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Short communication

Comparative study of antibody immobilization mediated by lipid and polymer fibers

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ABSTRACT

Antibody immobilization and function retention are important to a variety of applications, including proteomics, drug discovery, diagnostics, and biosensors. The present study investigates antibody immobilization mediated by cholesteryl succinyl silane (CSS) fibers, in comparison to hydrophobic polycaprolactone (PCL) fibers and hydrophilic plasma-treated PCL fibers. When incubated with a model protein, the formation of protein aggregates is observed on hydrophobic PCL fibers but not on the more hydrophobic CSS fibers, indicating that CSS fibers immobilize proteins through mechanisms other than hydrophobic interaction. When exposed to a limited amount of antibody, CSS fibers immobilize more antibodies than plasma-treated PCL fibers and no fewer antibodies than PCL fibers. The function retention of antibodies immobilized on the fibers is analyzed using a cell-capture assay, which shows that the antibody-functionalized CSS fibrous matrices capture 6- or 7-fold more cells than the antibody-functionalized PCL or plasma-treated PCL fibrous matrices, respectively. Data collected from the study show that the lipid fiber-mediated immobilization of antibody not only maintains the advantages of physical immobilization such as easiness and rapidness of operation but also improves function retention.

1. Introduction

Antibody immobilization is important in many clinical and laboratory settings, as antibodies are often the detection and/or capture element in immunosensors, targeted drug delivery systems, tissue scaffolds, and cell-capturing platforms [1–7]. Physical adsorption has been frequently exploited as a simple and versatile method to immobilize antibodies and proteins in general. However, a high percentage of physically immobilized antibodies can be denatured [8–10], and sometimes fewer than 3% of the binding sites remain functional [11]. It is widely accepted that the stable and oriented immobilization of proteins such as antibodies can greatly enhance analyte detection and/or capture [12–16]. A variety of coating and/or linker materials may functionalize surfaces, enhancing the stability, orientation and function retention of immobilized proteins. For instance, surfaces may be chemically modified using aldehydes, activated esters, maleimides, or other linkers that permit stable covalent immobilization of proteins [5,17–19]. However, heavy chemical manipulation involved in the process may compromise the integrity of immobilized proteins [20,21]. Bioactive molecules, such as biotin and nitriloacetic acid, may functionalize solid surfaces, enabling bioaffinity immobilization of proteins with optimal orientation [17]. This methodology can be costly and may suffer from a background noise due to nonspecific protein adsorption [22].

As a promising alternative, surfaces can be coated with self-assembling (SA) molecules, creating a protein-resistant layer that minimizes nonspecific protein adsorption but enables covalent or bioaffinity immobilization of proteins, including antibodies [2,23–27]. Among various SA molecules that have been explored as coating and/or linker materials to mediate protein immobilization, lipid polymers that are derived from cell membranes are particularly appealing. Like their natural counterparts, lipid polymers can be blood compatible, prevent nonspecific protein adsorption, and efficiently immobilize target proteins via linker lipids without compromising protein conformation [28–31]. Functional lipids can be patterned into micro- and/or nanostructures on solid surfaces, immobilizing proteins that enable the selective adhesion of target cells [32]. Despite these desired properties, lipid polymers usually have limited morphological and mechanical stabilities [33].

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Previously, we engineered cholesterol-derived lipids into stable fibers that are less than a micrometer in diameter [7]. The use of such cholesterol-derived fibers offers a biomimetic approach to immobilizing antibodies. In the cell membrane, cholesterol promotes the formation of liquid ordered microdomains [34], which are capable of anchoring a dizzying array of membrane-bound proteins [35,36]. Our studies show that lipid fibers stably and functionally immobilize antibodies that selectively target cancer cells [7], and that antibody-functionalized lipid fibers are more efficient in capturing cancer cells than their film counterparts [37]. The studies suggest that membrane-bound proteins may be embedded in lipid fibers in a manner similar to that seen in natural cell membranes, and that the large surface areas and packing defects of lipid fibers may enhance the embedding of membrane-anchoring regions of the proteins [37].

Herein a comparative study on antibody immobilization is performed on three fiber types: (1) lipid fibers that comprise cholesteryl succinyl silane (CSS) and immobilize antibodies through the embedding mechanism, (2) highly hydrophobic polycaprolactone (PCL) fibers that immobilize proteins via hydrophobic interaction, and (3) hydrophilic plasma-treated PCL fibers that adsorb antibodies through electrostatic interaction. The fibers possess comparable diameters, thereby minimizing the effects of surface topology on antibody immobilization. The function retention of immobilized antibodies on the fibers is evaluated by the capture of cancer cells, which requires the coordinated interactions of multiple immobilized antibodies with the surface receptors of a cell. The comparative study is to illustrate that lipid fibers possess significant advantages of physically immobilizing antibodies and retaining antibody functions over hydrophobic and hydrophilic polymer fibers.

2. Materials and methods

2.1. Fiber fabrication

CSS was synthesized, according to a procedure detailed in our previous studies [7,38]. An acidic solution of CSS at a concentration of 69% w/w was prepared in a mixed solvent that comprises 1 mL of tetrahydrofuran (Sigma–Aldrich) and 10 μL of 37% aqueous HCl. The solution was incubated overnight in a 40 °C water bath, permitting CSS hydrolysis and polymerization. The hydrolyzed and polymerized CSS solution was then electrospun into lipid fibrous membranes using a custom made device with a flow rate of 0.5 μL/min, a voltage of 12 kV, and a spinneret-to-ground distance of 12 cm. The fibers were collected on silicon chips that were placed on top of an aluminum foil collector plate.

A solution of PCL (MW: 80 kDa, Sigma–Aldrich) at a concentration of 10% w/v was prepared in 1,1,1,3,3,3-hexafluoro-2-propanol (MW: 168.04, Sigma–Aldrich). The solution was incubated at room temperature for a minimum of 6 h, briefly vortexed, and then electrospun into fiber with a flow rate of 20 μL/min, a voltage of 12 kV, and a spinneret-to-ground distance of 12 cm. The electrospun PCL fibers were collected on silicon chips that were placed on top of an aluminum foil collector plate. Approximately half of the collected PCL fibers immediately underwent an air-plasma treatment (Harrick Plasma, Model PDC-001) for 10 min under vacuum, generating plasma-treated PCL fibers.

2.2. Fiber characterization

SEM images were taken of CSS, PCL, plasma-treated PCL fibers using a Hitachi S-4800 field emission scanning electron microscope (FE-SEM). The fiber samples were first coated with platinum for 30 s using a sputter coating machine, and then imaged using SEM with accelerating voltage 5.0 kV. The average fiber diameter was calculated for each fiber type by measuring the diameter of 100 fibers via Image J.

The water contact angles were assessed for each fiber type. A 10 μL droplet of DI water was placed on each fiber matrix ($n = 3$) and measured with instrumentation from First Ten Angstrom (FTA-200, camera: RS-170). The contact angle was determined with FTA-32 software. From the collected measurements, the average water contact angle was determined for the CSS, PCL, and plasma-treated PCL fibers.

2.3. Antibody immobilization

A Bio-Rad protein assay was used to quantify the amount of anti-CD20 immobilized on the three fiber types. In brief, 0.25 cm^2 silicon chips were coated with electrospun CSS, PCL or plasma-treated PCL fibers. The matrices were each placed in a single well of a 48-well plate and rinsed with 1× PBS three times. The matrices were submerged in 200 μL of a working solution of anti-CD20 (10 μg/mL), consistent to the concentration previously determined for optimal antibody immobilization [7,37]. The fibers were allowed to incubate for 90 min at 37 °C. After the incubation period the solution was collected and placed in fresh wells of a new 96-well plate. As the scaffolds were submerged in the antibody solution, the fibers and wells were rinsed three times with PBS to ensure that all unbound protein was collected. The amount of unbound protein in the collected solution was detected with a Synergy 2 SL Luminescence Microplate Reader (BioTek, VT) at an excitation wavelength of 460/40 nm and an emission wavelength of 590/10 nm. The observed absorption shifts were then converted to unbound protein concentrations with the use of a known concentration ladder, and the amount of anti-CD20 immobilized on the fiber matrices was calculated.

2.4. Dissociation kinetics

Bovine serum albumin (BSA, Invitrogen) conjugated with Alexa Fluor 488 was chosen as a model protein to study the dissociation kinetics of immobilized proteins. All three fiber types were prepared on 1 cm^2 silicon chips. Each fiber specimen was placed in a 12-well plate. A 0.001% (w/v) solution was prepared and briefly centrifuged to remove any protein aggregates, which can lead to nonspecific background fluorescence. Samples were incubated in 2 mL of BSA solution containing 20 μg of BSA at 37 °C for 90 min, and then washed with 1× PBS three times. Fresh 1× PBS was added to each well before the relative intensity was read with the Synergy 2 SL Luminescence Microplate Reader (BioTek, VT). Samples were stored in the dark at room temperature between reads. The ability of each sample to sequester the proteins was evaluated for six days. The observed relative intensities of the immobilized BSA were converted to concentrations with the use of equations derived from a known concentration ladder.

2.5. Anti-CD20 immobilization and Granta-22 B-cell lymphoma cell capture

Each 0.25 cm^2 chip that was coated with electrospun fibers was placed in a single well of a 48-well plate, rinsed with 1× PBS three times, and incubated in a dilute solution of anti-CD20 (10 μg/mL) for 90 min. The samples were then washed with 1× PBS, incubated in a 0.1% BSA in 1× PBS solution for 60 min, washed again with 1× PBS, seeded with Granta-22 B-cell lymphomas at a concentration of 2×10^5 cells per sample, and incubated for 45 min to allow for cell capture. After cell capture the samples were washed again with PBS and subjected to a 15-min incubation in 4% paraformaldehyde. The captured cells were treated with Triton-x prior to being