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Poly(ethylene oxide) brushes prepared by the "grafting to" method as a platform for the assessment of cell receptor–ligand binding

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ABSTRACT

Poly(ethylene oxide) (PEO) with terminal alkyne and amino groups was grafted to a poly(glycidyl methacrylate) (PGMA) anchoring layer and the PEO/PGMA coatings were investigated as a non-fouling platform for the assessment of ligand–cell receptor interactions. The PEO/PGMA coatings deposited on Si/SiO_2 substrate were stable in phosphate buffered saline over a period of 8 days if the thickness of the PEO was less than 30 nm. The stability of the coating could be enhanced by an additional layer of 3-mercaptopropyltrimethoxysilane between the substrate and the PGMA layer. The grafted layers were shown to efficiently suppress nonspecific protein adsorption and cell adhesion. Based on the theoretical ligand-binding capacity, protein adsorption and stability data, the optimum thickness range of the PEO layer is 10–20 nm. The binding of an arginine–glycine–aspartic acid (RGD) ligand using azide–alkyne click chemistry demonstrated that the ligand surface density is controllable in the range from 10^0 pmol/cm^2 up to the capacity of the grafted layer of 10^2 pmol/cm^2 by varying the ligand concentration in the reaction mixture. Calf pulmonary artery endothelial cells adhered to and spread on ligand modified layers proportionally to the ligand surface density, thus demonstrating the applicability of these "grafted to" PEO brushes as a platform for cell receptor–ligand engagement studies.

1. Introduction

Synthetic peptide ligands derived from adhesion sites of extracellular matrix proteins are effective tools in guiding cell behavior on biomaterial surfaces [1,2]. Evaluating the interactions between cell membrane receptors and synthetic ligands is therefore a crucial issue in the development of advanced biomaterials for regenerative medicine and tissue engineering [3–5]. In order to elicit specific and controlled binding between ligands and cells, the ligands have to be presented against a background resistant to the adsorption of proteins from culture media, which would otherwise compromise the specificity of the assay [6,7].

Cell–ligand interactions are typically assessed on model planar substrates coated with well-defined self-assembled monolayers of functional thiols [8,9] or silanes [10]. More stable, versatile and substrate-independent coatings are prepared from low-fouling hydrophilic polymers [7]. These coatings have a great potential for use not only as testing surfaces but also as functional coatings in biomedical devices. Various structures of these polymer-based coatings have been developed, including interpenetrating

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networks [11], multicomponent cross-linked layers [12], polymer star layers [13], polymer brushes [14–16] and plasma polymer layers [17]. Among these, polymer brushes meet the requirements of cell–ligand interaction assays, namely reproducibility, predetermined ligand-binding capacity and good resistance to a non-specific protein adsorption. Polymer brushes are mostly prepared by a "grafting from" approach via surface initiated controlled radical polymerization [18], which provides precise control over the brush thickness and composition. Alternatively, they can be prepared by a "grafting to" approach, i.e., by attaching end-functionalized ready-made polymers to a chemically activated surface. The brush state can be reached if the grafting is performed either from a polymer melt or from a polymer film plasticized by solvent vapors (solvent-assisted grafting) [19,20], i.e., under conditions when the excluded volume interactions limiting the grafting density are efficiently screened. In comparison with "grafting from", the "grafting to" method is experimentally very straightforward. It avoids the polymerization step in a closed reactor and so it is a convenient way to prepare larger batches of samples.

Both grafting methods need surface functional groups that serve either as initiating sites or as coupling sites. To increase the independence from the substrate and the efficiency of the grafting, a *macromolecular anchoring layer* bearing reactive groups both for grafting and for anchoring to the substrate has been used [19,21,22]. A monolayer of the anchoring polymer bound to the surface provides reactive groups for grafting localized in tails and loops between the anchoring sites. This quasi three-dimensional layer has much higher grafting capacity and efficiency than native functional groups of the material surface or those introduced through a surface assembly of low-molecular weight modifiers, e.g. silanes.

The concept of the "grafting to" method, in combination with a macromolecular anchoring layer for preparing high-density polymer brushes, has been developed largely by the group of Luzinov [19,23,24]. They suggested the use of epoxy group-containing polymers such as poly(glycidyl methacrylate) (PGMA) for the anchoring layer [25]. Epoxy groups are able to react not only with typical nucleophiles such as amino and thiol groups but also with less reactive surface hydroxyl groups typically present on many inorganic materials, such as metals, semimetals and silicates [26,27].

Poly(ethylene oxide) (PEO), which is a well-established component of low-fouling coatings [28,29], has suitable properties for the "grafting to" method, in particular a low glass transition temperature (−10 °C) and a relatively low melting temperature (70 °C). Moreover, it can be prepared with high molecular uniformity and with a variety of terminal groups [30]. PEO brushes with high chain densities above 1 chain/nm^2 (167 pmol/cm^2) were prepared on a 2.5-nm-thick PGMA layer with PEO grafts of MW 5000 [31,32]. Assuming that a great part of the terminal groups in this PEO layer can be modified with the cell–adhesion ligands, the resulting ligand surface density would well cover the range of 10^{-1}–10^2 pmol/cm^2, which has been reported to elicit cell adhesion and spreading on ultrathin hydrophilic polymer coatings [11,15,18,33–36]. The employment of PEO brushes prepared by the "grafting to" method for cell–ligand interaction studies therefore seems to be highly attractive, due to the substrate-independency and straightforward preparation based on ready-made polymers, simple casting and thermal procedures.

The goal of our work was to evaluate PEO brushes prepared by grafting PEO from melt to a PGMA anchoring layer as a platform for cell receptor–ligand engagement studies. The brushes were prepared from linear PEO carrying terminal alkynyl groups for subsequent modification with cell–adhesion ligands via azide–alkyne cycloaddition. We demonstrate that PEO/PGMA coating on silicon is sufficiently stable in an aqueous environment for several days, and is highly resistant to non-specific protein adsorption. Finally, coatings functionalized with RGD integrin ligands were shown to promote cell adhesion that is dependent on ligand surface density.

2. Materials and methods

2.1. Polymers and reagents

Heterobifunctional PEOs α-(2-aminoethyl)-ω-methoxy-poly(ethylene oxide) (mPEO-NH_2) and α-(2-aminoethyl)-ω-(2-(but-3-ynoylamino)ethoxy)-poly(ethylene oxide) (alkyne-PEO-NH_2)(Rapp Polymere GmbH) were of M_n 5000 (M_p 4770, MALDI), and PDI of 1.1. Poly(glycidyl methacrylate) (PGMA), M_n 88,000, PDI 2.4 (PS calibration) was prepared by free radical polymerization. A mixture of 24 g of distilled glycidyl methacrylate, 36 ml of dry 1,4-dioxane and 240 mg of 2,2′-Azobis(2-methylpropionitrile) was purged with nitrogen and then stirred at 60 °C for 8 h in a closed reactor. The polymer was twice precipitated from a chloroform solution into diethyl ether. The solvents and low molecular reagents were purchased from Sigma–Aldrich and were used as received, unless stated otherwise.

2.2. RGD peptide ligand synthesis and radiolabeling

Model bioligand 5-azidopentanoyl-GGG**RDG**SGGGY-NH_2 was prepared by solid phase peptide synthesis using fluorenemethoxycarbonyl (Fmoc)/t-butyl chemistry [37] and Tenta Gel R Rink Amide resin (Rapp Polymere GmbH). The purity of the peptide was confirmed by HPLC analysis with a C18 reverse phase column and MALDI-TOF MS analysis. The peptide was radiolabeled with ^{125}I at the tyrosine residue using the chloramine T/ascorbic acid iodination method [38]. Iodination was performed on the resin-bound peptide with a selectively deprotected tyrosine residue [39]. The labelled peptide was cleaved from the resin and isolated by precipitation in diethyl ether. Its specific activity was 21 TBq/mol.

2.3. Preparation of substrates

Silicon wafers (single side polished, orientation ⟨100⟩, B-doped, resistivity 5–22 Ω cm) with a 45 nm wet thermal oxide overlayer (Siegert Consulting e.K.) were cut into 20 × 11 mm pieces, and were sonicated in methanol and