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Click chemistry on self-assembled monolayer of zeolite L crystals by microcontact printing – Applications in nanobiotechnology

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ABSTRACT

Self assembled monolayers (SAMs) of zeolite L crystals were functionalized with fluorescent dye molecules or bioactive carbohydrate using microcontact printing (mCP). Fluorescent molecules such as lissamine rhodamine (LRA) and sugars such as α-D-mannoside (α-D-Man) functionalized with a terminal acetylene spacer were immobilized by "click" reaction on the top side of an azide functionalized zeolite L monolayer. Patterned and non-patterned SAMs of α-D-Man functionalized zeolite were used for cell adhesion and cellular patterning.

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1. Introduction

Multivalent interactions, described by the interaction between multivalent receptors and ligands, play an important role in many biological recognition events [1] and have attracted significant interest in the field of biochemistry [2,3].

Carbohydrates, which define a class of biopolymers commonly found in nature as glycoconjugates, can form highly branched structures and contain much more structural information than nucleic acids and proteins which are linear assemblies [4]. Carbohydrates mediate many biological recognition and signaling processes [5], such as cell–cell communication [6], cell adhesion, interaction of pathogenic viruses, bacteria binding to cell membranes [7] and carbohydrate-binding protein (lectins) recognition [8–10].

SAMs of nanoparticles (NPs) functionalized with bioactive molecules have high importance and applications in the field of bio- and nanotechnologies [11]. These systems have been used as biosensors [12], molecular electronic devices [13], catalysis [14], or biocompatible surfaces to mimic extracellular matrix, ECM [15]. Nanoparticles and SAMs of NPs have also been used as an alternative to molecular systems to study carbohydrate mediated biological recognition and signalling processes due to their advantages such as unique size-dependent physical properties, large surface area, easy surface functionalization for targeting and imaging, and their chemical and photochemical stability [16]. In particular, the large surface area of self-assembled nanoparticles allows a larger number of contact points between the cell and the modified surface, resulting in more efficient information transfer [17]. The use of carbohydrates to functionalize NPs and nanomaterials has been shown to be a very successful approach for the detection, imaging [18–20], and sensing of proteins [21], cells and bacteria [22,23]. Also, carbon nanotubes coated with a biomimetic carbohydrate polymer were designed to mimic cell surface mucin glycoproteins [24].

Immobilization of carbohydrates on the surface of nanomaterials can be achieved by physi- and chemisorption in solution. However, more stable systems are obtained by covalent immobilization of the carbohydrate functionality by using bifunctional thiol- or alkoxysilane-based molecules [25]. The thiol and alkoxysilyl groups are responsible for the covalent fixation of these linkers to the surface of the NPs, while the second functional group is used to tether the desired functionalized carbohydrates.

We recently reported [26,27] that microcontact printing (mCP) [28] is an highly effective technique to functionalize NPs and SAMs of NPs under spatial control. Additionally, this technique reduces the reaction time of the functionalization and provides mild reaction conditions. mCP is a commonly used method for the replication of physical, chemical and biological patterns on solid surfaces and was also used for chemical synthesis on gold and silicon oxide surfaces [28e]. This method utilizes a microstructured elastomeric stamp that delivers ink molecules to a substrate surface in the

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contact area between the stamp and the substrate. Some selected examples of mCP are the syntheses of imines [29] and peptides [30] respectively. Also the mCP of acetylene derivatives onto azido-terminated SAMs by "click" chemistry on silicon oxide substrates [31,32] and of different esters onto amino functionalized SAMs of zeolites are known [26].

The click approach introduced by Sharpless and co-workers [33] often require the use of a Cu-catalyst [34]. Reinhoudt et al. have demonstrated that "click" chemistry can be applied by mCP of acetylenes onto azide-terminated substrates without a catalyst [32a]. The exclusion of the toxic Cu-catalyst is a very important result in particular for the immobilization of biomolecules. Additionally, they have demonstrated that the efficiency of immobilization of reactants for "click" reaction can be increased by introducing a Cu-coated elastomeric stamp, StampCat technique [35,36].

Although, carbohydrates can be fixed onto SAMs on glass substrates by mCP [31,37], to the best of our knowledge, there are no examples reported in the literature of using mCP to bind carbohydrates e.g. by "click" reaction onto the surface of *SAMs of nanoparticles*. As mentioned before, the use of nanopatterned surfaces, obtained by assembling nano-objects on the substrate, could lead to multifunctional surfaces as well as larger areas for multivalent interactions. In this study, we demonstrate that "click" chemistry can be applied in mCP to couple alkyne terminated carbohydrates to an azide terminated SAMs of zeolites L in the absence of an toxic Cu-catalyst. We describe an alternative pathway to functionalize nanoparticles with (bio)molecules. This strategy allows solvent-free, fast, cheap and spatial surface functionalization. Although, the efficiency of surface functionalization could be increased by StempCat technique, immobilization of biomolecules on SAMs of zeolites surface by mCP was efficient enough, in our study, for the recognition of biological systems (cell adhesion).

Zeolite L crystals have been used in a broad field of applications [38–41]. In this study zeolite L crystals were chosen due to the fact that zeolites are biocompatible, optically transparent, size tunable (from 30 up to 8000 nm) and in different shapes e.g. discs or cylinders, porous crystalline alumino–silicates. Their flat surface can be functionalized easily by silane chemistry and their channel entrances can be functionalized selectively in solution [42]. The unidimensional channels of zeolites L can be organized perpendicularly to the substrate upon monolayer formation [43]. These monolayers were prepared using the ultrasound aided strategy developed by Yoon et al. [44]. Moreover, SAMs of zeolites L provide homogenous functionalization by mCP due to their rather flat exposed surface. The functionalization of the SAMs of zeolite L crystals with mCP according to peptide coupling reaction [26] and the application of this system as a biocompatible surface for cell adhesion experiments and cellular patterning was demonstrated by us recently [27].

In this contribution, we describe the mCP of fluorescent molecules, lissamine rhodamine terminated with an acetylene group (LRA) and a carbohydrate, as α-D-mannoside (α-D-Man) on the top side of azide functionalized SAMs of disc-shaped zeolite L nanocrystals by a "click" reaction in the absence on Cu-catalyst. The obtained SAMs of α-D-Man functionalized zeolite L nanocrystals (α-D-Man-zeo) were used as models for biocompatible surfaces to study cell adhesion and cellular patterning. We demonstrated that the carbohydrate functionalized zeolite L monolayer obtained by mCP is a much better surface for cell adhesion than the precursor azide substrate. Additionally, the use of fluorescent molecules, *N,N′*-bis(2,6-dimethylphenyl)perylene-3,4,9,10-tetracarboxylicdiimide (DXP) inserted inside the channels of the zeolites allows us to easily visualize the position of the cell. Furthermore, we show that a defined zeolite pattern leads to the cellular patterning and spatial recognition of cells.

2. Experimental

2.1. Materials

3-(Bromopropyl)trichlorosilane (96%), 3-chloropropyltrimethoxysilane (CP-TMS, 97%), sodium azide (NaN_3), α-D-mannose pentaacetate, 4-pentyn-1-ol (purum, ⩾97.0%), potassium carbonate (BioXtra, ⩾99.0%), and *N,N′*-bis(2,6-dimethylphenyl)perylene-3,4,9,10-tetracarboxylicdiimide (DXP) were purchased from Sigma–Aldrich. Lissamine Rhodamine B sulfonyl chloride was purchased from Acrosss. 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI) was acquired from Polysciences Europe GmbH (Eppelheim, Germany).

Polydimethylsiloxane (PDMS) was obtained from Dow Corning Corporation (184 silicon elastomer base, SYLGARD). Toluene was purchased from Merck (stored over molecular sieves, puriss., H_2O_0.005%). Glass plates (1.8 cm × 1.8 cm) used for zeolite L monolayers were obtained from Servoprax GmbH. Disc-shaped (of approximately 1000 nm in diameter and 250 nm in height) zeolite L crystals were synthesized according to literature procedure [45] (zeolite synthesis have been described briefly in Supporting information) and X-ray powder diffraction (XRD) pattern of zeolite L (for details see Fig. S1) is comparable with literature results [46]. Loading of zeolite L crystals with DXP dyes were performed according to literature methods [47]. Preparation of SAMs of zeolites on glass surface, formation of patterned zeolite L monolayer, synthesis of alkinyl substituted LRA and alkinyl substituted α-D-Man (Fig. S2), and mCP of molecules on azide functionalized SAMs of zeolites are described in Supporting information.

2.2. Functionalization of zeolites with azide unit

Twenty milligrams of potassium exchanged disk-shaped zeolite L crystals was suspended in 3 mL of dry DMF in a Teflon centrifuge tube, and 20 μL of bromopropyl trichlorosilane was added. After sonication for 30 min at 40 °C, the sample was centrifuged and washed with EtOH twice. In the next step, NaN_3 (1 mg, 0.015 mmol) was added to the zeolite in 3 mL of DMF and stirred at room temperature overnight. Afterwards, the sample was centrifuged and washed with water twice and EtOH twice.

2.3. Cell experiments

HeLa cells were obtained from ATCC/LGC Standards GmbH (Wesel, Germany), cultivated according to the provider's protocol and stained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI).

2.3.1. Seeding of HeLa cells

HeLa cells were taken out of the cryo-conservation (−196 °C) and stored for 20 min at −20 °C. Under gently shaking the cells were resuspended in pre-heated (37 °C) HeLa cell medium (F12 medium, 10% fetal calf serum (FCS), 100 U/ml Penicillin, 100 μg/ml Streptomycin). After centrifugation at 800 rpm for 10 min the cell pellet was resuspended in fresh pre-heated HeLa cell medium. The amount of cells was counted by use of a Neubauer® counting chamber. 50,000 cells/cm^2 were seeded and incubated for 12 h at 37 °C and 5% CO_2.

2.3.2. HeLa cell counting

After staining with DAPI the cells were counted by using the program Image J from the National Institute of Health, USA.

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