BIOMIMMOBILIZATION USING HUISGEN [1,3]-DIPOLAR CYCLOADDITION ON VAPOR-BASED POLYMER COATINGS

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Introduction

By merging reactive coatings technology based on chemical vapor deposition polymerization with Huisgen [1,3]-dipolar cycloaddition, biomedical coatings were deposited on many substrates relevant for biotechnology, which can provide precise immobilization capabilities for biomolecules.

Continuous technological progress in biology and medicine have fueled the needs for well-defined biointerfaces. For instance, in the area of BIOMEMS, the availability of precise immobilization methods for peptides, proteins, and DNA via exactly controlled surface reactions is often paramount for device performance. Future advances in the design of biologically-active interfaces require novel strategies for the robust and specific attachment of biological ligands onto surfaces. Chemical vapor deposition polymerization has been used to create reactive coatings with a range of different chemical side groups for further surface modification [1,2]. While Huisgen [1,3]-dipolar cycloadditions have been used for controlled surface modification of a series of different substrates including gold and silicon, much less is known about methods that can be applied to a wide range of different substrates without the need for custom-tailoring the surface chemistry for each substrate. Herein, a new class of biofunctional surfaces based on alkyne-containing vapor-based polymer coatings will be discussed [3].

Experimental

Materials. [2.2]paracyclophane (1) has been purchased from Sigma-Aldrich. Prior to polymer deposition using CVD polymerization, the starting materials di-ethyl [2,2]paracyclophane (2a) and ethynyl[2,2]paracyclophane (2b) were prepared from the commercially available paracyclophane 1, which was first converted to the respective di- and mono-formyl derivatives followed by Bestmann’s acetylene synthesis. Synthesis of paracyclophane 4 involved conversion of cyclo-octyn-3-ol and [2.2]paracyclophane-4-carboxylic acid anhydride in the presence of DMAP and TEA in anhydrous THF for 10 hrs.

CVD polymerization. Depositions were carried out in a custom-built chemical vapor deposition system. The source consists of a quartz tube encased in a 3-zone tube furnace. A system pressure of 0.5 mbar and sublimation temperatures between 90-110 ºC were used to ensure sublimation. Argon carrier gas was used to independently control the flow velocity of sublimated monomers. The sample holder was set 2 inches below the position of the source. Rotation of the sample holder ensured formation of homogeneous copolymer surfaces. The sample holder remained at 15 ºC. Deposition occurred on silicon, gold, and glass substrates. To minimize wall deposition, the chamber wall was heated to 120 ºC (1).

Surface characterization. Thicknesses were recorded at a wavelength of 532 nm using an EP3-SW imaging ellipsometry (Nanofilm Technologie GmbH, Germany). Both, nulling (four zones) and mapping experiments were performed at an angle of incident of 70º, and an anisotropic Cauchy model was used to model the ellipsometric parameters psi and delta. Infrared spectroscopy was performed on a Nicolet 6700 spectrometer utilizing the grazing angle accessory (Smart SAGA) at a grazing angle of 85º.

Results and Discussion

Our initial studies on alkyne-containing vapor-deposited polymer coatings demonstrated that azide-functionalized CVD polymers can be deposited on a wide range of different substrates thereby opening up the potential of the Huisgen [1,3]-dipolar cycloaddition reaction to a versatile group of substrate materials [3]. Successful polymerization resulted in well-defined polymer films with thicknesses between 50 and 200 nm. The identity of the polymers was confirmed using a combination of surface analytical methods including X-ray photoelectron spectroscopy, FTIR spectroscopy and imaging ellipsometry.

Following our initial approach [3], Huisgen 1,3-dipolar cycloaddition between the reactive polymer coating 3b and an azide-containing biotin-based ligands was conducted in the presence of copper(ii) sulfate and sodium ascorbate. Sodium ascorbate acts as a reductant, generating Cu ions in situ from CuSO4, which functions as the active catalyst of the cycloaddition. In addition to the previously used biotin-azide ligand [3], an azide-functionalized laminin-derived peptide (YIGSR), as well as 1-azido-1-deoxy-β-D-glucopyranoside and 1-azido-1-deoxy-β-D-galactopyranoside were used. Spatial control over the cycloaddition reaction onto polymer 3b was achieved via microcontact printing (µCP) of the Copper catalyst onto preadsorbed ligand films. Surface patterning was confirmed by XPS imaging monitoring the spatial distribution of N1s signal. In addition, imaging ellipsometry revealed peptide patterns with an average thickness of about 1 nm. When the biotin-azide ligand was used, subsequent surface reaction with fluorescence-labeled streptavidin resulted in characteristic surface patterns as determined by fluorescence microscopy. Similarly, selective 1-azido-1-deoxy-β-D-glucopyranoside and 1-azido-1-deoxy-β-D-galactopyranoside was imaged by subsequent reaction with fluorescence-labeled lectins. In the case of 1-azido-1-deoxy-β-D-galactopyranoside, Concanavalin was used, while 1-azido-1-deoxy-β-D-galactopyranoside was reacted with PNA.

In the case of laminin peptide ligands, biological activity was confirmed by incubating films consisting of polymer 3b with and without immobilized laminin petide with human umbilical vein endothelial cells (HUVECs). Cells were initially incubated without medium for 6 hrs and were seeded onto substrates that were modified with the laminin epitop peptide. A representative image is shown in Figure 1 along with a reference, which was
not modified with the peptide.

One of the potential disadvantages of reactive coating 3b for use in biomedical application is the need to use a Copper catalyst, which may cause cytotoxicity of the modified surfaces, if the Copper cannot be quantitatively removed after surface reaction. Ring-constrained alkynes have recently been shown to undergo Huisgen [1,3]-dipolar cycloaddition without the need for copper catalysts [4]. Following this concept, we developed a surface modification approach that uses ring-constrained alkynes for copper-free Huisgen [1,3]-dipolar cycloaddition.

Using 4-hydroxymethyl-[2,2]paracyclophane as starting material, we initially attempted to synthesize a cyclooctyne-functionalized [2,2]paracyclophane following literature-described procedures. However, most likely due to the low solubility of 4-hydroxymethyl-[2,2]paracyclophane, the reaction did not yield detectable desired product. Ultimately, the synthesis was achieved by reaction of [2.2]paracyclophane-4-carboxylic acid anhydride with cyclo-octyn-3-ol to result in paracyclophane 4 with 28% yield. Subsequent CVD polymerization of 4 resulted in well-defined polymer films. The chemical composition of the polymer film based on XPS was in good agreement with the theoretical composition obtained on the basis of the composition of starting material 4. As expected, polymer 5 underwent Huisgen [1,3]-dipolar cycloaddition, even without the use of a Copper catalyst.

Conclusions

Alkinyl-modified poly-p-xylylene coatings can be prepared by CVD polymerization on a wide range of different surfaces. They enable further surface modification with azide modified ligands with or without use of Copper catalyst. The introduction of a generic surface modification approach is significant, because many of the widely used biomaterials do not have intrinsic functional groups for direct modification. Moreover, synthesis and CVD polymerization of [2.2]paracyclophane 4 establishes a significant advancement towards biomedical coatings because it eliminates the need for using Copper. These novel materials may find applications in microfluidics, sensors, BIOMEMS, or as biomedical implant coatings.

References

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