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Comparative Study of Antibody Immobilization Mediated by Lipid and Polymer Fibers

Celine Cohn^a, Siu Ling Leung^b, Zhengbao Zha^b, Jessica Gamboa^a, Weibing Teng^b, Xiaoyi Wu^{*,a,b}

^aBiomedical Engineering GIDP, University of Arizona, Thomas W. Keating Bioresearch Building, 1657 E Helen Street, Tucson AZ 85721, United States of America

^bDepartment of Aerospace and Mechanical Engineering, University of Arizona, 1130 N Mountain Ave, Tucson, AZ 85721, United States of America

Abstract

Oriented antibody immobilization is 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 support a proposed embedding mechanism, through which CSS fibers immobilize antibodies. 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 antibody orientation and function.

Keywords

Antibody immobilization; lipid fibers; physical adsorption; function retention; cholesterol

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

*Corresponding Author: xwu@email.arizona.edu; Tel: 1-00-520-626-5854; Fax: 1-00-520-621-8191.

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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].

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 μ L 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 μ L/min, a voltage of 12 kV, and a spinneret-to-ground distance of 12 cm. The fibers were collected on silicon chips.

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 μ L/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 the 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 seconds 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 μ L 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

Silicon chips coated with CSS, PCL or plasma-treated PCL fibers were each placed in a single well of a 48-well plate, rinsed with phosphate buffered saline three times, then exposed to 200 μ L of a solution containing 2 μ g of anti-CD20, and 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. Each specimen was rinsed three times with fresh 1×PBS; the rinsed solution was added to the corresponding well so that all solution collected from one chip went into the same well. A Bio-Rad protein assay kit was used to quantify the amount of anti-CD20 remaining in the solution. The amount of anti-CD20 immobilized on the fiber specimens was then 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. A 0.001% (w/v) solution was prepared and briefly centrifuged to remove any protein aggregates, which can lead to nonspecific background fluorescence. Fiber samples on 1 cm² silicon chips were placed in a 12-well plate, incubated with 20 µg of BSA 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 cm² 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 2×10⁵ 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 stained with Alexa Fluor phalloidin against actin and ProLong® Gold Antifade with DAPI (Life Technologies) against the cell nuclei, and imaged the following day with fluorescent microscopy (Nikon).

2.6. Statistical analysis

Student's t-test was computed between each fiber type to determine statistical significance for average fiber diameters, and antibody immobilization and cell capture efficiencies. P-values less than 0.05 were considered statistically significant whereas anything greater than 0.05 was considered insignificant.

3 Results and Discussion

3.1. Fiber Surface Morphology and Hydrophobicity

Electrospun fibers possess large specific surface areas that may enhance protein immobilization. Since fiber diameter is a key factor that defines fiber surface areas, SEM images were taken of the three fiber types and processed using IMAGE-J to obtain fiber diameter (Fig. S1). Distributions of fiber diameter were plotted for the three fiber types in

Fig. 1. The average diameters of PCL, Plasma-treated PCL, and CSS fibers were determined to be $2.725 \pm 0.047 \mu\text{m}$, $0.915 \pm 0.034 \mu\text{m}$, and $0.993 \pm 0.038 \mu\text{m}$, respectively. The p values were calculated to be less than 0.001 for PCL vs. CSS fibers, less than 0.001 for PCL vs. plasma-treated PCL fibers, and 0.13 for CSS vs. plasma-treated PCL fibers, respectively. Difference in the average diameters of plasma-treated PCL and CSS fibers is not statistically significant. However, the average diameters of all three fiber types fall within the same microscale. The comparable diameters of the fibers would minimize the effects of surface morphology on protein immobilization.

Surface hydrophobicity is another key parameter in determining physical immobilization of proteins. To determine surface hydrophobicity, water contact angles were analyzed for the three fiber types, and optical images of water drops on the fibrous matrices were presented in Fig. 1 (see inserts). The water contact angles were determined to be $93.3 \pm 2.3^\circ$, $35.1 \pm 2.2^\circ$, and $136.5 \pm 2.3^\circ$ for electrospun PCL, plasma-treated PCL, and CSS fibers, respectively. The water contact angle analysis suggests that plasma-treated PCL fibrous matrices have hydrophilic surfaces while the surfaces of PCL and CSS fibrous matrices are highly hydrophobic.

Water contact angles can provide insight on the molecular polarity as well as the underlying nanostructure of a material. The shape of a water droplet results from the forces pulling at the three-phase contact line where the droplet meets the material surface. However, achieving an exaggerated water contact angle is only possible with the presence of underlying surface architectures [39]. When a surface is moderately rough, the surface area fractions are composed of two elements, the liquid phase and the solid surface phase. When a roughness threshold is passed, the introduction of air creates a third surface area fraction. The inclusion of air causes a decrease in the solid-liquid contact, ultimately causing an increase in the observed hydrophobicity of a rough surface [40]. As a result, a micro- or nanoscale topology is often the root cause of super-hydrophobicity [41,42]. This is illustrated with the PCL fibers. While PCL films generally have water contact angles of 70° [43], PCL fibrous matrices show an increased water contact angle of 93.3° due to the enhanced surface roughness over their film counterparts.

3.2. BSA Immobilization and Dissociation

The protein immobilization on and dissociation from the electrospun fibers was evaluated using bovine serum albumin (BSA), which can be nonspecifically adsorbed onto a solid surface [44]. BSA is often used to block surfaces and can be used as a model protein to study the protein interactions at the solid-liquid interface [45]. Here, the three fiber types were exposed to the working solution of BSA conjugated with Alexa Fluor 488 for 90 min, then removed from the BSA solution and placed in fresh PBS for the study of protein dissociation. Within the 90 minutes of immobilization, BSA aggregated on PCL fibers (Fig. S2). It is likely that BSA was hydrophobically adsorbed on PCL fibers and largely denatured, causing the denatured BSA to aggregate. In contrast, no BSA aggregation was observed on CSS fibers, although CSS fibers appeared to be more hydrophobic than PCL fibers. This indicates that CSS fibers immobilize BSA through mechanisms other than hydrophobic interactions.

We limited the quantitative analysis of BSA dissociation to plasma-treated and CSS fibers. After the 90 minutes of exposure, plasma-treated PCL and CSS fibers immobilized $1.78 \pm 0.20 \mu\text{g}$ and $1.41 \pm 0.31 \mu\text{g}$ of BSA, respectively. As shown in Fig. 2, the amount of BSA on the fibers dropped sharply within the first day. After 31 hours the plasma-treated PCL and CSS fibers retained $0.86 \pm 0.15 \mu\text{g}$ and $0.73 \pm 0.16 \mu\text{g}$ of BSA, respectively. By the second day the sequestered BSA began to stabilize at terminal amounts of $0.58 \pm 0.15 \mu\text{g}$ for plasma-treated PCL fibers and of $0.58 \pm 0.17 \mu\text{g}$ for CSS fibers. Over a 6-day period, the dissociation of BSA on plasma-treated PCL and CSS fibers follow the first-order kinetics model

$$y = y_t + y_d e^{-\beta t}$$

in which y is the amount of immobilized BSA, t is time, y_t is the terminal amount of BSA that remains on the fibers, y_d is the amount of dissociated BSA, and β is the dissociation rate constant. Parameters used in the model of BSA dissociation from plasma-treated PCL and CSS fibers were listed in Table 1. It is found that, the terminal amounts of BSA immobilized on plasma-treated PCL and CSS fibers were identical, but the amount of dissociated BSA on plasma-treated PCL was higher than on CSS fibers, and the rate constant of BSA dissociation was lower on plasma-treated PCL fibers than on CSS fibers. It is noted that the sum of y_t and y_d equals to the amount of BSA that is initially immobilized on the fibers. Although plasma-treated PCL fibers initially immobilized more BSA than CSS fibers did, they also dissociated more BSA than CSS fibers. As a result, both fiber types retained a comparable amount of BSA.

3.3. Anti-CD20 Immobilization

Murine anti-CD20 monoclonal antibody, which specifically recognizes CD20 phosphoprotein expressed on the surfaces of normal B lymphocytes and B-cell lymphomas [46,47], was chosen for a study of protein immobilization on the three fiber types. Specifically, each fiber sample was exposed to $2 \mu\text{g}$ of anti-CD20 from a working solution of $10 \mu\text{g}/\text{ml}$ and incubated for 90 min at 37°C . After incubation, the solution was removed from the samples and placed in a new, sterile 96-well plate. The amount of anti-CD20 remaining in the solution was evaluated to be $0.15 \pm 0.37 \mu\text{g}$, $0.83 \pm 0.49 \mu\text{g}$, and $0 \pm 0.0514 \mu\text{g}$ for plasma-treated PCL, PCL, and CSS fibrous matrices, respectively. Accordingly, the percentage of immobilized anti-CD20 was computed for each fiber type. As shown in Fig. 3, plasma-treated PCL fibers immobilized the lowest amount of antibody, at $59 \pm 24.5\%$ ($1.17 \pm 0.49 \mu\text{g}$ out of $2 \mu\text{g}$); PCL fibrous matrices were capable of immobilizing approximately $92 \pm 18.5\%$ of antibody ($1.85 \pm 0.37 \mu\text{g}$ out of $2 \mu\text{g}$); CSS fibers immobilized $100 \pm 2.6\%$ of anti-CD20, virtually all of the antibody present.

It is understood that proteins (e.g., anti-CD20 and BSA) are immobilized on hydrophilic plasma-treated PCL fibers through electrostatic interaction and on PCL fibers via hydrophobic interaction. In contrast, anti-CD20 is presumably immobilized on CSS fibers by embedding its membrane-anchoring domains in the lipid fibers. It is noted that no statistical difference was found in the amount of anti-CD20 that was immobilized on CSS and PCL fibers. Given that CSS fibers appear to be more hydrophobic than PCL fibers, a

further study is necessary to verify that antibodies with membrane-anchoring domains are immobilized on CSS fibers through the proposed embedding mechanism rather than hydrophobic interactions.

3.4. Evaluation of Immobilized Anti-CD20 Using a Cell-Capture Assay

The function retention of immobilized anti-CD20 was analyzed using a cell-capture assay. Specifically, PCL, plasma-treated PCL, and CSS fibers were functionalized by anti-CD20, and used to capture Granta-22 B-cell lymphomas. The cells express surface receptors, CD20 phosphoprotein, which target anti-CD20 antibody. Plasma-treated PCL, PCL, and CSS fibers immobilized a comparable amount of anti-CD20, ranging from $1.17 \pm 0.49 \mu\text{g}$ to $2.00 \pm 0.05 \mu\text{g}$ per sample. The three fiber types thus provide a relatively fair system for a comparative study of function retention of antibodies immobilized on hydrophilic, hydrophobic, and lipid fibers. The captured Granta-22 cells were stained with DAPI against the cell nuclei and imaged using fluorescent microscopy. Representative fluorescent images of the Granta-22 cells captured on the anti-CD20 functionalized PCL, plasma-treated PCL, and CSS fibrous matrices were shown in Fig. 4. It was found that the functionalized CSS fibers were able to capture substantially more cells than either the anti-CD20 functionalized PCL or plasma-treated PCL fibers.

A quantitative analysis of the obtained images reveals that the functionalized PCL and plasma-treated PCL fibrous matrices captured 227 ± 67 cells per mm^2 and 173 ± 60 cells per mm^2 , respectively (Fig. 5). Difference in the number of captured cells on the two fiber types is statistically insignificant ($p = 0.56$). In contrast, the functionalized CSS fibrous matrices captured a substantially higher number of cells than the functionalized PCL and plasma-treated PCL fibers (The p values were less than 0.01 for CSS vs. PCL fibers and CSS vs. plasma-treated PCL fibers). The number density of captured cells on the CSS fibers was determined to be 1330 ± 272 cells per mm^2 , which is nearly six-fold higher than that on the PCL fibers. It is worthwhile noting that the amounts of immobilized anti-CD20 on the PCL and CSS fibers were statistically indifferent ($1.85 \pm 0.37 \mu\text{g}$ vs. $2.00 \pm 0.05 \mu\text{g}$ per sample). Compared to the functionalized PCL fibers, therefore, the higher capture efficiency of the functionalized CSS can be attributed solely to better retention of the antigen-binding functions of immobilized anti-CD20.

3.5. Comparison of Antibody Physical Immobilization Mechanisms

Mechanisms responsible for physical immobilization of antibodies are dictated by the characteristics of a solid surface. As illustrated in Fig. 6, antibodies such as anti-CD20 are immobilized mainly through hydrophobic interactions on the highly hydrophobic surfaces of PCL fibers. Further, entropic forces due to conformational changes in immobilized antibodies may provide additional forces for antibody immobilization. Results from the BSA immobilization study and the anti-CD20 immobilization and cell capture assays support this review. When each fiber sample was exposed to $20 \mu\text{g}$ of BSA in solution, large protein aggregates that were visible to naked eyes formed on PCL fibers (Fig. S2). When each fiber sample was exposed to $2 \mu\text{g}$ of anti-CD20 in solution, PCL fibers immobilized $1.85 \pm 0.37 \mu\text{g}$ of anti-CD20. Statistically, difference in the amounts of anti-CD20 immobilized on PCL and CSS fibers was insignificant. Compared to CSS fibers, however, PCL fibers were less

capable of retaining the antigen-binding functions of anti-CD20, as evident by the cell-capture assay. This is consistent with the view that antibodies are hydrophobically adsorbed on PCL fibers and that a large number of the immobilized antibodies are improperly oriented and/or denatured, leading to a heavy loss of functions.

Plasma-treated PCL fibers are hydrophilic and immobilize antibodies largely through electrostatic interactions. As a result, charged residues of both the Fab fragments and the Fc regions of antibodies may electrostatically interact with the surfaces of plasma-treated PCL fibers, leading to randomly oriented antibodies on the fibers (Fig. 7). The antigen-binding Fab regions of improperly oriented antibodies have no or limited accessibility to antigens or surface receptors of a cell. In addition, improperly oriented antibodies may generate steric hindrance for adjacently immobilized and properly oriented antibodies. Consequently, randomly oriented antibodies that are immobilized on plasma-treated PCL fibers through electrostatic interactions have a poor ability to bind to antigens or surface receptors of a cell.

As illustrated in Fig. 7, antibodies are physically immobilized on CSS fibers through the embedding mechanism. Specifically, lipid molecules are not perfectly packed on the surfaces of CSS microfibers due to the curvature effects; the imperfect packing of CSS molecules creates nanoscale defects on fiber surfaces; the crystallizable Fc regions of antibodies can be embedded in the nanoscale defects of CSS fibers. Like natural cholesterol, CSS molecules allow the anchoring and subsequent crystallization of membrane-bound domains of proteins, such as the Fc regions of antibodies. The anchoring of the Fc regions of antibodies, which is mediated by the nanoscale defects of CSS fibers, has two implications. First, antibodies immobilized on CSS fibers will be oriented and can better retain antigen-binding functions, compared to the randomly oriented antibodies that are immobilized on plasma-treated PCL fibers. Indeed, the anti-CD20 functionalized CSS fibrous matrices are able to capture $7.7\times$ more cells than the anti-CD20 functionalized plasma-treated PCL fibers. Second, the Fab regions of antibodies or the relatively hydrophilic regions of proteins, which are immobilized on CSS fibers, will cover fiber surfaces, creating a monolayer protective coating. The protective coating may prevent the formation of antibody or protein aggregates on CSS fibers, which are highly hydrophobic.

4 Conclusion

Lipid fibers comprising CSS, PCL fibers, and plasma-treated PCL fibers with average diameters in the range of $0.9\sim 2.7\ \mu\text{m}$ were electrospun. As revealed by water contact angle analysis, plasma-treated PCL fibrous matrices are hydrophilic and can immobilize antibodies via electrostatic interactions, PCL fibrous matrices are highly hydrophobic and presumably immobilize antibodies through hydrophobic interactions, and CSS fibrous matrices are more hydrophobic than PCL fibrous matrices. When exposed to a large amount of BSA (i.e., $20\ \mu\text{g}$ per fiber sample), protein aggregates were formed on PCL fibers but not on more hydrophobic CSS fibers; CSS and plasma-treated PCL fibers stably immobilized a similar amount of BSA. When exposed to a limited amount of anti-CD20 antibody (i.e., $2\ \mu\text{g}$ per fiber sample), CSS, PCL, and plasma-treated PCL fibers immobilized $100 \pm 2.6\%$, $92 \pm 18.5\%$, and $59 \pm 24.5\%$ of anti-CD20, respectively. Further, a cell-capture assay showed that the anti-CD20 functionalized CSS fibrous matrices captured $5.9\times$ more cells than the

functionalized PCL matrices, and $7.7\times$ more cells than the functionalized plasma-treated PCL matrices. Collectively, results from these studies strongly support that oriented immobilization of antibodies and better retention of antibody functions can be achieved on CSS lipid fibers through a proposed embedding mechanism. The lipid fibers capable of functionally immobilizing antibodies hold strong promise for the development of highly sensitive proteomics and cell capture biotechnologies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- [1]. Talbert JN and Goddard JM, Colloids and Surfaces B-Biointerfaces 93 (2012): 8–19.
- [2]. Briand E, Salmann M, Compere C, and Pradier CM, Colloids and Surfaces B-Biointerfaces 53 (2006): 215–224.
- [3]. Trilling AK, Beekwilder J, and Zuithof H, Analyst 138 (2013): 1619–1627. [PubMed: 23337971]
- [4]. Jung Y, Jeong JY, and Chung BH, Analyst 133 (2008): 697–701. [PubMed: 18493668]
- [5]. Leung S, Zha Z, Cohn C, Dai Z, and Wu X, Colloids and Surfaces B: Biointerfaces 121 (2014): 141–149. [PubMed: 24967549]
- [6]. Guex AG, Hegemann D, Giraud MN, Tevaearai HT, Popa AM, Rossi RM, and Fortunato G, Colloids and Surfaces B-Biointerfaces 123 (2014): 724–733.
- [7]. Zha ZB, Cohn CM, Dai ZF, Qiu WG, Zhang JH, and Wu XY, Advanced Materials 23 (2011): 3435–3440. [PubMed: 21721057]
- [8]. Gibbs J, Corning ELISA Technical Bulletin (2001): 8.
- [9]. Paborji M, Pochopin NL, Coppola WP, and Bogardus JB, Pharm Res 11 (1994): 764–71. [PubMed: 8058650]
- [10]. Wang W, Singh S, Zeng DL, King K, and Nema S, J Pharm Sci 96 (2007): 1–26. [PubMed: 16998873]
- [11]. Butler JE, Ni L, Nessler R, Joshi KS, Suter M, Rosenberg B, Chang J, Brown WR, and Cantarero LA, J Immunol Methods 150 (1992): 77–90. [PubMed: 1613260]
- [12]. Mansur HS, Orefice RL, Vasconcelos WL, Lobato ZP, and Machado LJ, J Mater Sci Mater Med 16 (2005): 333–40. [PubMed: 15803278]
- [13]. Vareiro MM, Liu J, Knoll W, Zak K, Williams D, and Jenkins AT, Anal Chem 77 (2005): 2426–31. [PubMed: 15828777]
- [14]. Pei Z, Anderson H, Myrskog A, Duner G, Ingemarsson B, and Aastrup T, Anal Biochem 398 (2010): 161–8. [PubMed: 19962366]
- [15]. Peluso P, Wilson DS, Do D, Tran H, Venkatasubbaiah M, Quincy D, Heidecker B, Poindexter K, Tolani N, Phelan M, Witte K, Jung LS, Wagner P, and Nock S, Anal Biochem 312 (2003): 113–24. [PubMed: 12531195]
- [16]. Liu YS, Zhang YY, Zhao YN, and Yu J, Colloids and Surfaces B-Biointerfaces 121 (2014): 21–26.
- [17]. Rusmini F, Zhong ZY, and Feijen J, Biomacromolecules 8 (2007): 1775–1789. [PubMed: 17444679]
- [18]. Cho H and Jaworski J, Colloids and Surfaces B-Biointerfaces 122 (2014): 846–850.
- [19]. Corman ME, Armutcu C, Uzun L, Say R, and Denizli A, Colloids and Surfaces B-Biointerfaces 123 (2014): 831–837.

- [20]. Zucca P and Sanjust E, *Molecules* 19 (2014): 14139–94. [PubMed: 25207718]
- [21]. Hartmann M and Kostrov X, *Chem Soc Rev* 42 (2013): 6277–89. [PubMed: 23765193]
- [22]. Argarana CE, Kuntz ID, Birken S, Axel R, and Cantor CR, *Nucleic Acids Res* 14 (1986): 1871–82. [PubMed: 3951999]
- [23]. Kwon Y, Han ZZ, Karatan E, Mrksich M, and Kay BK, *Analytical Chemistry* 76 (2004): 5713–5720. [PubMed: 15456290]
- [24]. Kausaite-Minkstiniene A, Ramanaviciene A, Kirlyte J, and Ramanavicius A, *Analytical Chemistry* 82 (2010): 6401–6408. [PubMed: 20669994]
- [25]. Bonroy K, Frederix F, Reekmans G, Dewolf E, De Palma R, Borghs G, Declerck P, and Goddeeris B, *Journal of Immunological Methods* 312 (2006): 167–181. [PubMed: 16674973]
- [26]. Valsesia A, Colpo P, Meziani T, Lisboa P, Lejeune M, and Rossi F, *Langmuir* 22 (2006): 1763–1767. [PubMed: 16460103]
- [27]. Hodneland CD, Lee YS, Min DH, and Mrksich M, *Proceedings of the National Academy of Sciences of the United States of America* 99 (2002): 5048–5052. [PubMed: 11959956]
- [28]. Ishihara K, Nomura H, Mihara T, Kurita K, Iwasaki Y, and Nakabayashi N, *Journal of Biomedical Materials Research* 39 (1998): 323–330. [PubMed: 9457564]
- [29]. Park J, Kurosawa S, Takai M, and Ishihara K, *Colloids and Surfaces B-Biointerfaces* 55 (2007): 164–172.
- [30]. Vikholm I, Viitala T, Albers WM, and Peltonen J, *Biochimica Et Biophysica Acta-Biomembranes* 1421 (1999): 39–52.
- [31]. Cashion MP and Long TE, *Acc Chem Res* 42 (2009): 1016–25. [PubMed: 19453103]
- [32]. Sekula S, Fuchs J, Weg-Remers S, Nagel P, Schuppler S, Fragala J, Theilacker N, Franueb M, Wingren C, Ellmark P, Borrebaeck CAK, Mirkin CA, Fuchs H, and Lenhert S, *Small* 4 (2008): 1785–1793. [PubMed: 18814174]
- [33]. Lian T and Ho RJ, *J Pharm Sci* 90 (2001): 667–80. [PubMed: 11357170]
- [34]. Mukherjee S and Maxfield FR, *Annu Rev Cell Dev Biol* 20 (2004): 839–66. [PubMed: 15473862]
- [35]. Simons K and Ikonen E, *Nature* 387 (1997): 569–572. [PubMed: 9177342]
- [36]. Korade Z and Kenworthy AK, *Neuropharmacology* 55 (2008): 1265–1273. [PubMed: 18402986]
- [37]. Zha ZB, Jiang LN, Dai ZF, and Wu XY, *Applied Physics Letters* 101 (2012): 193701. [PubMed: 23213266]
- [38]. Zhang JH, Cohn CM, Zha ZB, Dai ZF, and Wu XY, *Applied Physics Letters* 99 (2011): 103702. [PubMed: 21990942]
- [39]. Blossey R, *Nat Mater* 2 (2003): 301–6. [PubMed: 12728235]
- [40]. Zen Yoshimitsu AN, Toshiya Watanabe, and Kazuhito Hashimoto, *Langmuir* 18 (2002): 4.
- [41]. Bormashenko EY. *De Gruyter studies in mathematical physics*. xvii, 170 pages.
- [42]. Gao L and McCarthy TJ, *Langmuir* 25 (2009): 14105–15. [PubMed: 19627073]
- [43]. Xu FJ, Wang ZH, and Yang WT, *Biomaterials* 31 (2010): 3139–47. [PubMed: 20117830]
- [44]. Sadana A and Sadana N. 1st ed Amsterdam; Boston: Elsevier. x, 361 p., 2008.
- [45]. Ge S, Kojio K, Takahara A, and Kajiyama T, *J Biomater Sci Polym Ed* 9 (1998): 131–50. [PubMed: 9493841]
- [46]. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R, Newman RA, Hanna N, and Anderson DR, *Blood* 83 (1994): 435–445. [PubMed: 7506951]
- [47]. Davis TA, Czerwinski DK, and Levy R, *Clinical Cancer Research* 5 (1999): 611–615. [PubMed: 10100713]

Highlights

- Lipid fibers were made from electrospun cholesteryl succinyl silane (CSS).
- The immobilization of anti-CD20 antibody and the retention of anti-CD20 function were examined on CSS fibers, in comparison to hydrophobic polycaprolactone (PCL) fibers and hydrophilic plasma-treated PCL fibers.
- The anti-CD20 functionalized CSS fibrous matrices captured 6- or 7-fold more cells than the anti-CD20 functionalized PCL or plasma-treated PCL fibrous matrices, respectively.
- CSS fiber-mediated immobilization of antibody not only maintains the advantages of physical immobilization (e.g., easiness and rapidness of operation) but improves function retention.

Fig. 1. Diameter distributions of PCL (a), plasma-treated PCL (b), and CSS fibers (c). Inserts show representative optical images of water drops on the surfaces of the fibrous matrices.

Fig. 2. BSA dissociation analysis. The amount of immobilized BSA remaining on the plasma-treated PCL (a) and CSS fibrous matrices (b) over a period of 6 days. Symbols with error bars represent experimental data, and the solid lines represent the curve fitting using the first-order kinetics model.

Fig. 3. Antibody immobilization analysis. The percentage of anti-CD20 immobilized on each fiber type was calculated.

Fig. 4. Cell capture. Optical images of Granta-22 cells captured on the PCL (a), plasma-treated PCL (b), and CSS fibrous matrices (c) that were functionalized using anti-CD20. The cells were stained with DAPI against cell nuclei.

Fig. 5.
Density of Granta-22 cells captured on the PCL, plasma-treated PCL, and CSS fibrous matrices that were functionalized with equal amount of anti-CD20.

Fig. 6.

A schematic presentation of antibody immobilization on polymer and lipid fibers. Highly hydrophobic surfaces such as PCL fibers immobilize antibodies mainly through hydrophobic interactions, leading to antibody denaturation and random orientation. Hydrophilic surfaces such as plasma-treated PCL fibers immobilize antibodies through electrostatic interactions, resulting in random orientation. Antibodies are immobilized on biomimetic lipid fibers such as CSS fibers through the embedding of the crystallizable Fc regions in the nanoscale defects of the fibers. This leads to oriented antibody immobilization and improved function retention.

Table 1.

Parameters used in the first-order kinetics model of BSA dissociation

	y_t (µg per sample)	y_d (µg per sample)	Dissociation rate constant (per hour)
Plasma-treated PCL	0.58	1.174	0.0327
CSS	0.58	0.828	0.0506