THE DETECTION OF ANTIBODIES SECRETED BY MICROFLUIDICALLY TRAPPED BIOLOGICAL CELLS *VIA* EXTRAORDINARY OPTICAL DETECTION BASED NANOSCALE IMMUNOBIOSENSING ARRAYS

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ABSTRACT

We present the trapping of 17/9 hybridoma cells, and the subsequent optical detection of their secreted antibodies, using a poly(dimethyl siloxane)-based multi-level microfluidic chip. Cells are trapped in polymer microwells. The binding of secreted antibodies to immobilized antigen is sensed *via* shifts in the surface plasmon resonance of nanohole array based immunobiosensors. With ~200 cells/trap, we find that the secreted 17/9 antibodies specifically bind to a synthetic peptide sequence derived from the Hemagglutinin A of the influenza virus, yielding a larger shift than that produced by the nonspecific binding to a 2F5 control peptide.

KEYWORDS: antibody, antibody-secreting cell, hybridoma cell, antigen, peptide, cell trapping, microwell, immunobiosensing, surface plasmon, extraordinary optical detection, nanohole array, focused ion beam

INTRODUCTION

Therapeutic monoclonal antibodies (MAbs) are a medically and economically important class of biotechnological drug [1]. MAb production usually involves isolating and screening hundreds to thousands of antibody-secreting cells (ASCs) for 4-6 weeks to identify 1-10 hybridoma cell lines producing the desired high-affinity antibodies (Abs) [2]. Hybridoma cells are produced *via* fusing human or murine B cells with myeloma cells [3] and/or transforming them with a virus [4]. The resulting cultures are tested for the Abs that bind the desired antigen (Ag) and stable ASC lines are produced from positive clones. Alternatively, ASCs can be isolated directly from lymphocytes derived from immunized human or animal blood. The genes encoding the Abs are then cloned and transferred into DNA vectors that are used to transform cultured cell lines into lines producing the said MAbs in large quantities.

We are developing a system to sense the Abs produced by each of thousands of ASCs on a single slide, to afford real-time identification of those secreting high-affinity Abs against a target Ag. We previously reported polymeric flow-through and microwell cell traps, trapping hybridoma cells into biocompatible poly(dimethyl siloxane) (PDMS) microwells, and removing extraneous cells with minimal trapped cell displacement [5]. In this paper, 17/9 MAbs secreted by trapped hybridoma cells bind to its Ag, a peptide (HA) whose sequence was derived from influenza virus hemagglutinin, as immobilized onto a immunobiosensing gold surface. This binding shifts the surface plasmon (SP) resonance (SPR) of the immunobiosensors, affording secreted MAb detection.

THEORY

Love *et al.* [2] and Muraguchi *et al.* [6] have both reported successfully trapping fluid-suspended cells, settling under the influence of gravity, using arrays of microwell cell traps. Inspired by this success, we have developed arrays of 30-200 μm diameter, 60-80 μm deep, and 3.77 mm periodic microwells inset into the surface of 400-700 μm thick PDMS films (Figure 1a) [5]. We have also developed arrays of 1 mm wide and 3.77 mm periodic square through holes perforating 150-400 μm thick PDMS films (Figure 1b). These perforated films are to be aligned atop the microwell arrays, to act as a spacer between the microwells and the immunobiosensing gold surface (Figure 1b).

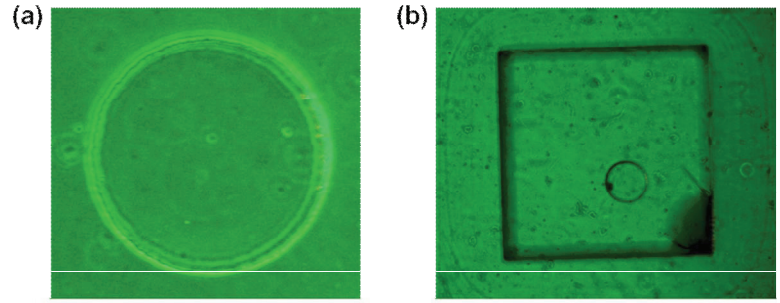

Figure 1: (a) A 200 μm diameter and 60-70 μm deep microwell inset into the surface of a ~670 μm thick PDMS film. (b) A 1-mm-x-1-mm through hole in a ~270 μm thick PDMS film is aligned atop the aforementioned microwell.

Each immunobiosensor is composed of an array of 200 nm diameter and 450 nm periodic nanoholes milled, *via* a focused ion beam, through 100 nm thick gold films adhered to 1"-x-1"-x-1-mm glass slides *via* 5 nm thick titanium films (Figure 2). These nanohole arrays produce SPs when optically excited, with a gold-media dielectric contrast sensitive SPR [7]. Ag is immobilized onto these gold surfaces (see the Experimental section). These immunobiosensing gold surfaces are then placed atop a through hole array which is itself aligned atop a microwell array, so as to be exposed to the MAbs secreted by the cells trapped within the microwells. MAb-Ag binding is detected *via* shifts in the extraordinary optical transmission (EOT) spectra through the nanohole arrays, a SPR sensing mode that facilitates miniaturization more readily than the more commonly used reflective Kretschmann mode [7].

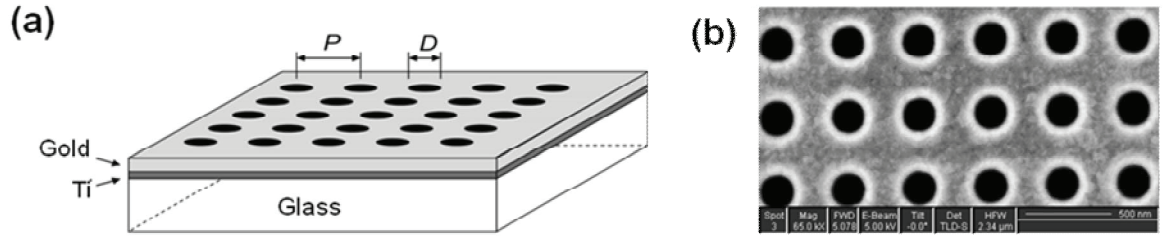

Figure 2: (a) Arrays of 200 nm diameter D and 450 nm periodic P nanoholes milled, via a focused ion beam, through a 100 nm thick gold film adhered to a 1"-x-1"-x-1-mm glass substrate via a 5 nm thick titanium adhesion layer. (b) A scanning electron micrograph of one such milled nanohole array.

EXPERIMENTAL

Milled gold surfaces were cleaned in Piranha (an aqueous solution of H_2SO_4 and H_2O_2) and then serially incubated, in ambience, for: (i) ~24 hr in 10 mM 11-mercaptoundecanoic acid (MUA); (ii) ~45 min in 75 mM 3-(*N*,*N*-dimethylamino)propyl-*N*-ethylcarbodiimide (EDC) and 15 mM *N*-hydroxysuccinimide (NHS); (iii) ~1 hr in 25 μg/mL of streptavidin (SA) in 10 mM sodium acetate (pH 5.5); (iv) ~15 min in 1M ethanolamine; and (v) ~1 hr in 25 μg/mL of biotinylated peptide Ag in pH 7.4 phosphate buffered saline (PBS) (Figure 3). Bio-HA, bearing the sequence (NH_3^+)-Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Orn(biotin) Gly Cys-($CONH_2$) and specifically binding to 17/9 MAb, was used for the sample surface (Figure 3a). Bio-2F5, derived from the HIV envelope protein gp41 subunit, bearing the sequence (NH_3^+)-Glu Gln Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp Ser Gly Lys (biotin)-Gly Cys-($CONH_2$), and specifically binding to the 2F5 MAb, was used for the Ag control surface (Figure 3b). Pre- and post-incubation EOT spectra were measured in air, following DI H_2O rinsing and N_2 drying, for each of these steps.

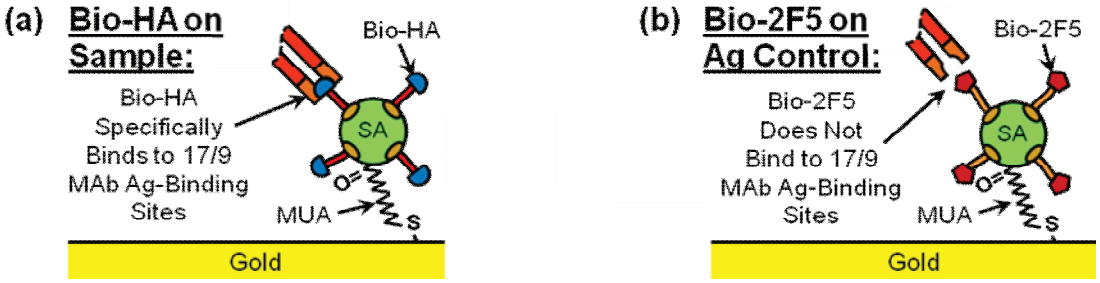

Figure 3: (a) Sample surface: MUA-gold bond via thiol, SA-MUA bond via NHS (EDC catalyzed), and Bio-HA-SA bond at 4-biotin sites. (b) Ag control surface: Bio-2F5 is used instead of Bio-HA.

PDMS films were incubated for ~24 hr at 4°C in PBS with 25% v/v fetal bovine serum to block nonspecific binding, then rinsed and sonicated in PBS. $4x10^4$ 17/9 MAb secreting hybridoma cells, suspended in Dulbecco's modified Eagle's medium, were deposited onto through holes aligned atop 200 μm wide microwells, yielding ~200 cells/trap following ~10 min of settling. The sample and Ag control surfaces were each placed atop one of these PDMS stacks and incubated for ~4 hr at 37°C in 5% CO_2. EOT spectra were then measured, following DI H_2O rinsing and N_2 drying, in air.

RESULTS AND DISCUSSION

Figure 4 shows the EOT spectrum through one of the sample surface nanohole arrays as measured in air: (a) after the initial Piranha cleaning of the sample surface; (b) after the subsequent MUA, EDC/NHS, and SA incubations; (c) after the subsequent ethanolamine and Bio-HA peptide incubations; and (d) after the final incubation aligned atop ~200 trapped hybridoma cells secreting 17/9 MAbs. (In each case, the depicted EOT spectrum is normalized to its peak value.) Each subsequent incubation resulted in the binding of additional mass near the sample surface, and thus resulted in an induced shift in the SPR of the nanohole array.

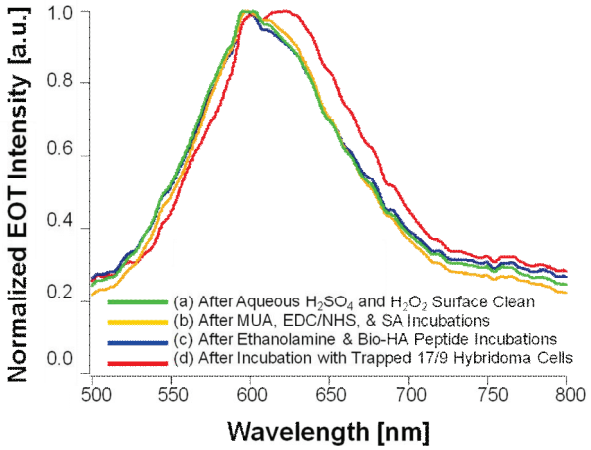

Figure 4: The EOT spectrum through one of the sample surface nanohole arrays, as measured in air, at various stages in this experiment. In each case, the EOT spectrum is normalized to its peak value.

Table 1 lists the pre- to post- secreted 17/9 MAb incubation SPR shifts for two sample surface nanohole arrays and two Ag control surface nanohole arrays (all four of which were aligned above ~200 cells/trap during the incubation). The sample surface yielded a greater bound 17/9 MAb mass, and thus a greater pre- to post- secreted 17/9 MAb incubation SPR shift, than the Ag control surface.

Table 1. Pre- to post- secreted 17/9 MAb incubation SPR shifts, as measured in air

Surface	Ag Layer	No. of Nanohole Arrays Probed	SPR Shift (± Expt. Error) [nm]
Sample	Bio-HA	2	20 ± 3
Ag control	Bio-2F5	2	10 ± 1

CONCLUSIONS

This work indicates that the Abs secreted by ~200 cells/trap can be detected, *via* the SPR shift induced by the Ab-Ag binding; confirmation of this effect will require further replications of this experiment. With further refinement, this system may detect the Ag-specific binding by Abs secreted by thousands of individually trapped ASCs on a single slide.

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