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Micropatterned Surfaces with Covalently Grafted Unsymmetrical Polyoxometalate-Hybrid Clusters Lead to Selective Cell Adhesion

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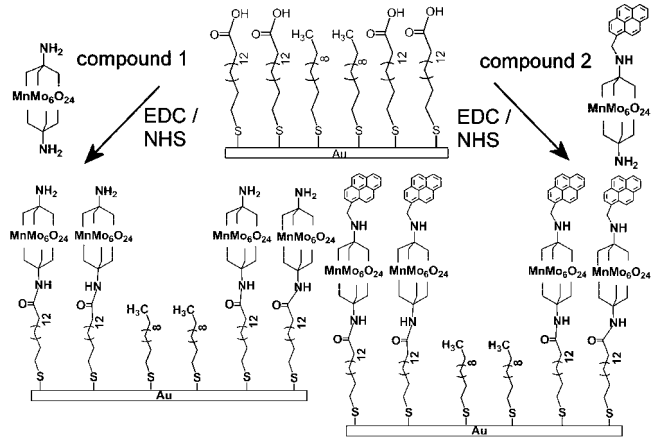
Polyoxometalates (POMs) are molecular metal oxides with a wide variety of structural types and have diverse physical properties including catalytic, electronic, and biological activity.[1] Therefore POMs are attractive for the development of materials and functional devices[2] and the surface patterning of POMs would be an important step forward to gain spatial control, yet POMs have rarely been covalently anchored to surfaces.[3] One approach would be to anchor the POM to the surface via self-assembled monolayers (SAMs), which exhibit a high degree of structural order, and can be patterned easily.[4] Further, SAMs of alkanethiolates on gold are an important class of model substrates for mechanistic investigation of biomolecules including proteins and cells, and they have been widely utilized as platforms for studies of cell adhesion due to their flexibility.[5]

Since we are interested in developing POM-containing hybrids, we thought it would be of great interest to pattern POMs onto SAMs, see Scheme 1, as this could lead to a high degree of spatial control leading to many applications.[1] This is because POMs are rigid, anionic, and have interesting redox properties,[6] can be coated in organic ligands,[7] and have applications as diverse as in catalysis,[8] directing self-assembly at the surface using the POM scaffold, the development of sensors, or electronically active surface coatings.[2] Further, the development of pyrene-POM hybrids[9] should result in systems that are highly charged, yet hydrophobic. In this work we are particularly interested in developing new types of POM-based micropatterned surfaces for biomolecule or cell interactions.

The 16-mercaptohexadecanoic acid (MHA) moieties were stamped using a patterned poly(dimethylsiloxane) (PDMS) on gold surface, to which MHA molecules are linked via Au−S bonds (Scheme 1). The terminal carboxylic acid groups, which are directed away from the surface relative to the substrate, are available for covalent modification by coupling with symmetric Mn-Anderson clusters of compound **1** via N_3-(dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC)/*N*-hydroxysuccinimide (NHS). It should be noted that the development of such a step-by-step protocol has allowed us to lable these patterns with different terminal groups onto SAMs.

The SEM results (Supporting Information, Figure S3) show that micro-patterns (μP) with circular features of 50 μm in diameter have been transferred from the PDMS stamp inked with MHA to the gold surface through the formation of S−Au bonds. Analysis using time-of-flight secondary ion mass spectrometry (TOF-SIMS) has been used to analyze the composition of the patterned material. The results (Figure S2) confirm the coupling reaction between MHA and compound **1**. The significance of the result presented herein lies with the fact that this provides the first example of micropatterning a POM cluster onto SAMs via a covalent attachment and at the same time, it opens a completely new pathway for POM-based surface science, and the interaction of cells with metal oxide/POM-based surfaces. To test the cell response to μP(**1**-SAM), cell adhesion assays were performed using fibroblast cells (hTERT-BJ1), and the SEM results showed that cells have no preference to the patterned regions of this new assembly.

Scheme 1. Schematic Representation of Patterned Au Surface[a]



[a] Schematic shows covalent modification of areas terminated in carboxylic acid groups by attaching a symmetric Mn-Anderson cluster of compound **1** (LHS) and asymmetric Mn-Anderson cluster of compound **2** (RHS) onto SAMs via amide bond formation to give μP(**1**-SAM) and μP(**2**-SAM), respectively. (EDC 2 mM, NHS 5 mM, MES 50 mM, pH 6.5, solvent MeCN:H_2O = 1:4). Mn-Anderson = $MnMo_6O_{24}$.

A recent study of cell adhesion to areas with fluoroscein and rhodamine[10] demonstrated that although the rest of the surface was covered with aliphatic molecules terminated with methyl group, human fibroblasts showed an unexpected response to align themselves to the aromatic patterns, despite the presence of poly(ethylene glycol) (PEG), a protein resistant molecule in the fluorescent regions. Therefore, the nonadhesive cell behavior of μP(**1**-SAM) inspired us to develop a POM-organo hybrid further decorating **1**-SAMs by attaching a pyrene group to give μP(**2**-SAM) as shown in Scheme 1.[9] This was achieved by attaching the pyrene group to one side of the Mn-Anderson cluster. The composition of compound **2** has been established by ESI-MS spectrometry in MeCN. Interestingly, the free amine group at one side of compound **2** allows further "postsynthetic covalent modification" of the chemical properties on SAMs. It is noteworthy that the aromatic pyrene ring is composed solely of hydrogen and carbon, which is a good contrast to those compounds reported in literature,[10] where

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carboxylic acid group and hydroxyl group were present on the fluoroscein and rhodamine platforms which demonstrate cell adhesion properties.

After studying the interactions of μP(**2**-SAM) and fibroblasts, SEM results showed that cells specifically adhere to the patterned areas containing the aromatic pyrene platforms, see Figure 1. In some cases cells do spread beyond the patterned area but only to locate themselves on a patterned area adjacent.

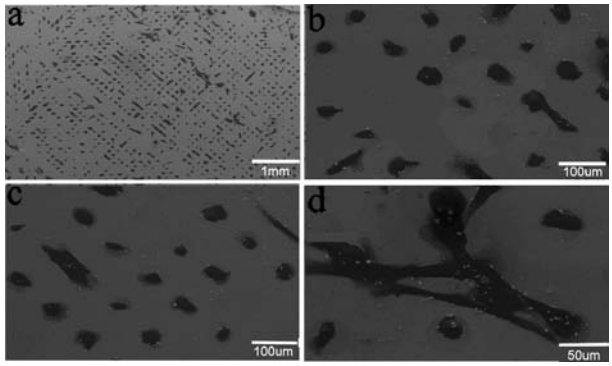
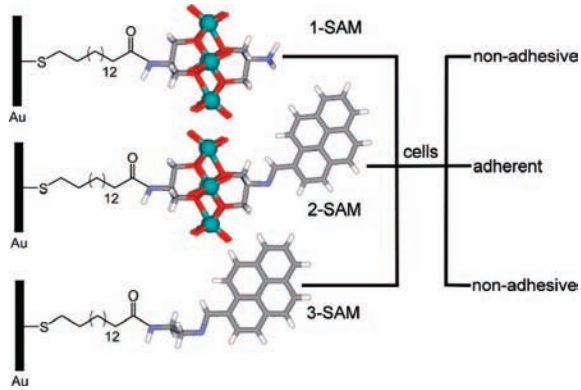


Figure 1. SEM images of the interactions between hTERT cells and the pyrene group modified Anderson cluster linked to the SAMs. Cells locate themselves exclusively on the patterned areas: (a) full overview on the surface; (b−d) enlarged images.

These results show that human fibroblasts have high affinity to the pyrene platform, and the observation of the cells spreading exclusively on pyrene terminated regions is significant since it highlights the opportunity to use model substrates that present different functionalities to modify cell adhesion behavior. The different cell response behavior to μP(**1**-SAM) and μP(**2**-SAM) systems provides a fundamental question regarding to the function of the POM-based Mn-Anderson cluster. To establish the role of the cluster, compound **3** was designed and patterned onto SAMs, μP(**3**-SAM), via an ethylenediamine linker (Scheme 2). Preliminary results of the cell response to μP(**3**-SAM) (Figure S5) showed that the immobilized substrate of pyrene group does not show obvious cell adhesion, which in turn unambiguously suggests that Mn-Anderson cluster does play an important role in the unique cell adhesion behavior of μP(**2**-SAM).

Scheme 2. Summary of the Results Reported in This Study[a]



[a] Rectangle = Au surface; N, blue; O, red; C, grey; H, white; Mo, green; Mn not visible.

The fact that cells preferentially adhere to the pyrene platform of μP(**2**-SAM) assembly is interesting and could be associated with the local negative charge associated with the POM cluster, and/or the size of the cluster which in turn prevents association between adjacent pyrene moieties in μP(**2**-SAM).[11] To help understand this complex process contact angle measurements were performed which showed the hydrophobicity of the surfaces increased gradually in order control-SAM < **1**-SAM < **2**-SAM < **3**-SAM. These preliminary measurements show the differences between the surfaces and they indicate that the physical properties of the surface-grafted POMs can be tuned by grafting different functional groups. Importantly they show that the **2**-SAM has a intermediate hydrophobic property between the control and **3**-SAM. Since previous studies on the interaction of POMs with biological systems show that parameters like POM-type, size, composition, etc. can also influence the interactions, it appears that electrostatic effects will be the most important part.[12]

In summary, we have demonstrated, for the first time, patterning of a polyoxometalate clusters onto SAMs via a simple, fast microcontact printing technique. By changing the terminal functional group, an asymmetric POM-containing framework with a pyrene group grafted onto Mn-Anderson cluster of compound **2** has been constructed and patterned successfully onto SAMs. We believe this is important since a SAM-POM-PYRENE hybrid system shows specific cell adhesion behavior on the patterned areas, but the function of Mn-Anderson cluster core is not fully understood. A preliminary investigation of cell response to μP(**3**-SAM) indicates that cells show no preference to the patterned areas, suggesting that Mn-Anderson cluster plays important roles in cell adhesion behavior of μP(**2**-SAM).

The attachment of the cells on patterned SAMs has been previously observed after the substrates were precoated with fibronectin[13] and adhesion peptides.[14] Herein, no adhesive proteins were employed and yet the cells responded strongly to the patterned areas. Our current research efforts are aiming at establishing a deeper understanding of the mechanisms driving the cell adhesion behavior of the POM-SAMs interactions by adjusting the functional groups at the tails of SAMs and applying novel polyoxometalate clusters into SAMs.

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Supporting Information Available: Preparation of SAMs, POM-SAMs, cell culture and SEM analysis, and synthetic procedures of compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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