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Layer-by-layer generation of PEG-based regenerable immunosensing surfaces for small-sized analytes

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ABSTRACT

Small molecules (haptens) like pharmaceuticals or peptides can serve as targets for antibody binding in competitive immunoassay-based flow-through assays. In this work, a strategy for preparing polyethylene glycol (PEG) coatings for subsequent hapten immobilization on glass-type silica surfaces is presented and characterized in detail. Two substrates bearing terminal silanol groups were utilized, a glass slide and a silicon wafer. First, surfaces were thoroughly cleaned and pretreated to generate additional silanol groups. Then, a silane layer with terminal epoxy groups was created using 3-glycidyloxypropyltrimethoxysilane (GOPTS). Epoxy groups were used to bind a layer of diamino-poly(ethylene glycol) (DAPEG) with terminal amino groups. Finally, the low molecular weight compound diclofenac was bound to the surface to be used as model ligand for competitive biosensing of haptens. The elementary steps were characterized using atomic force microscopy (AFM), water contact angle measurement, grazing-angle attenuated total reflection (GA-ATR) FT-IR spectroscopy, and X-ray photoelectron spectroscopy (XPS). The data collected using these techniques have confirmed the successive grafting of the molecular species, evidencing, that homogeneous monolayers were created on the silica surfaces and validated the proposed mechanism of functionalization. The resulting surfaces were used to investigate polyclonal anti-diclofenac antibodies recognition and reversibility using quartz crystal microbalance with dissipation (QCM-D) measurements or an automated flow-through immunoassay with chemiluminescence (CL) read-out. For both techniques, recognition and reversibility of the antibody binding were observed. The stability of sensors over time was also assessed and no decrease in CL response was observed upon 14 days in aqueous solution. The herein presented strategy for surface functionalization can be used in the future as reproducible and reusable universal platform for hapten biosensors.

1. Introduction

Immunosensors are analytical devices incorporating an antigen-specific immunoglobulin integrated within a physicochemical transducer (Luppa et al., 2001). They are employed to automate immunoassays in microfluidic systems also known as lab-on-a-chip (Han et al., 2013). These systems are sensitive analytical techniques for many applications in clinical diagnosis (Mohammed and Desmulliez, 2011; Shiddiky et al., 2012), as well as food (Boujday et al., 2008; Rau et al., 2014; Salmain et al., 2012, 2011) and environmental analysis (Boujday et al., 2009a, 2010; Prieto-Simón and Campàs, 2009). There exist immunosensors for label-free and label-based competitive assay systems. In label-free assays, the antibody binding is detected directly by the transducer such as quartz crystal microbalance (QCM) sensors (Su et al., 2000). In label-based assays, labels can be introduced *via* tracers or secondary antibodies. For example, chemiluminescence (Mirasoli et al., 2014) (CL) labels can be used.

One of the greatest challenges in the field of immunosensors is the controlled and reliable surface functionalization (Piehler et al., 2000). This is particularly true in the case of small-sized target analyte (hapten) sensing, where the accessibility and reliability of the target molecules layer definitively impact the sensor response (Boujday et al., 2010; Shankarana et al., 2007). On silica

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and silica-like surfaces, various strategies, mostly involving silane grafting on silanol groups, were described (Gooding and Ciampi, 2011; Liebes et al., 2009; Ulman, 1996). The control of this process is crucial as the silane layer may be unstable in the aqueous media used for biological applications (Dekeyser et al., 2008). However, for high throughput analysis the substrate should be stable and regenerable. In this way, the biosensing devices become economical, reproducible and cost-efficient (Jiang et al., 2008; Su et al., 2000). In the present context, the term regeneration describes the dissociation of the immunocomplex and liberation of the ligand on the silica surface. Such surface regeneration procedures are already described for a surface plasmon resonance (SPR) (Shankarana et al., 2007) and QCM-D (March et al., 2009) immunosensing, as well as for microflow-based chemiluminescence immunoassay (Oswald et al., 2013). To immobilize the antigen on the transducer surface, a protein–hapten conjugate can be immobilized or the hapten can be coupled covalently direct to a shielding layer. The latter has been used successfully for reproducible mycotoxin determination in wheat extract (Oswald et al., 2013). For this application, a polyethyleneglycol (PEG) based coating was attached to the silane layer on silica glass slides. The PEG allows a high surface coverage by the hapten and low non-specific binding what makes the surface quite resistant to biofouling (Mehne et al., 2008; Piehler et al., 2000; Wolter et al., 2007).

The next step towards a reliable high throughput analysis with these silica glass slide modifications is the control and reproduction of the promising surface chemistry preparation. Moreover, it can be transferred to other silicate surfaces like silicon with native oxide layer and additional transduction techniques. In the present study, we investigate this surface chemistry in-depth, with a molecular approach to control every elementary step of the surface functionalization.

The first step for the silica surface modification (Fig. 1) is the cleaning and oxidation of the silica surface in order to produce silanol groups (Cras et al., 1999). Then, a 3-glycidyloxypropyltrimethoxysilane (GOPTS) layer is grafted on the silica surface, followed by attachment of an epoxysilane and the diamino-poly (ethylene glycol) (DAPEG) with a molecular weight of ~2000 Da. The terminal amino groups are available to immobilize haptens or derivatives with appropriate chemical functions directly on the surface. These haptens may serve as competing analytes for free antibody binding sites using competitive immunoassays. They can be liberated from bound antibodies by using an appropriate regeneration buffer.

For the presented characterization of this surface preparation, two substrates bearing terminal silanol groups were utilized, glass slides and silicon wafers with a native silica layer on their polished surface. For the investigation of the roughness and morphology on these two types of silica surface, atomic force microscopy (AFM) was used in the peak force mode under air. Water contact angle measurements were carried out on both surface types (Si wafer and glass slides) to assess the interfacial properties of the resulting surface. The chemical composition of the surface was analyzed by grazing-angle attenuated total reflection FT-infrared spectroscopy (GA-ATR FT-IR) on silicon wafers (Lummerstorfer and Hoffmann, 2004), X-ray photoelectron spectroscopy (XPS) allowed ultimate analysis of the surface coating and enabled also estimating the layer thickness after each preparation step. Finally, the pharmaceutical diclofenac was used as a model ligand for the immobilization on DAPEG. A highly selective polyclonal antiserum (Deng et al., 2003) from rabbit was used for studying the regeneration procedure by a label-free QCM with dissipation measurement (QCM-D). Furthermore, an automated flow-through platform (Kloth et al., 2009) with chemiluminescence readout by a CCD camera was used to study the specificity, regenerability and stability of DAPEG-based diclofenac microarrays.

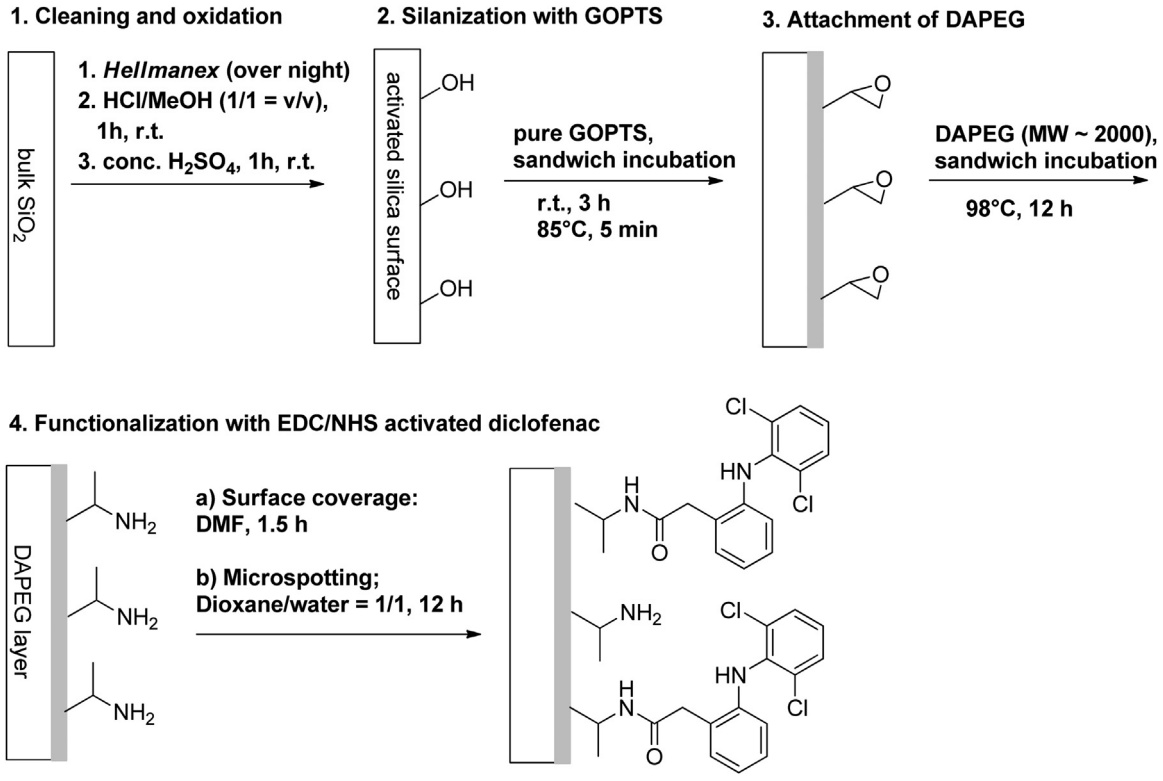

Fig. 1. Overall procedure for the preparation of a PEG based diclofenac biosensor chip or microarray.

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