" is one of the "bioorthogonal reactions" defined by Sletten and Bertozzi.[26] "Bioorthogonal" is a refinement of the "click" concept, requiring the reactions not only to proceed reliably, highly efficient, and specific under physiological conditions but also to be strictly selective so that they do not interfere with any of the biological processes *in vitro* or *in vivo*.[26] Bioorthogonal copper free click chemistry has been successfully used in many applications such as biomolecule and cell labelling, *in vivo* imaging, etc.[26,38]

Active Ester and Amine Reactions

Active ester reactions with amines are broadly used in organic synthesis, peptide synthesis, and surface functionalization.[39,40] Compared to anhydrides, active esters are not as highly reactive but still readily reactive with amine groups under mild conditions and they are usually more resistant to hydrolysis and more stable for storage. The most well-known active ester is the N-Hydroxysuccinimide (NHS) ester, which is commonly seen in the 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) or N′,N′-dicyclohexyl carbodiimide (DCC)



Scheme 2. Different spatial arrangements for functional groups of multifunctional reactive CVD coatings. Green and red color pixels each represent a different type of functional group. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

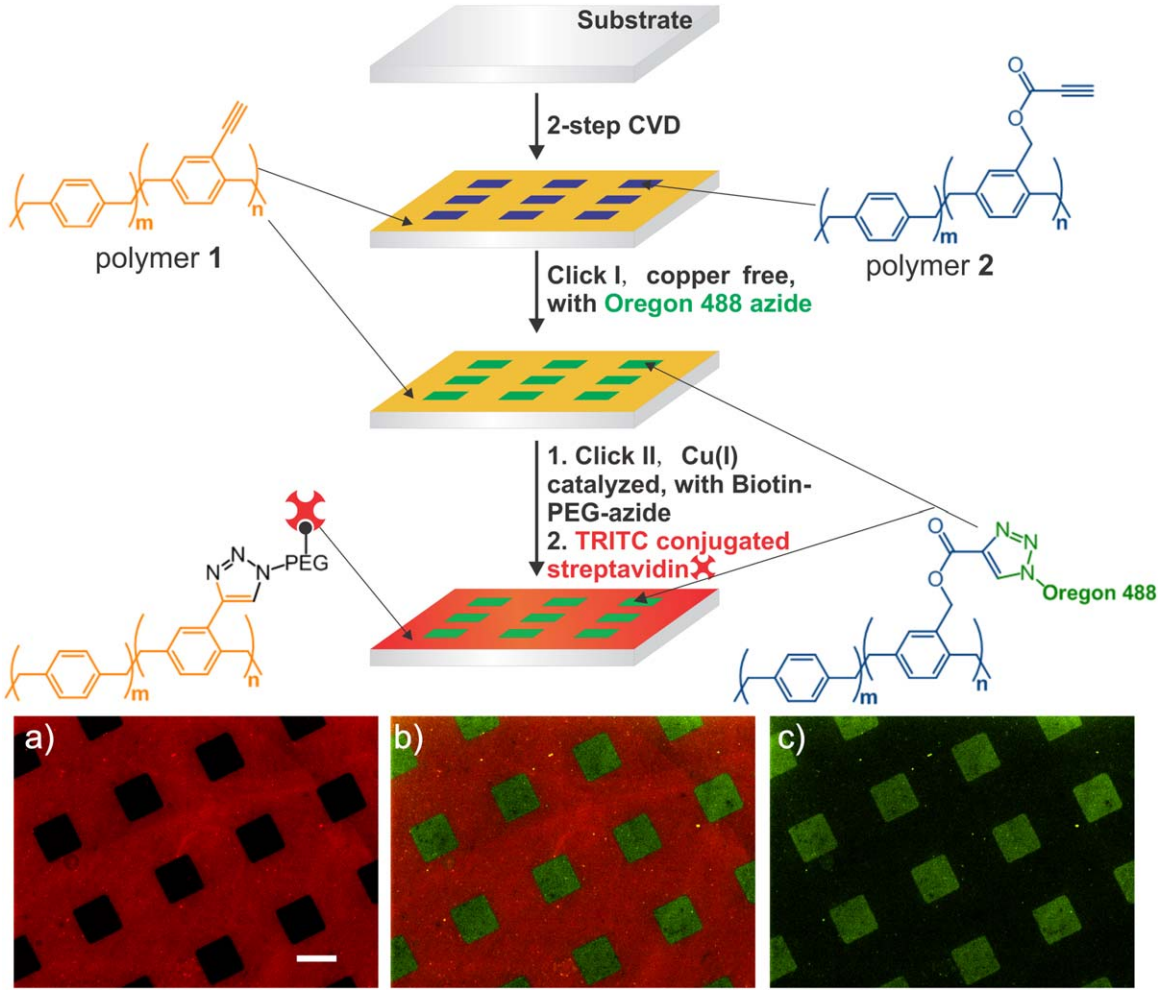


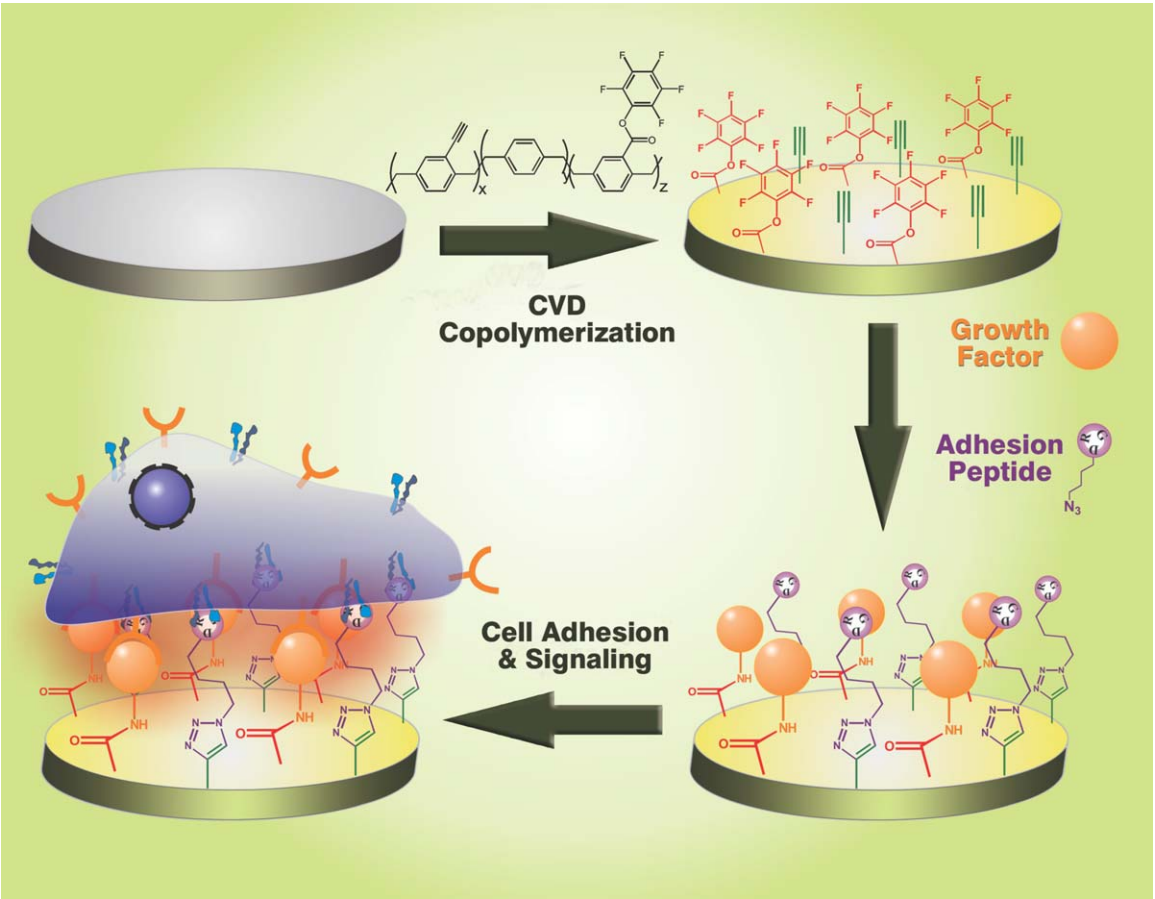
Figure 1. "Double-click" cascade using the different reactivity of activated and nonactivated alkyne with azide groups. The surface is coated with different CVD polymers in different selected areas. (a), (b), (c) Fluorescence images of samples prepared exactly as shown in the schematic representation. (b) overlay image of green and red channels shown in (a) and (c). Scale bar represents 200 μm. (Reproduced from [Ref. 15], with permission from Wiley (Copyright 2011 Wiley). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

crosslinker chemistry for carboxylic acids and primary amines.[41] The disadvantage of the NHS ester is that it is prone to hydrolysis, sensitive to moisture, and difficult for long-term storage.[42]

Pentafluorophenyl (PFP) ester is another active ester with better stability in aqueous solution than NHS esters, but can still readily react with amine functionalized molecules.[11] Lahann et al.[43] have first used CVD-based PFP ester coatings for immobilizing amino-functionalized linkers or for direct covalent binding of proteins. Similarly, Gleason and co-workers[44,45] incorporated PFP ester into the iCVD polymer coatings by copolymerization with pentafluorophenyl methacrylate (PFM) in 2007. Amine functionalized fluorescence dyes, Polyethylene glycol (PEG) or peptide can subsequently be immobilized on the coating surfaces.[44–46] Potential applications include generating protein/cell patterns and immobilizing the protein antibodies of viral vectors for gene delivery.[47,48] In a more recent publication, the Lahann group reported using the PFP ester–amine chemistry together with the alkyne–azide click chemistry to orthogonally immobilize two different biomolecules on the surface (Scheme 3).[14] The bioactivities of the co-immobilized biomolecules were verified. PFP-ester was used for immobilizing epidermal growth factor in this specific work and it is generally applicable to all types of proteins.[14,48] For example, CVD-based PFP-ester coatings have been successfully used for binding anti-adenovirus antibodies on the surface of tissue engineering scaffolds for viral gene delivery.[48]

Aldehyde/Ketone Reactions with Hydrazide and Alkoxyamine

Aldehyde and ketone groups are useful reactive sites for organic synthesis and biomolecule modifications.[11] Usually biomolecules contain no naturally occurring aldehyde residues, which need to be introduced by chemical modifications, such as periodate oxidation of carbohydrates.[11] Under physiological condition, aldehydes and ketones react with amine nucleophiles enhanced by the α-effect, such as alkoxyamines, hydrazides, or hydrazines.[26] The reactions of aldehyde or ketone with alkoxyamine or hydrazide derivatives form stable oxime and hydrazone linkages, respectively.[26] Although oximes have better hydrolytic stability than hydrazines, more biomolecules with hydrazide groups are commercially available, while



Scheme 3. Scheme of orthogonally immobilizing two different biomolecules on the CVD polymer surface with both alkyne and PFP-ester functional groups. (Reproduced from [Ref. 14], with permission from Wiley (Copyright 2012 Wiley). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

choices of aminoxy functionalized molecules are limited.[11,49] Maynard and co-workers[50,51] reported using the oxime chemistry for immobilizing α–oxoamide or levulinate-modified proteins onto electron-beam generated patterns of aminooxy functionalized PEG on silicon. Lahann and co-workers[16,52] used aldehyde-functionalized CVD polymers for binding sugar or sugar-containing molecules, such as heparin on the surface, usually with the help of a dihydrazide linkers as these sugar molecules are also functionalized with aldehyde. Other hydrazide-functionalized molecules like biotin hydrazide or PEG-hydrazide are also commonly used to react with the aldehyde functionalized surface and other biomolecules can be immobilized afterwards.[52,53] The aldehyde-hydrazide reaction has also been used together with the alkyne–azide click reaction for orthogonally immobilizing two different biomolecules on the multifunctionalized CVD film.[16] As shown in Scheme 1, ketones and in particular the more reactive fluorinated ketones are commonly used functional groups in CVD polymer coatings. The fluorinated ketones introduce both hydrophobicity and surface reactivity toward hydrazide or aminoxy functionalized molecules.[54] For example, radicals generated from trifluoroacetyl-functionalized precursor show excellent penetration into microstructures during the CVD polymerization and have been used as polymer matrix in polymer/carbon nanotube nanocomposites.[22] The nanocomposites can be further functionalized with hydrazide/aminoxy containing molecules.[22]

Thiol Reactions

Orientated and site-specific peptide immobilization is of particular importance for understanding protein and peptide functions and their interactions with material surfaces.[55,56] Site-specific orientation and immobilization is achievable if uniquely reactive groups are accessible at the surface of biomolecules.[57,58] Thiol groups is a particularly interesting reactive group that is naturally occurring in proteins and peptides. Unlike the commonly exploited amino groups, free thiol groups are rarely present in native proteins and cysteine is the only amino acid with sulfhydryl group.[11,57] Free thiol groups can however be obtained biochemically, generated through biopolymer chemical synthesis, or introduced by genetic engineering.[57] They can also be intentionally introduced to specific sites during peptide synthesis, aiming for orientated, and site-specific immobilization.

The classical thiol-ene or thiol-yne reactions are highly useful bioconjugation reactions with the benefit of their tolerance to water and oxygen, though they usually need be photochemically or thermally activated.[59–61] Similarly, maleimide-thiol reactions can proceed under a milder reaction condition more compatible with

delicate biomolecules, with an ideal reaction pH range of 6.5–7.5.[11,61] Maleimide is a popular functional group for commercially available crosslinking agents or linker molecules. As listed in Scheme 1, CVD polymer coatings can carry the thiol reactive functional groups such as alkene, alkyne, and maleimide for reacting with the thiol-containing biomolecules. Chen and coworkers[61,62] have used the thiol-maleimide reaction and photochemical thiol-ene and thiol-yne reactions on reactive CVD polymer coatings for generating peptide, protein, PEG, and cell patterns.

Atom Transfer Radical Polymerization Reactions

Atom transfer radical polymerization (ATRP) belongs to the category of "living" polymerizations, a concept introduced in 1956, by Szwarc, in which chain termination and chain transfer are absent.[63,64] All controlled living radical polymerization (CRP) methods are "based on establishing a rapid dynamic equilibration between a minute amount of growing free radicals and a large majority of the dormant species".[65] ATRP is the most extensively studied CRP with the advantages of precise control over polymer molecular weights, molecular weight distributions, complex architectures, and functionality.[66] Similar as the situation of click chemistry, lots of recent efforts to improve the ATRP technique aimed at reducing the amount of toxic transition metal catalysts (most commonly copper salt) added in the polymerization process. The new improvements include simultaneous reverse and normal initiation (SR&NI), activators generated by electron transfer (AGET), initiators for continuous activator regeneration (ICAR), activators regenerated by electron transfer (ARGET), and electrochemically mediated atom transfer radical polymerization (eATRP).[67–71] Several excellent reviews from Matyjaszewski group have summarized the recent development of ATRP.[66,72,73] The new techniques not only significantly lowered the transition metal catalyst amount, reduced the need for catalyst removal after polymerization, but also increased the oxygen tolerance of the ATRP process, which can facilitate its industrialization.

For bioactive surface functionalization, polymer brushes prepared through surface-initiated ATRP (SI-ATRP) is indispensable and have been used in myriad applications.[73–75] Polymer thin films with bromoisobutyrate groups for ATRP initiation can be prepared through CVD polymerization.[76] PEG brushes are among the most commonly used polymer brushes prepared by SI-ATRP to effectively prevent protein adsorption and cell adhesion.[21,76] We recently reported the utility of CVD coatings for the fabrication of an ultrasmall implantable composite microelectrodes[21] (Figure 2). A layer of 800 nm poly(*p*-xylylene) without functional groups was used as the insulation layer for the polymer/carbon fiber composite microelectrode. Another 50 nm polymer coating functionalized with bromoisobutyrate groups was coated on top of the insulation layer followed by SI-ATRP of poly(ethylene glycol) methyl ether methacrylate (PEGMA), which effectively prevented protein adsorption. This study is a good example showing how CVD polymer coatings can be easily integrated into the micro-fabrication processes. For the SI-ATRP process, a wide variety of properties (different composition, topology, functionality, charge etc.) can be introduced through choice of the monomers or macromonomers that make up the polymer brushes and through the end groups that can be converted into other functional groups for subsequent surface modifications.[66] Used

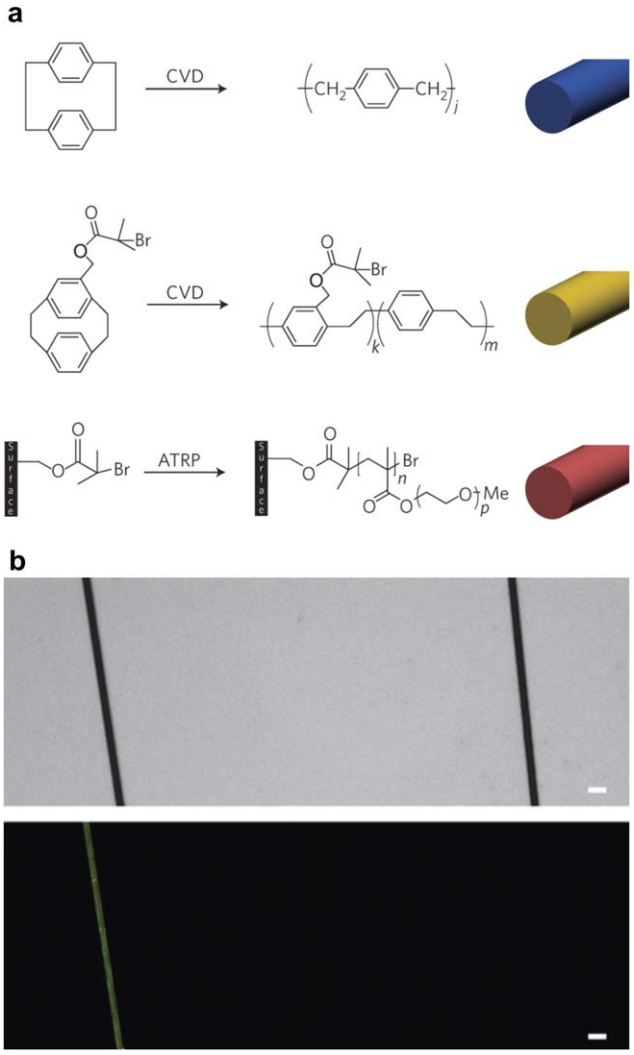
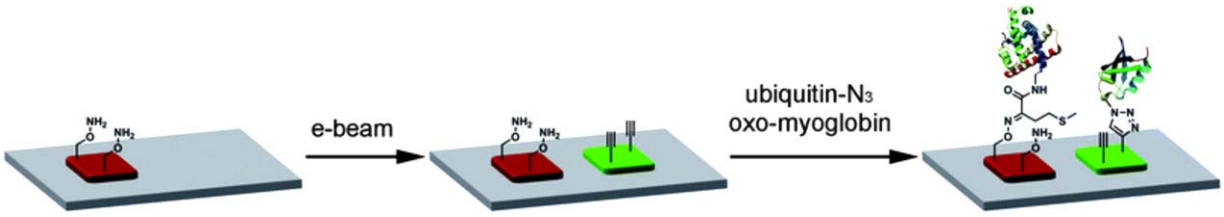


Figure 2. (a) Scheme of applying CVD polymer coatings in the fabrication of the microthread electrodes. Carbon fibers were coated with 800 nm poly(*p*-xylylene) followed by a 50-nm-thick layer of poly((*p*-xylylene-4-methyl-2-bromoisobutyrate)-co-(*p*-xylylene)). PEG methyl ether methacrylate (PEGMA) brushes were then grafted onto the doubly coated fiber by SI-ATRP. (b) Bright-field and fluorescence images of FITC–albumin adsorbed onto a poly(*p*-xylylene)-coated device (left) and an ATRP-PEGMA coated device (right). Scale bar, 20 μm. The images show less protein adsorption onto the PEGMA surface (right) compared to nonfunctionalized poly(*p*-xylylene) coating surface (left). (Modified from [Ref. 21], with permission from Nature Publishing Group (Copyright 2012 Nature Publishing Group). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

together with the SI-ATRP technique, the surface functionalization capability of the CVD polymers can be significantly expanded and even more diversely applicable.

Orthogonal Immobilization Strategies for Multifunctionalized Surfaces

To mimic the complex extracellular environment of cells and tissues, multiple biomolecules need to be immobilized on surfaces with a reproducible, efficient, biocompatible, and precisely controlled method.[2,77] In attempts to address this challenge, the development



Scheme 4. Protein patterning by two orthogonal reactions. Aminooxy and alkyne PEGs are patterned next to each other by e-beam lithography. α-Ketoamide-myoglobin followed by azide-modified ubiquitin are then conjugated to the surface. (Reproduced from [Ref. 51], with permission from RSC. (Copyright 2011 Royal Society of Chemistry). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of orthogonal immobilization strategies have made considerable progress either alone or in conjunction with micro-/nanopatterning techniques.[50,51,78,79] The patterning techniques include dip-pen nanolithography (DPN), electron-beam (e-beam) lithography, capillary force lithography (CFL), soft lithography, photolithography, nanoparticle-based or supramolecular self-assembly, Langmuir–Blodgett patterning, etc.[10,50,77,78,80–82]

Besides reactive CVD polymer coatings, there exist alternative ways for orthogonal surface modifications. For instance, Chi and co-workers developed an interesting approach using nanostripe patterns using Langmuir–Blodgett patterning with orthogonal groups in different pattern areas functionalized by silane chemistry.[81] The chemistries chosen in this study, CuAAC and thiol-ene reactions, need to be applied sequentially to ensure their orthogonality. Jonas et al.[83] synthesized photosensitive silanes to protect amino, hydroxyl, and carboxylic acid groups. The latter two are incompatible with the silane anchor group and need to be protected before silane reacts with the glass or SiO_2 surface. The possibility of orthogonal deprotection by controlling the photoactivation condition was demonstrated in the article.[83] Maynard and co-workers[50] used CuAAC and oxime chemistry together to orthogonally immobilize two different proteins functionalized with azide and α–oxoamide, respectively (Scheme 4).[50] PEGs are able to cross-link to the native oxide layer on silicon or to PEG film itself when exposed to focused electron beams, which is how the surface shown in Scheme 4 were patterned.[50] One good feature of this method is that PEG can minimize nonspecific binding.[50] In addition, the reactive functional groups on the proteins are artificially introduced for site specific immobilization and bioactivity retention.[50]

Despite the presence of many good alternative methods for surface orthogonal multifunctionalization, reactive CVD polymer coatings combine a number of remarkable features: First, they are highly reproducible, without the issues of moisture sensitive silane chemistries. Moreover, the spatial distribution of functional groups can in many cases be accurately controlled with various options available as shown in Scheme 2, Figure 1, and Scheme 3. The CVD method is simple and straightforward with often only one or at the most two reaction steps for multifunctionalization. The functional groups are applied in the form of a well-controlled, nanoscale thin film generated by a solvent-free process, which needs no post-polymerization treatment for solvent removal and thus more biocompatible. Lastly, there is usually no over complicated organic synthesis and the cost is lower than the expensive e-beam lithography.

CONCLUSIONS AND OUTLOOK

The rapid development of synthesis, chemical modification, and commercialization of biomolecules, such as proteins, peptides, sugars, DNA, or RNA has provided a wide selection of easily accessible choices for bioactive surface functionalization. These functionalized biomolecules can be immobilized onto the substrate-independent reactive CVD polymer coatings to accommodate the demanding needs of various biomedical applications.

The vapor-based reactive polymer coatings discussed in this review provide a wide selection of bioactive functional groups but still not every desired functional group is compatible with the CVD process. To address this limitation, in addition to the common bioconjugation reactions, multifunctionalized linker molecules and techniques like SI-ATRP can further expand the surface functionalization capacity of the CVD polymer coatings significantly. Bioactive surface functionalization is a highly multidisciplinary area with an increasing bulk of contributions and input from organic chemistry, polymer science, biology, instruments development, and other science/engineering areas. With the collective effort, we can better understand the biointerfaces and control the biomaterial surfaces with more precise and biomimetic approaches.

ACKNOWLEDGMENTS

The authors gratefully acknowledge support from DTRA, under project HDTRA1-12-1-0039 and the Army Research Office (ARO) under Grant W911NF-11-1-0251.

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