Micro-assembly of Functionalized Particulate Monolayer on C_{18}-Derivatized SiO_2 Surfaces

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Abstract: This work describes a simple approach to immobilize functionalized colloidal microstructures onto a C_{18}-coated SiO_2 substrate via specific or non-specific biomediated interactions. Biotinylated bovine serum albumin pre-adsorbed onto a C_{18} surface was used to mediate the surface assembly of streptavidin-coated microbeads (2.8 μm), while a bare C_{18} surface was used to immobilize anti-*Listeria* antibody-coated microbeads (2.8 μm) through hydrophobic interactions. For a C_{18} surface pre-adsorbed with bovine serum albumin, hydrophobic polystyrene microbeads (0.8 μm) and positively charged dimethylamino microbeads (0.8 μm) were allowed to self-assemble onto the surface. A monolayer with high surface coverage was observed for both polystyrene and dimethylamino microbeads. The adsorption characteristics of *Escherichia coli* and *Listeria monocytogenes* on these microbead-based surfaces were studied using fluorescence microscopy. Both streptavidin microbeads pre-adsorbed with biotinylated anti-*Listeria* antibody and anti-*Listeria* antibody-coated microbeads showed specific capture of *L. monocytogenes*, while polystyrene and dimethylamino microbeads captured both *E. coli* and *L. monocytogenes* non-specifically. The preparation of microbead-based surfaces for the construction of microfluidic devices for separation, detection, or analysis of specific biological species is discussed. *Biotechnol Bioeng* **83**: 416–427, 2003.
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INTRODUCTION

Microfluidic devices interfaced with efficient sample preparation and delivery systems are capable of providing rapid identification of specific biological species such as nucleic acids, protein, drugs, or other important biological compounds (Khandurina and Guttman, 2002). Functionalized microbeads have been used extensively in chromatography as packing material for analytical bioseparations (Leonard, 1997). There has been a growing interest, as pointed out by Andersson et al. (2001, 2002), to combine microbead-based surfaces with microfluidic devices, to provide bead-based DNA analysis (Andersson et al., 2000; Bruckner-Lea et al., 2000), and to carry out chromatographic separations (Oleschuk et al., 2000) as well as protein analysis in immunoassays (Buranda et al., 2002; Sato et al., 2000).

Self-assembly of microstructures onto a surface can be achieved through electro-static self-assembly (Tien et al., 1997), electric-field-mediated self-assembly (Lee et al., 2002), template-assisted self-assembly (Yin et al., 2001), and many other well-established techniques, including the use of capillary forces (during solvent evaporation), gravitational forces, or magnetic forces. At biological interfaces, Mucic et al. (1998) have demonstrated the synthesis of binary nanoparticle network materials through DNA-direct

assembly strategies. Hiddessen et al. (2000) have shown the assembly of binary colloidal structures via specific carbohydrate–selectin interactions. Patterning of self-assembled beads in silicon channels has been demonstrated by Andersson et al. (2001) through micro-contact stamping of biotinylated bovine serum albumin (BSA) and followed by self-assembly of streptavidin-coated microbeads. This paper reports fabrication of functionalized colloidal microstructures onto a C_{18}-coated SiO_2 substrate via self-assembly or adsorption.

The C_{18} surface (Fig. 1), created by self-assembly of octadecyltrichlorosilane (ODTS), has been commonly used as a surface modifier for reverse-phase chromatography stationary phases (Ladisch, 2001; Wirth et al., 1993). It consists of a self-assembled and closely packed alkylsiloxane monolayer that gives an extremely hydrophobic neutrally charged surface (contact angle ~110°C using deionized water). C_{18} surfaces have found applications as boundary lubricants that reduce surface stiction in MEMS devices (Clear and Nealey, 2001), nanolithography (Jeon et al., 1997), ultrathin imaging layers (Peters et al., 2000), and semiconductor coatings (Angst et al., 1991). When incubated with a protein at aqueous phase, a C_{18} surface can rapidly capture a monolayer of protein through hydrophobic interactions. Lysozyme, insulin, albumin, and hexokinase all form a protein film of approximately a monolayer when adsorbed onto a C_{18}-modified SiO_2 surface at a concentration of 0.25 mg/mL in pH 7.4 PBS buffer (Lee and Laibinis, 1998). Most importantly, a C_{18} surface to which biotinylated BSA, BSA, or a non-ionic surfactant such as Tween 20 is pre-adsorbed effectively blocks non-specific adsorption of proteins or bacterial cells (Huang et al., 2002, 2003).

Chemical modification of microchannel surfaces (in situ or ex situ) requires surface cleaning, pretreatment steps, and post-treatment steps in order to achieve surface chemistries that will enable functional groups to be attached but which will exhibit minimal non-specific adsorption when the procedure is completed. The benefit of using microbeads is that the chemistry can be carried out separately, using conditions that might otherwise degrade the surfaces and microstructures on the chip. For commercially available microbeads, many of the bead chemistries have been developed by the manufacturers to minimize non-specific adsorption and to selectively bind the desired proteins or biomolecules. The third benefit of the use of microbeads is increased surface area and therefore enhanced adsorption capacity over what can be achieved on a microfabricated chip with modifications carried out on the surface of the chip. Furthermore, the beads can be prepared with controlled porosities, and since their pore path lengths are 1 micron or less, rapid equilibrium can be achieved.

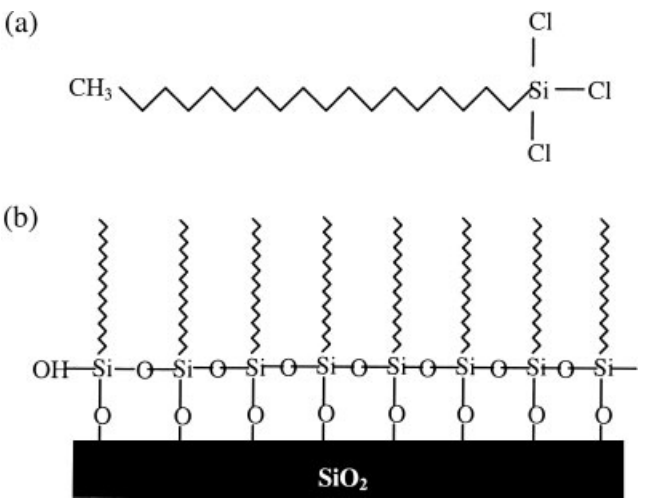

Figure 1. (**a**) Structure of octadecyltrichlorosilane (C_{18}). (**b**) Possible structures of C_{18} monolayer on the SiO_2 surface. (Drawing is not to scale.)

A method to immobilize functionalized microbeads onto a C_{18}-modified SiO_2 surface through bio-mediated interactions is described in this paper. A microchip with a C_{18} surface pre-adsorbed with biotinylated BSA enables rapid self-assembly of streptavidin-coated microbeads through specific biotin–streptavidin interaction. When coated with BSA, this microchip surface immobilizes polystyrene or dimethylamino beads through possible non-specific hydrophobic or electrostatic interactions. Protein-coated microbeads such as ones coated with anti-*Listeria* antibody can also be immobilized onto a bare C_{18} surface through hydrophobic interactions. A microbead-patterned surface results where the only area capable of binding proteins or microbes is the microbead itself, since biotinylated BSA or BSA pre-adsorbed onto a C_{18} surface blocks non-specific adsorptions (Huang et al., 2002, 2003). Fluorescence microscopy was used in this work to study the adsorption of *Escherichia coli* and the food pathogen *Listeria monocytogenes* onto various types of microbeads immobilized on microchip surfaces.

MATERIALS AND METHODS

The preparation of microchips capable of detecting target organisms or proteins requires systematically engineered, prepared, and validated surfaces. The Methods section of this paper describes the preparation of surfaces, assembly concepts, and validation of functionalities.

Materials: Microbeads, Proteins, Buffer, Bacteria, and Antibody

Polystyrene magnetic beads coated with streptavidin, 2.8 μm in diameter (cat. no. 112.05), and polystyrene magnetic beads coated with anti-*Listeria* antibody, 2.8 μm in diameter (cat. no. 710.06), were purchased from Dynal Biotech (Oslo, Norway). Polystyrene beads, 0.8 μm in diameter (cat. no. PP-08-10), and polystyrene beads functionalized with dimethylamino, 0.8 μm in diameter (cat. no. DP-08-10), were purchased from Spherotech (Libertyville, IL). Biotinylated BSA (cat. no. 29130, lot no. CC48629) with 8 mol of biotin attached to each mole of BSA was purchased from Pierce (Rockford, IL). BSA (cat. no. A-0281, lot no.

100K7415) was purchased from Sigma-Aldrich (St. Louis, MO). PBS buffer (pH 7.2) is made from a reagent package purchased from Sigma-Aldrich (cat. no. 1000-3).

Bacterial cultures of *E. coli* ATCC51379 and *L. monocytogenes* V7 were used in this study. *L. monocytogenes*, a Gram-positive, rod-shaped food-borne pathogen, causes serious food poisoning (Farber and Peterkin, 1991) and is the subject of studies for its rapid detection (Bashir et al., 2001; Bhunia et al., 2001; Gomez et al., 2001; Li and Bashir, 2002). Monoclonal antibody (MAb) C11E9 belongs to IgG2b subclass and is produced from mice. This antibody binds to the surface proteins of *L. monocytogenes* and *Listeria innocua* in immunoassays and does not cross-react with secondary antibody (anti-rabbit IgG) (T. Geng, Molecular Food Microbiology Laboratory, Purdue University, personal communications, 2003) or other microorganisms tested (*Enterococcus* spp., *Staphylococcus* spp., *Bacillus* spp., *E. coli*, *Salmonella*, and *Pseudomonas* spp.) (Bhunia, 1997; Bhunia et al., 1991). Purification of C11E9 was performed by protein G immunoaffinity chromatography using an Acta Prime Liquid Chromatography System (Pharmacia Corp., Peapack, NJ).

Once purified, MAb C11E9 was conjugated with biotin to form biotinylated anti-*Listeria* antibody. The procedure for antibody biotinylation follows the instructions from Pierce and as modified by Hnatowich et al. (1987). Final antibody/protein concentration was determined to be 1.2 mg/mL by measuring its absorbance at 280 nm. FITC-labeled goat anti-mouse IgG (Fab specific) purchased from Sigma-Aldrich (cat. no. F5262, lot no. 050K4888) was used to confirm the functionality of biotinylated anti-*Listeria* antibody that was pre-immobilized by the streptavidin-coated microbeads.

Culture and Labeling of *Listeria* and *E. coli* Microbial Cells

E. coli and *L. monocytogenes* were grown in Brain Heart Infusion broth (Becton Dickinson and Company, Cockeysville, MD) at 37°C for 16–18 h. *E. coli* and *Listeria* cell concentrations were determined to be approximately 5×10^9 and 5×10^8 CFU/mL, respectively, using serial dilution of samples, with 100 μL of each dilution plated out. Cells harvested at this stationary phase are assumed to have similar cell surface properties, which are responsible for ligand and receptor interactions.

The cells were labeled with a green fluorescent dye, FITC, purchased from Sigma-Aldrich (cat. no. F-4274, lot no. 70K2617). FITC was dissolved in 0.1 *M* sodium bicarbonate buffer (pH 9.6) at a concentration of 2 mg/mL. After filter sterilization using a 0.45-μm size filter (Gelman Sciences Inc., Ann Arbor, MI), 1 mL of FITC solution was combined with centrifuged cells (from 10^8–10^9 cells/mL) in a 1.5-mL Eppendorf® tube and incubated at 37°C for 30 min. After incubation, the cells were centrifuged again and then washed three times with 1 mL of PBS buffer to remove unbound FITC molecules.

Preparation of Microchip With C_{18} Surface

A 3-inch silicon wafer with surface crystal orientation <100> was oxidized in a furnace to grow a layer of SiO_2. To activate the SiO_2 surface with a high concentration of hydroxyl groups, the wafer was immersed in piranha solution (7:3 concentrated H_2SO_4/30% H_2O_2) for 30 min. *Caution! Piranha solution reacts violently with organic compounds and is severely exothermic during reaction. It should be handled with extreme care.* Afterward, the wafer was rinsed with deionized water for 15 min. Bulk water on the surface of the SiO_2 wafer was then blown away with nitrogen because free water may cause undesirable polymerization to ODTS. A thin film of water that remains on the surface is thought to enhance the quality of the C_{18} coating (Flinn et al, 1994; Hair and Tripp, 1995; Le Grange and Markham, 1993). Recent studies on growth mechanisms of ODTS formation on silica substrates can be found in the literature (Liu et al., 2001; Peters et al., 2002; Richter et al., 2000). The wafer was transferred into a sealed glass jar in a 0.1% (v/v) solution of ODTS (Sigma-Aldrich, cat. no. 10,481-7, lot no. 13326MO) in anhydrous toluene (Sigma-Aldrich, cat. no. 24,451-1, lot no. JO03646EO), in a nitrogen-purged glove box and at room temperature. The wafer was immersed for 24 h in the toluene solution, rinsed afterward three times with fresh toluene, and finally subjected to ultrasonic cleaning in toluene for 5 min. This effectively removes the residual ODTS left on the surface. The wafer was then blown dry with nitrogen and baked in an oven at 120°C for 30 min to complete formation of the Si–O bond.

The ODTS-silanized wafer was placed in a Petri dish covered with aluminum foil and stored in a nitrogen-purged box until it was used. Microchips were prepared shortly before adsorption experiments. The microchips were approximately 3 mm × 3 mm in size and were prepared by carefully cleaving the wafer using a diamond scribe. Contact angle measurements made on the ODTS-silanized surface using deionized water gave a contact angle between 105° and 110° (Huang et al., 2002).

Methods: Proteins, Microbeads, Antibody, and Bacteria Adsorption Protocols

Adsorption protocols involved incubation of protein, microbeads, antibody, and bacterial cells with the chips at room temperature in a 48-well polystyrene plate purchased from Fisher Scientific (Pittsburgh, PA) (cat. no. 08-772-1C). This was followed by a washing step with PBS buffer (pH 7.2) to rinse off any unbound or excess material from the chip surface. Biotinylated BSA and BSA were diluted to a concentration of 2 mg/mL using PBS buffer (pH 7.2). For streptavidin-coated microbeads (2.8 μm), the concentration was 10 mg/mL (6.7×10^8 beads/mL) based on the data provided by the manufacturer. For anti-*Listeria* antibody-coated microbeads (2.8 μm), however, this data was not given by the manufacturer. Using serial dilution with PBS buffer and by visually counting the number of beads with an

Table I. Summary of various types of microbead-coated surfaces.

Type of microbead surface	C_{18}	Biotinylated BSA	BSA	Streptavidin microbeads	Anti-*Listeria* microbeads	Polystyrene microbeads	Dimethylamino microbeads
Streptavidin	•	•		•			
Anti-*Listeria*	•				•		
Polystyrene	•		•		•		
Dimethylamino	•		•				•

optical microscope, we estimated the concentration of anti-*Listeria* antibody-coated beads to be ~10^8 beads/mL. The concentrations of polystyrene and dimethylamino-functionalized microbeads were both calculated to be 1.78 × 10^{11} beads/mL based on information provided by the manufacturer. The biotinylated anti-*Listeria* antibody was diluted with PBS buffer, and the final antibody concentration was calculated to be 50 μg/mL. FITC-labeled goat

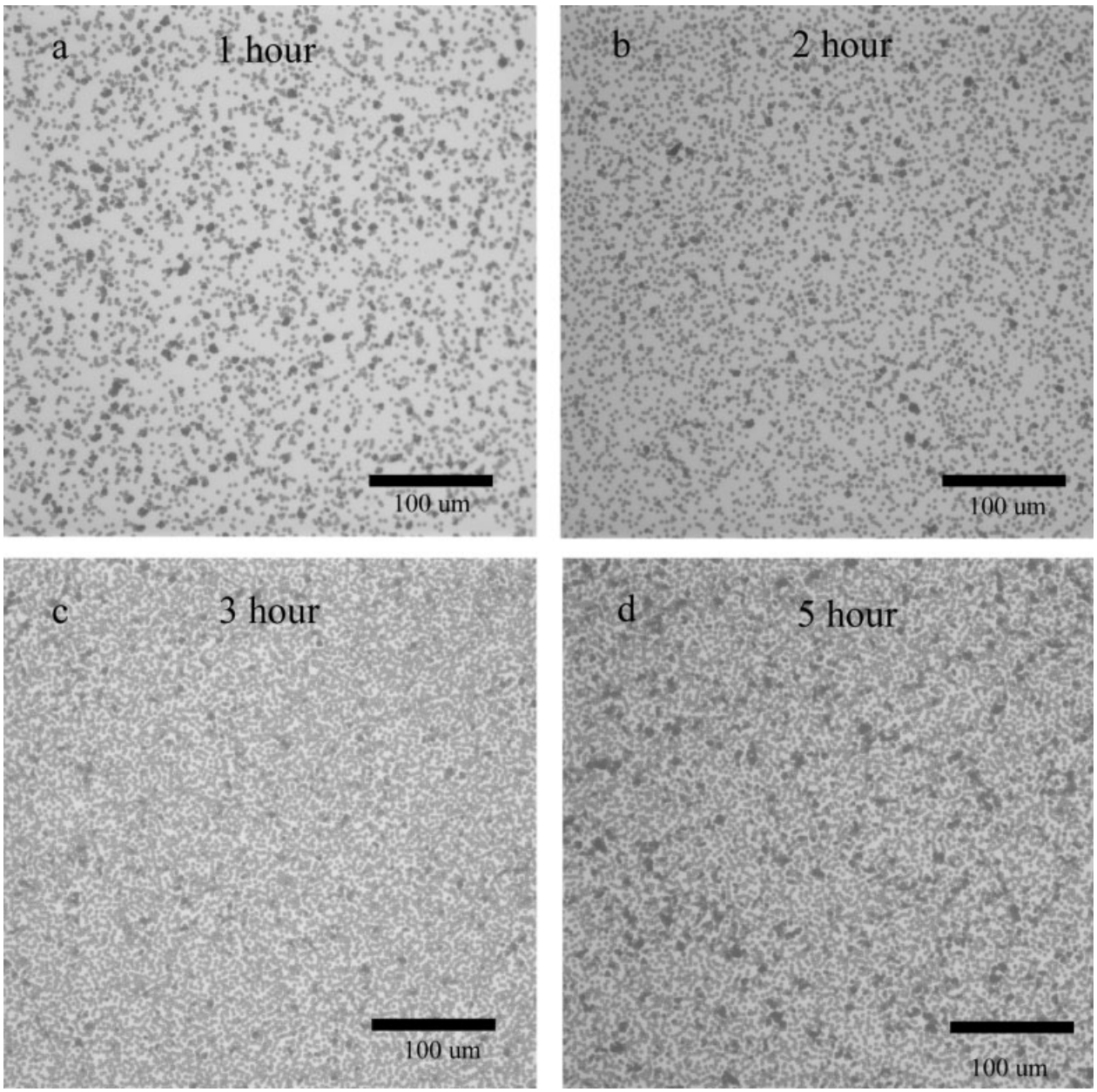

Figure 2. Bright field of images of 2.8-μm streptavidin-coated microbeads in PBS buffer (pH 7.2) in contact with C_{18} surface pre-adsorbed with biotinylated BSA for (**a**) 1 h, (**b**) 2 h, (**c**) 3 h, and (**d**) 5 h. (Scale bar is 100 μm.)

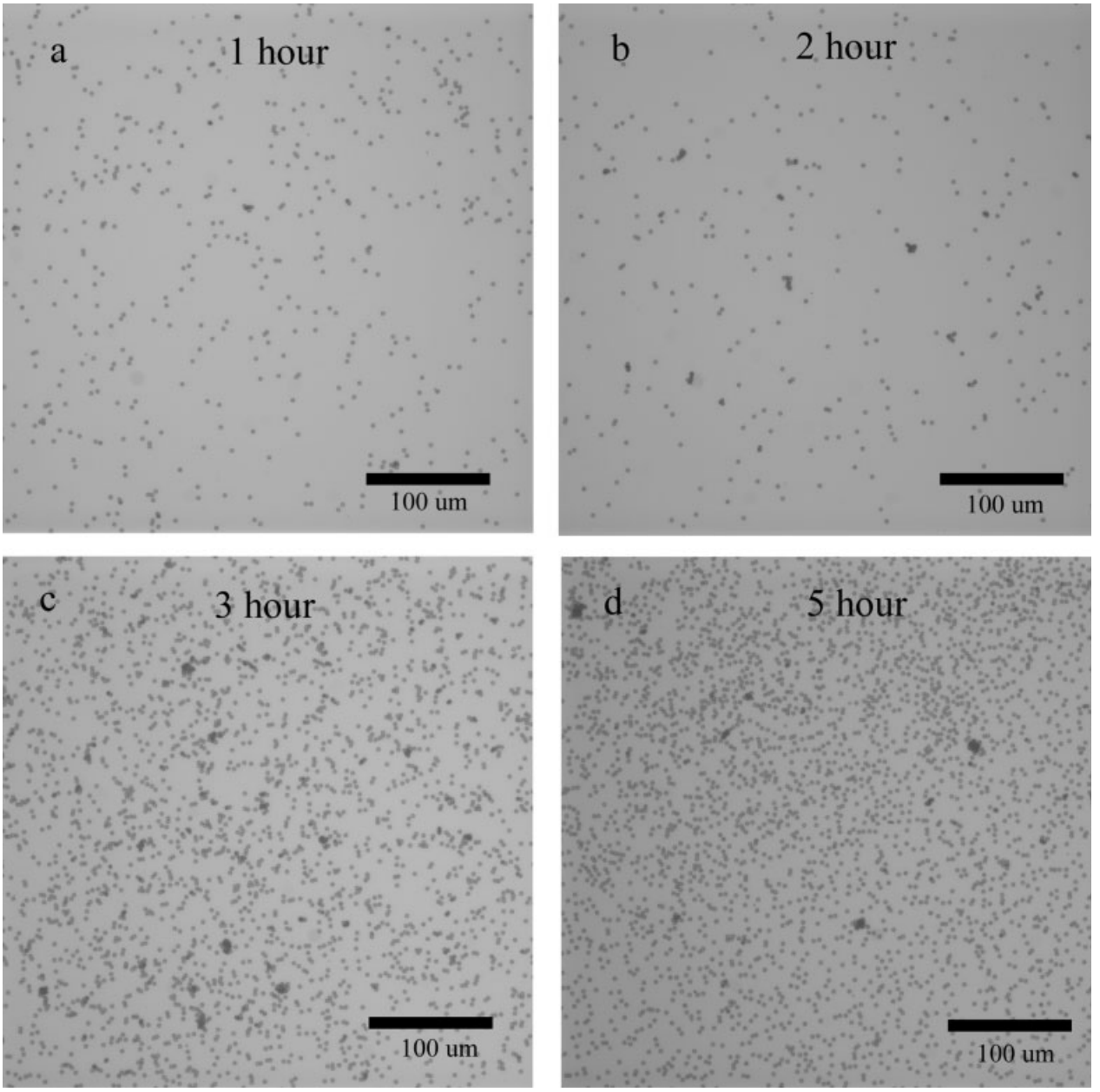

Figure 3. Bright field of images of 2.8-μm anti-*Listeria* antibody-coated microbeads in PBS buffer (pH 7.2) in contact with a bare C_{18} surface for (**a**) 1 h, (**b**) 2 h, (**c**) 3 h, and (**d**) 5 h. (Scale bar is 100 μm.)

anti-mouse antibody (IgG) at 5 mg/mL was diluted 1:100 using PBS buffer to give a concentration of 50 μg/mL. FITC-labeled bacterial cells were diluted 10-fold with PBS buffer to approximately 10^7–10^8 cells/mL before the incubation.

Each C_{18} microchip was immersed in 150 μL of respective wash buffer in a 1,400-μL well of a 48-well plate. To initiate the incubation, 50 μL of respective protein, antibody, bacterial cells, or 10 μL of respective microbeads was added into the well and incubated at room temperature using a rotary shaker at 120 rpm. Washing steps were done by placing the chips into different wells after incubation. For protein, antibody, and bacterial cell adsorption, chips were washed three times with PBS buffer, for 5 min each, after each treatment. For the studies on immobilization of microbeads, the chips were rinsed one time in PBS buffer for 5 min. In each washing step the microchip was in contact with the solution for 5 min while the 48-well plate was agitated on the rotary shaker at 120 rpm. The amount of solution used for each microchip was 300 μL for every washing step. In this manner, microchips with different combinations of microbead surfaces were prepared as summarized in Table I.

The incubation time for BSA or biotinylated BSA was 30 min. The times required for streptavidin microbeads to be immobilized on a biotinylated BSA surface and anti-*Listeria* microbeads to be immobilized on a bare C_{18} surface

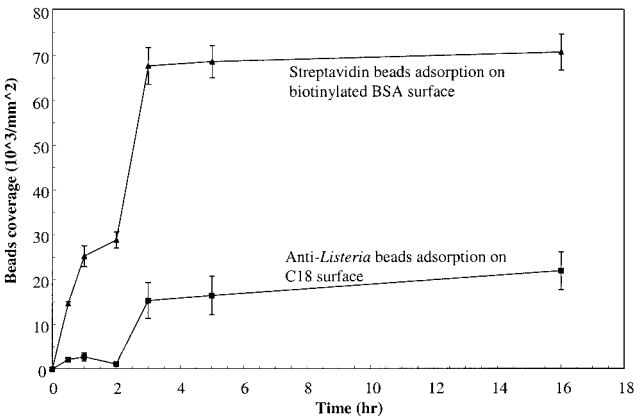

Figure 4. Adsorption kinetics of streptavidin microbeads on a C_{18} surface pre-adsorbed with biotinylated BSA and of anti-*Listeria* antibody-coated microbeads on a bare C_{18} surface. (Error bar represents one standard deviation in 10^3 beads/mm^2 using data in triplicate.)

were studied by varying the incubation time at 0.5, 1, 2, 3, 5, and 16 h. The incubation time for polystyrene and dimethylamino microbead immobilization on BSA-coated microchips was 2 h. Prior to contact with bacterial cells, the microchip surface immobilized with anti-*Listeria* microbeads was incubated with BSA for 30 min in order to block the exposed C_{18} surfaces from non-specific adsorptions. The microchip surface immobilized with the streptavidin microbead surface was incubated with biotinylated anti-*Listeria* antibody for 30 min. To confirm the functionality of the biotinylated anti-*Listeria* antibody captured by streptavidin microbeads, the surface was incubated with FITC-labeled goat anti-mouse IgG for 30 min.

Living *E. coli* or *L. monocytogenes* cells were contacted with microbead surfaces for 1 h. Four types of microbead-coated surfaces were used: (1) streptavidin microbead surfaces pre-adsorbed with biotinylated anti-*Listeria* anybody;

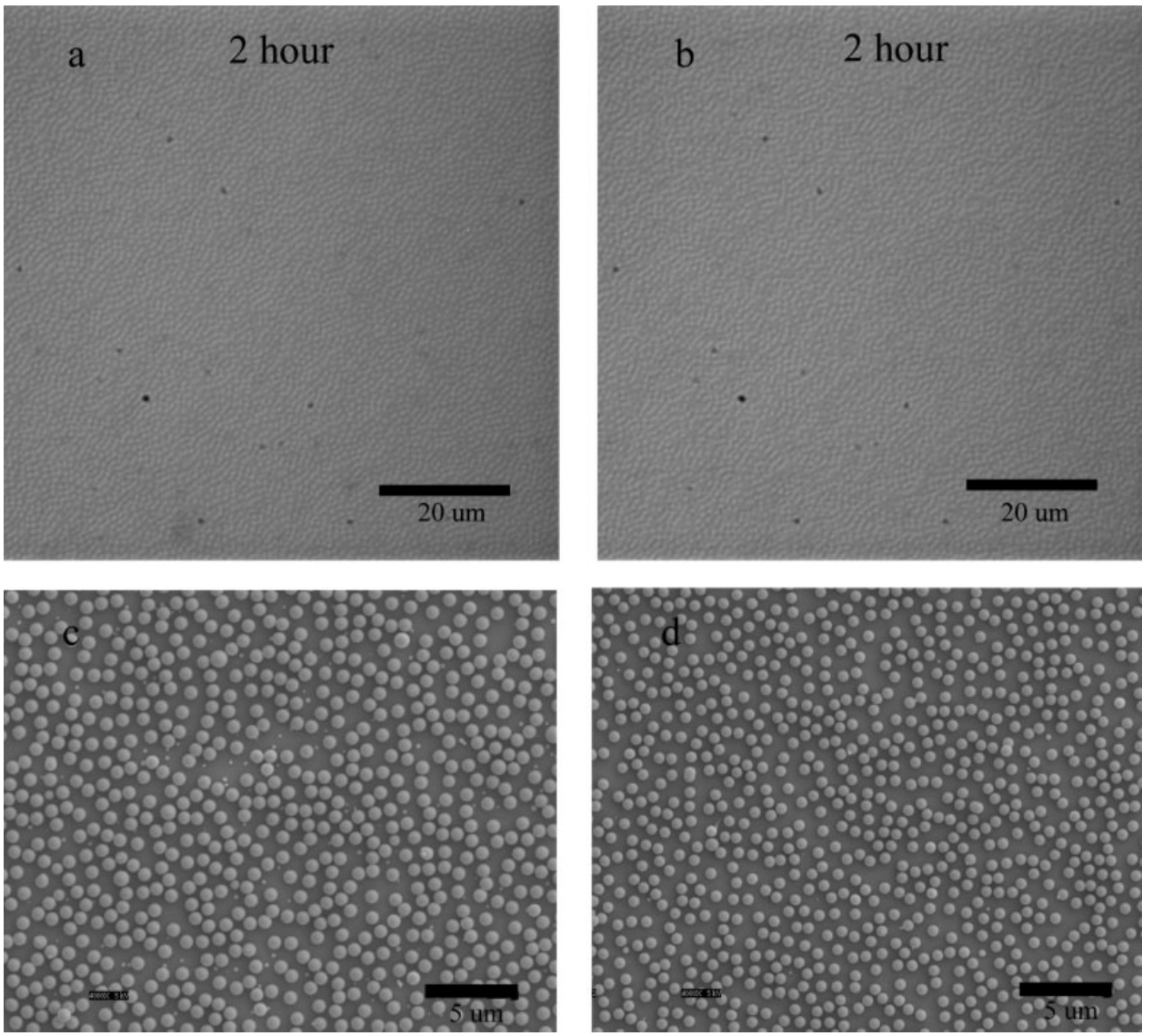

Figure 5. Phase contrast images of (**a**) 0.8-μm polystyrene beads and (**b**) 0.8-μm dimethylamino beads in PBS buffer (pH 7.2) in contact with C_{18} surface pre-adsorbed with BSA for 2 h. (Scale bar is 20 μm.) SEM of (**c**) polystyrene beads and (**d**) dimethylamino beads. (Scale bar is 5 μm.)

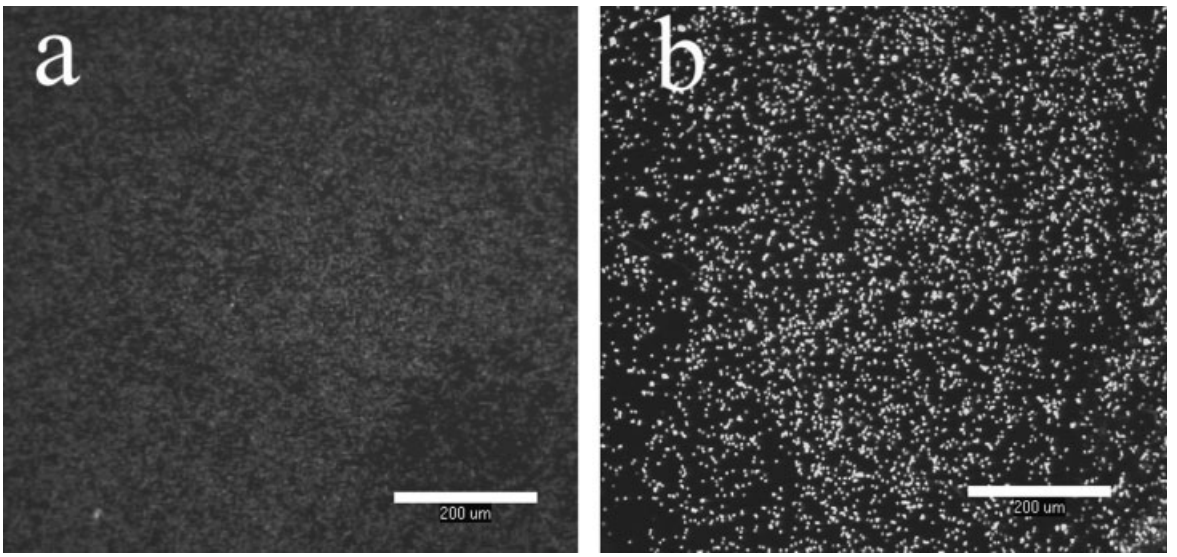

Figure 6. Fluorescence images of (**a**) streptavidin microbeads pre-adsorbed with biotinylated anti-*Listeria* antibody and (**b**) anti-mouse IgG adsorption from PBS buffer (pH 7.2) to streptavidin microbeads pre-adsorbed with biotinylated anti-*Listeria* antibody. (Scale bar is 200 μm.)

(2) anti-*Listeria* microbead surfaces pre-blocked with BSA; (3) polystyrene microbead surfaces; and (4) dimethylamino microbead surfaces. The streptavidin or anti-*Listeria* microbeads were incubated for 3 h with the biotinylated BSA surfaces or the bare C_{18} surface, while polystyrene or dimethylamino microbeads were kept in contact for 2 h with surfaces that previously had BSA pre-adsorbed.

Microscopy

After being washed, the microchips were placed into chambers that were made from a durable silicone. The silicone gasket was patterned with squares and then pressed against a microscope slide to form an imaging chamber. To keep the proteins, microbeads, or bacterial cells from drying, a drop of PBS buffer was placed onto the microchip surface. A cover slip was used to seal the imaging chambers before the optical images were collected. Bright field or phase contrast images were taken using Nikon Eclipses L150 microscope with an Insight digital color camera. In order to interpret results involving the adsorption of microbeads versus time, the number of streptavidin beads or anti-*Listeria* beads adsorbed or bound was obtained by visually counting the number of beads after the chips had been rinsed one time with 300 μL of PBS buffer. A 100 μm × 100 μm area on the picture was used for counting the number of beads. Any beads inside this area were counted. The final number was an average from a chip on which microbeads from a total of three randomly picked 100 μm × 100 μm areas were counted.

The fluorescence images were observed in a Nikon Labophot fluorescence microscope with a FITC filter and an Optronics 470T CCD camera and acquired with MetaMorph software (Universal Imaging Corp., Downingtown, PA). The fluorescence label (FITC) has an emission peak between 515 and 525 nm, and this was the range of wavelengths over which the samples were imaged. In order to interpret results involving the adsorption of bacterial cells on microbead-coated surfaces, the number of *E. coli* and *L. monocytogenes* cells adsorbed or bound were counted using Quantity One Version 4.2.3 software from Bio-Rad Corp. (Hercules, CA). A 435 μm × 435 μm area on the fluorescence image was used for counting the number of bacterial cells. For scanning electron microscopy (SEM), the samples were fixed in 2% glutaraldehyde in 0.1 *M* phosphate buffer for 30 min at 4°C. The fixation was followed by washing the samples with phosphate buffer and then dehydrating in a graded series of EtOH at room temperature (30%, 50%, and 70% EtOH for 10 min each). The samples were kept in 70% EtOH solution until they were critical-point dried before SEM.

RESULTS AND DISCUSSION

The adsorption of 2.8-μm streptavidin microbeads onto the biotinylated BSA-coated surface is shown in Fig. 2. As can be seen, full coverage was achieved after 3 h of incubation and was expected, given the strong affinity between biotin and streptavidin (K_d value on order of 10^{-15} *M*). Beyond 3 h, surface aggregation is indicated. This is compared with immobilization of 2.8-μm anti-*Listeria* microbeads on a bare C_{18} surface (Fig. 3) through hydrophobic interactions, where the coverage was less. Based on the results in Figs. 2 and 3 and on two additional sets of data at 0.5 and 16 h (pictures not shown), the microbead surface coverage was plotted versus time in Fig. 4. There is a step increase in the number of beads adsorbed within 3 h followed by a leveling off. The adsorption characteristics of smaller 0.8-μm polystyrene and 0.8-μm dimethylamino microbeads on a C_{18} surface pre-adsorbed with BSA are shown in Fig. 5. Uni-

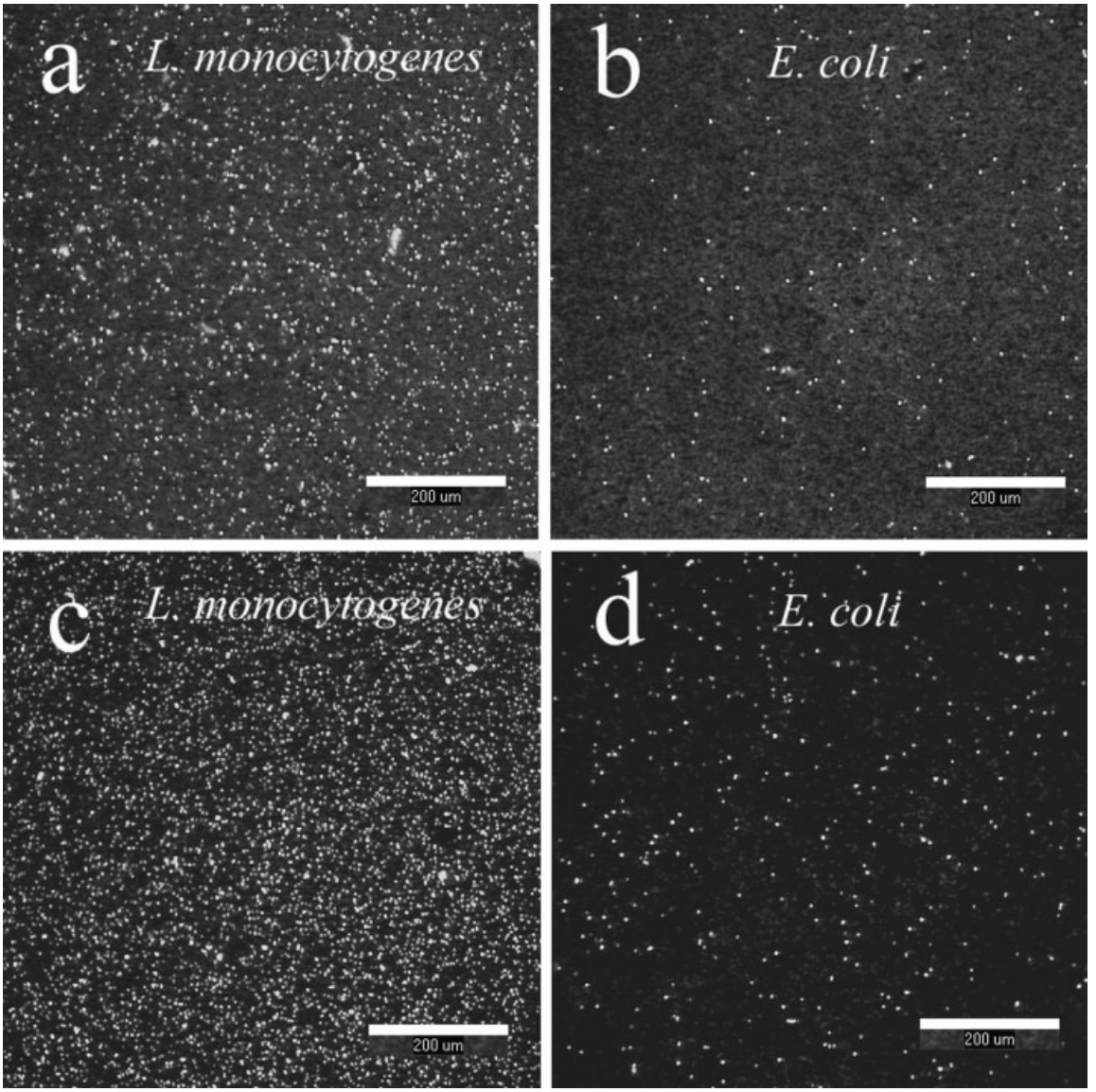

Figure 7. Fluorescence images of *L. monocytogenes* (~10^7 cells/mL in PBS buffer) adsorbed on (**a**) streptavidin microbead surface pre-adsorbed with biotinylated anti-*Listeria* antibody and (**c**) anti-*Listeria* antibody-coated microbead surface previously blocked with BSA and of *E. coli* (~10^8 cells/mL in PBS buffer) adsorbed on (**b**) streptavidin microbead surface pre-adsorbed with biotinylated anti-*Listeria* antibody and (**d**) anti-*Listeria* antibody-coated microbead surface previously blocked with BSA. (Scale bar is 200 μm.)

form coverage was approached for both polystyrene and dimethylamino microbeads within 2 h of incubation. Because polystyrene microbeads are hydrophobic and dimethylamino microbeads are positively charged, the surface self-assembly processes are likely to be driven by hydrophobic and electrostatic interactions.

After biotinylated anti-*Listeria* antibody were immobilized onto the streptavidin microbeads, FITC-labeled anti-mouse IgG was used to probe the functionality of the biotinylated antibody. The activity of biotinylated anti-*Listeria* antibody to bind anti-mouse IgG (Fab specific) was confirmed by fluorescence images in Fig. 6. Streptavidin beads pre-adsorbed with biotinylated anti-*Listeria* antibody covered the surface (Fig. 6a). Because the fluorescently labeled anti-mouse IgG binds the biotinylated anti-*Listeria* antibody, this results in the bright fluorescent dots (Fig. 6b). Fluorescence images of bacterial adsorption on antibody-coated microbeads are contrasted in Fig. 7. In order to demonstrate specific capture of bacterial cells, *L. monocytogenes* and *E. coli* were incubated with the surface shown in Fig. 2c, where the streptavidin microbead surfaces were pre-adsorbed with biotinylated anti-*Listeria* antibody, and also with the surface of Fig. 3c, where the anti-*Listeria* microbeads on bare C_{18} surfaces were pre-blocked with BSA. For specific capture, a greater number of *L. monocytogenes* cells were adsorbed onto anti-*Listeria* antibody-coated surfaces compared to *E. coli* alone (see Fig. 7 and Table II). On the other hand, the polystyrene and dimethylamino microbeads captured *L. monocytogenes* strongly and non-specifically when compared to *E. coli* alone (see Fig. 8 and Table II). This confirms our previous work, which showed that the Gram-positive *L. monocytogenes* ex-

Table II. Number of bacterial cells adsorbed on various surfaces in a 435 μm × 435 μm (0.189 mm^2) area.

	E. coli.	Listeria monocytogenes
Microbead-coated surface[a]		
Streptavidin beads with biotinylated anti-*Listeria* antibody	66	550
Anti-*Listeria* beads blocked with BSA	105	755
Polystyrene beads	290	1592
Dimethylamino beads	580	1590
Protein-coated surface[b]		
Biotinylated BSA	1	2
Streptavidin	25	45
Streptavidin blocked by BSA	9	20
Streptavidin with biotinylated anti-*Listeria* antibody	No data	60

[a]Data from Figs. 7 and 8.
[b]Data obtained from Huang et al. (2003).

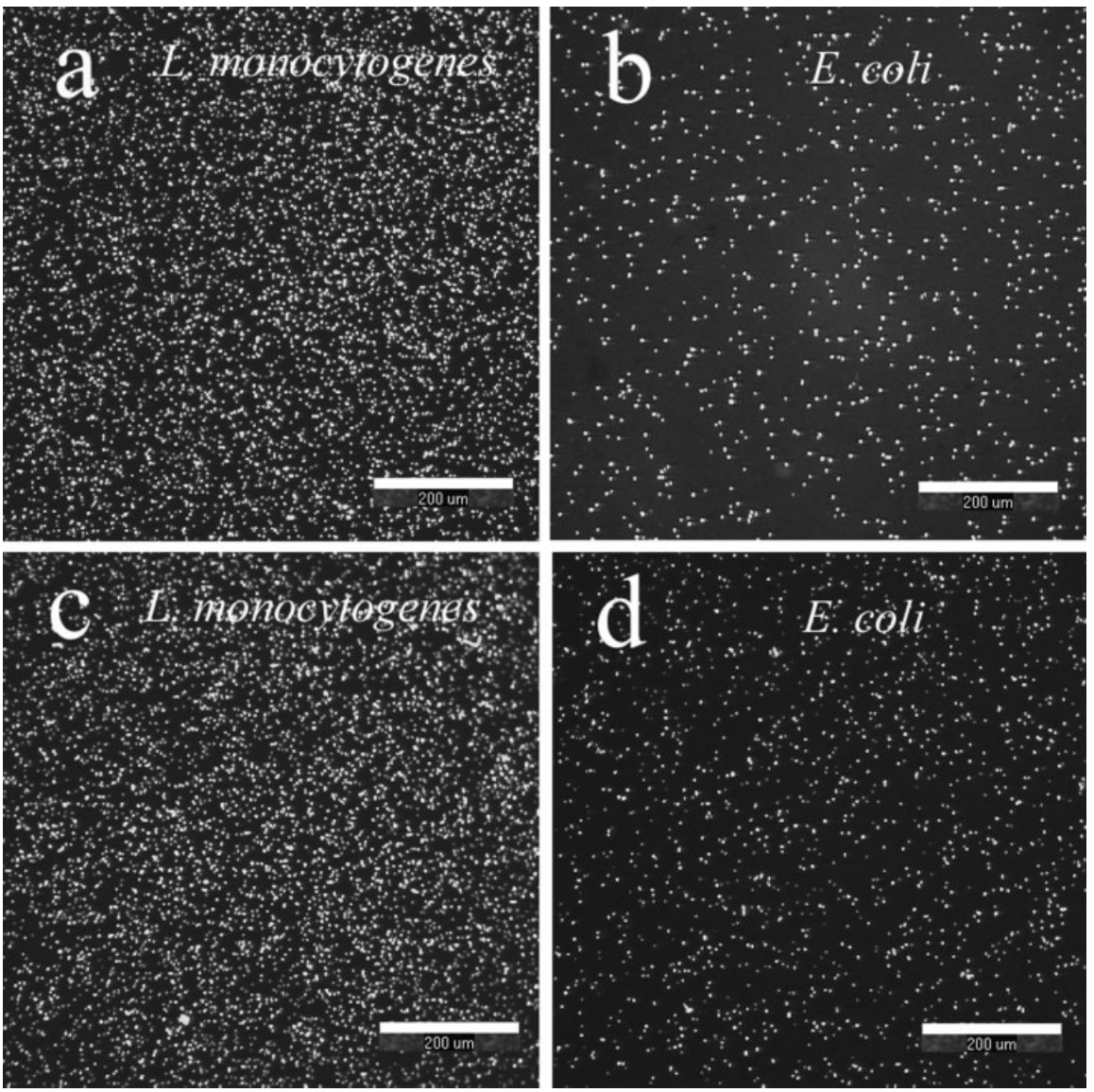

Figure 8. Fluorescence images of *L. monocytogenes* (~10^7 cells/mL in PBS buffer) adsorbed on surfaces coated with (**a**) polystyrene microbead surface and (**c**) dimethylamino microbead surface and of *E. coli* (~10^8 cells/mL in PBS buffer) adsorbed on (**b**) polystyrene microbead surface and (**d**) dimethylamino microbead surface. (Scale bar is 200 μm.)

hibits stronger adsorption on a surface with hydrophobic properties when compared to the Gram-negative *E. coli* (Huang et al., 2002). The large difference in the number of *E. coli* and *L. monocytogenes* captured by a polystyrene and dimethylamino microbeads surface might be associated with differences in the surface properties of these bacterial cells and could be used to further differentiate between the two. The capture of bacterial cells by microbead-coated surfaces and protein-coated surfaces is compared in Table II; microbead-coated surfaces (streptavidin microbeads adsorbed with biotinylated anti-*Listeria* antibody) captured 9 times more *L. monocytogenes* cells than the protein-coated surface (streptavidin adsorbed with biotinylated anti-*Listeria* antibody).

Bacterial cell attachment to the microbead surface was validated using SEM and are shown in Fig. 9. SEM micrographs show that bacterial cells are captured by both polystyrene and dimethylamino beads (Fig. 9a–d). Since the beads are either hydrophobic (i.e., polystyrene) or anion exchanging (dimethylamino), we postulate that possible mechanisms that could explain capture could include hydrophobic or steric interactions. From the results shown in Fig. 9, it is apparent that a surface based with functionalized microbeads is effective in capturing bacterial cells either specifically or non-specifically. Specific capture is a necessary condition for detection of a target organism by an antibody previously placed on the surface. Non-specific capture is useful if detection or quantification of bacteria regardless of type is the goal.

These results show that a microfluidic device with C_{18}-derivatized surfaces may be coated with an interfacing protein such as biotinylated BSA or BSA to provide a scaffold or foundation for capturing a monolayer of functionalized microbeads. There are at least 10 manufacturers that make beads ranging from 100 nm to 3 μm in diameter and with various types of chemistry. Using the approach described in

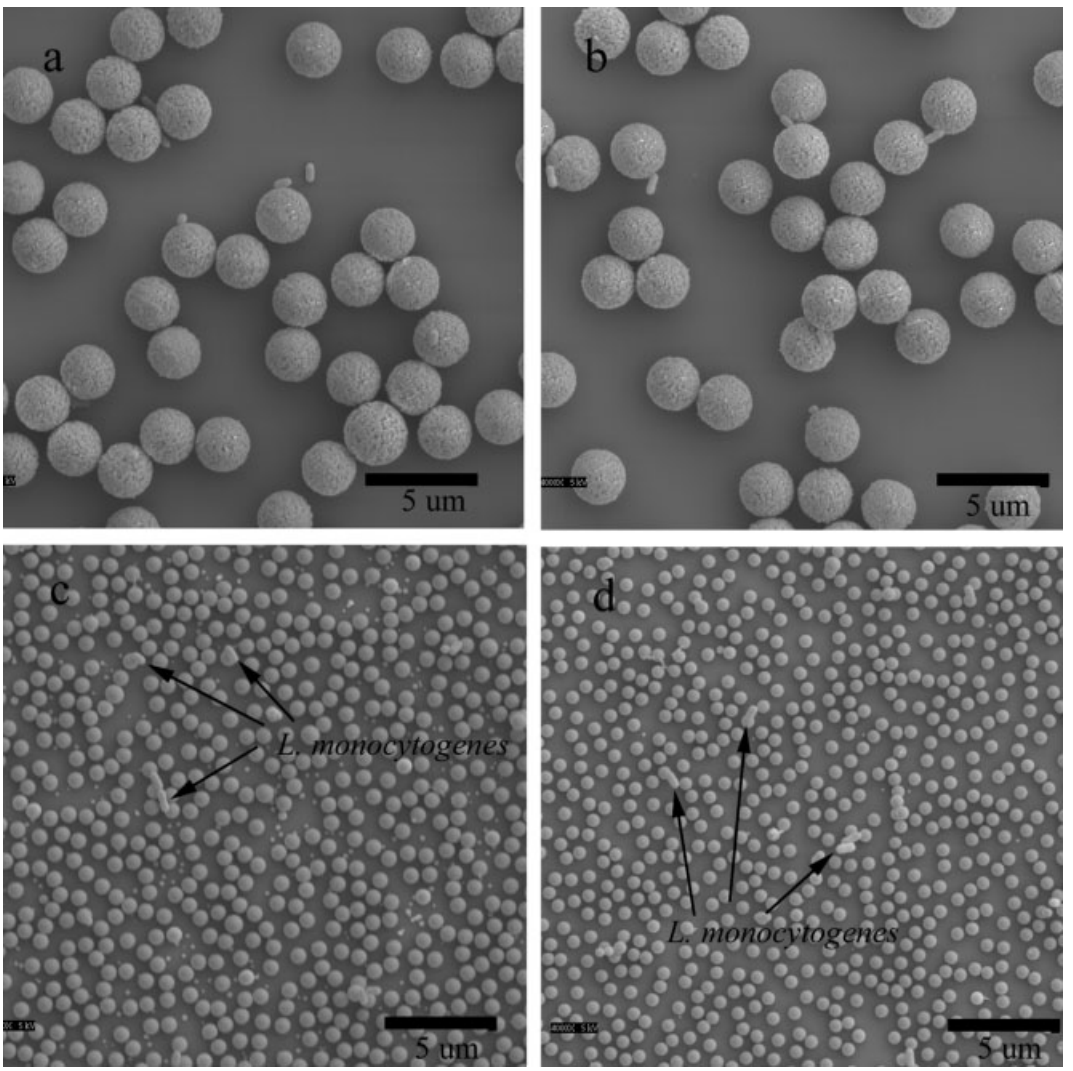

Figure 9. SEM of *L. monocytogenes* cells on (**a**) streptavidin microbeads pre-adsorbed with biotinylated anti-*Listeria* antibody; (**b**) anti-*Listeria* antibody-coated microbeads previously blocked with BSA; (**c**) polystyrene microbead surface; and (**d**) dimethylamino microbead surface. In (**c**) and (**d**), *L. monocytogenes* cells are not easily seen due to the background. Locations of some cells are revealed at the tips of the pointed arrows. (Scale bar is 5 μm.)

this paper, an array of different surfaces may be quickly prepared. The combined use of functionalized microbeads for specific capture and biotinylated BSA or BSA for blocking non-specific adsorption enables development of fully functional microfluidic devices for separation, detection, or analysis of specific biological species.

CONCLUSIONS

This paper illustrates principles for systematic design and fabrication of surfaces for the construction of microfluidic devices. A simple approach to immobilize colloidal microstructures onto a C_{18}-coated SiO_2 substrate via specific or non-specific biomediated interactions has been described herein. Streptavidin-coated microbeads (2.8 μm) formed a highly packed monolayer when self-assembled onto a C_{18} surface pre-adsorbed with biotinylated BSA. Anti-*Listeria* antibody-coated microbeads (2.8 μm), on the other hand, formed a surface with lower surface coverage when immobilized onto a bare C_{18} surface. High surface coverages were observed for both polystyrene (0.8 μm) and dimethylamino (0.8 μm) microbeads, when they were allowed to self-assemble into a monolayer on a C_{18} surface pre-adsorbed with BSA. Biotinylated anti-*Listeria* antibody, an IgG-type antibody that binds *Listeria* spp., was anchored onto the streptavidin-coated beads. Its functionality was confirmed using a fluorescently labeled anti-IgG as reporter molecules. Streptavidin microbeads pre-adsorbed with biotinylated anti-*Listeria* antibody and anti-*Listeria* antibody-coated microbeads showed specific capture of *L. monocytogenes*, while polystyrene microbeads and dimethylamino microbeads captured *E. coli* and *L. monocytogenes* non-specifically.

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