Detection of One-Bead-One-Compound (OBOC) Combinatorial Library Using Oblique Incidence Reflectivity Difference (OI-RD) Microscopy

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Abstract

The utilization of an Oblique-Incidence Reflectivity Difference (OI-RD) microscope to investigate a One-Bead-One-Compound (OBOC) combinatorial chemical library is described. A six thousand random small molecule library was previously prepared for proof-of-principle experiments. This paper describes the initial results of these experiments.

I. Introduction

Detection of biomolecules has been of interest in the biological and life sciences community. The Oblique-Incidence Reflectivity Difference (OI-RD) microscope is a good candidate for measuring and detecting biomolecules and biomolecular reactions in microarrays because of its high-throughput capabilities, real-time measurements, and label-free detection.

Microarrays are composed of ~100 μm diameter spots of immobilized biomolecules that are arranged in a regular pattern [1]. Oblique-Incidence Reflectivity Difference is a type of ellipsometry that scans microarrays and measures a change in polarization due to a reflection from a microarray spot. Using OI-RD microscopy, biomolecular reactions can be observed.

The goal of this experiment is to use OI-RD microscopy to examine a random library consisting of six thousand small molecules, namely, a one-bead-one-compound (OBOC) combinatorial library. The OBOC combinatorial library method synthesizes millions of random molecules such that each bead displays unique compounds. It has been developed to study small molecules that specifically react with cellular proteins [2]. This library is a new platform that is fast to produce and ideal for high-throughput screening. We intend to show that OI-RD is an efficient method for screening such a library.

II. Optical Setup

The optical setup of the OI-RD microscope is shown in Figure 1. An s-polarized Nd:YAG laser (wavelength $\lambda = 532$ nm) passes through a photoelastic modulator which causes the light to oscillate between s-polarization and p-polarization at a frequency of 50 kHz. The laser then passes through a phase shifter, which introduces an adjustable phase between s-polarization and p-polarization. This adjustable phase is used to zero the signal from the bare glass slide. The light then passes through an F-theta lens, and then reflects off of the microarray at an oblique angle of incidence theta. The laser then passes through an objective and an analyzer. A photodiode detector measures the resulting oscillating transmitted intensity. The first harmonic component of the intensity is measured with a digital lock-in amplifier. At oblique incidence angles, s-polarization and p-polarization reflectivities change disproportionately due to the presence of a thin film. The OI-RD signal is proportional to d/λ, where d is the thickness of the spot and λ is the wavelength of the laser. The thickness of the spot increases when the immobilized target biomolecules react with solution phase probe molecules, yielding a measurable change in the OI-RD signal [3].

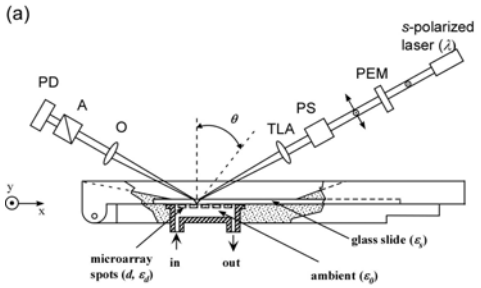


FIG 1: OI-RD microscope for microarray imaging. PEM: Photoelastic Modulator, PS: Phase Shifter, TLA: F-Theta Lens Assembly; O: Objective Lens, A: Analyzer, PD: Photodiode.

III. Methods and Materials

Figure 2(a) shows a schematic of a Tentagel bead and its compounds synthesized using the one-bead-one-compound combinatorial library method. A 100μm diameter polymer bead is conjugated to three random small molecules (R_1, R_2, R_3) via a molecular scaffold. The molecular scaffold is composed of a backbone section, on which R_1, R_2, and R_3 are synthesized. A linker section separates the backbone from additional functional groups used to attach the compounds to surfaces. A cleavable disulfide bond is used to attach the scaffold to the bead [2]. After cleaving the disulfide bond, the biotin group is used to immobilize the scaffold on a streptavidin coated glass slide.

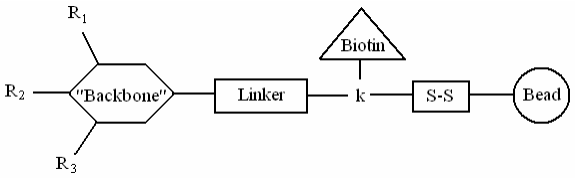


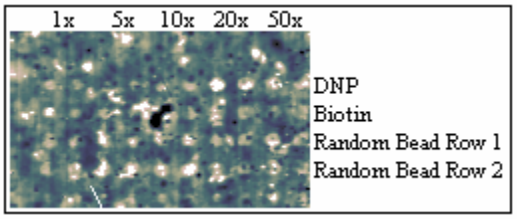
FIG 2: Schematic of a bead and its compounds.

A six thousand random sample OBOC library was prepared. However, before scanning the entire library, controlled experiments had to be conducted to ensure that the library synthesis procedure was successful and to determine optimal conditions for printing the small molecules. Tentagel beads soaked in ethanol were placed in a petri dish, and single beads were manually transferred into wells of a 384-well microplate using an electric pipettor and a stereo microscope; 20μL of ethanol were picked up by the pipettor and placed into the wells. Control beads containing DNP (Dinitrophenol) and biotin instead of the scaffold structure were also included. After the ethanol evaporated, 10 μL of TCEP (Tris[2-carboxyethyl] phosphine) was pipetted into the wells containing the beads. The TCEP cleaved the disulfide bonds between the scaffold and the polymer bead. The beads were left in the TCEP solution for sixteen hours before printing. Microarrays were printed using a split-pin contact printing robot (Omnigrid 3000). The deposited compounds are immobilized by the biotin group binding to the streptavidin coating on the glass slide. Before scanning, the microarrays were washed to remove excess un-immobilized compounds from the spots.

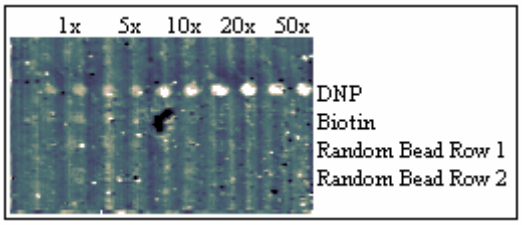
IV. Results

Figure 3(a) shows the image of the microarray before reaction. A titration series of 1x, 5x, 10x, 20x and 50x of TCEP was used. 1x of TCEP signifies one TCEP molecule for every small molecule present. There are 1 x 10^{12} small molecules on each bead. The first and second rows consist of DNP and biotin, respectively. The third and fourth row, labeled Random Bead Row 1 and Random Bead Row 2, respectively, are different molecules taken from the random OBOC combinatorial library. The TCEP solution from each well was printed twice; therefore there are two spots for 1x DNP and so on. Figure 3(b) shows that change in OI-RD signal due to reacting the microarray with 0.011 μM anti-DNP antibody. Figure 3(c) shows the change due a second reaction of the same microarray with 0.045 μM streptavidin. In the first reaction, only DNP reacted with the anti-DNP. Thus anti-DNP and DNP reacted specifically, demonstrating that the cleavage procedure has successfully released the compound from the beads. In the second reaction, all of the samples, except the previously reacted DNP, reacted with streptavidin.

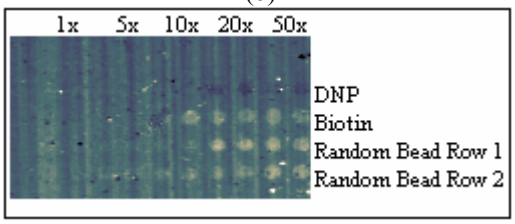
To understand why streptavidin reacted with all of the microarray spots except DNP, we reacted a second microarray from the same printing batch with streptavidin. Figure 4 shows the change due to the streptavidin reaction. It is clear that all of the samples, including DNP, reacted with streptavidin. We interpret these results as follows: For some reason, streptavidin binds to all the compounds cleaved from the beads. However, when the DNP spots are initially reacted with anti-DNP, the larger (150 kDa) antibody molecules prevent the smaller streptavidin molecules (55 kDa) from accessing the binding sites on the spot.



(a)



(b)



(c)

FIG 3: Microarray of two controlled beads, namely, DNP and Biotin, and ten random beads from the OBOC random library before and after reaction. The TCEP titration series increases from left to right. Two microarray spots were printed from every well.

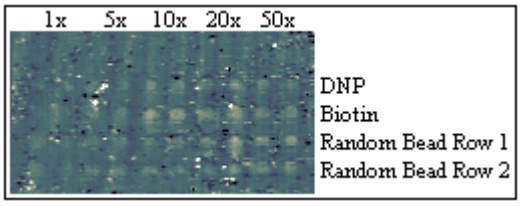


FIG 4: Microarray of two controlled beads, namely, DNP and Biotin, and ten random beads from the OBOC random library after reaction with streptavidin. The TCEP titration series increases from left to right. Two microarray spots were printed from every well.

From figures 3 and 4, we hypothesize that streptavidin may have bound to the microarray spots because of two reasons: 1) a nonspecific reaction with the scaffold, or 2) a specific reaction with free biotin. As seen in the figure 3(a), a reaction between an antibody and its antigenic spot is specific. Anti-DNP, an antibody, is specific to its antigen, DNP.

We reacted the streptavidin coated slide with a 0.07 μM anti-biotin antibody. All of the samples reacted with the anti-biotin antibody (Figure 5), confirming the presence of free-biotin in all of the spots.

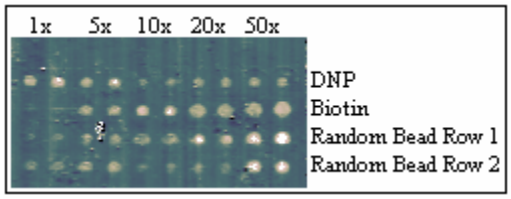


FIG 5: Microarray of two controlled beads, namely DNP and Biotin, and ten random beads from the OBOC random library before and after reaction with Anti-Biotin.

V. Discussion

There are three possibilities that can explain the free biotin on the glass surface. The first possibility concerns the formation of disulfide bonds. After the TCEP cleaves the disulfide bonds, the sulfhydryls bind to each other, producing disulfide bonds once more. This would cause free biotin on the surface, as seen in Figure 6.

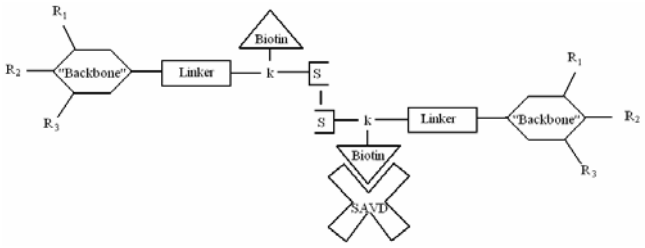
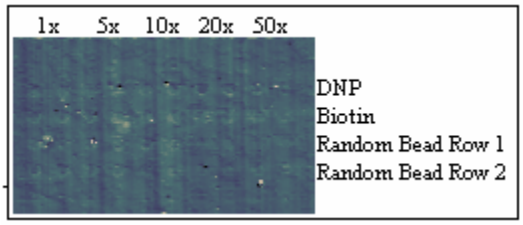
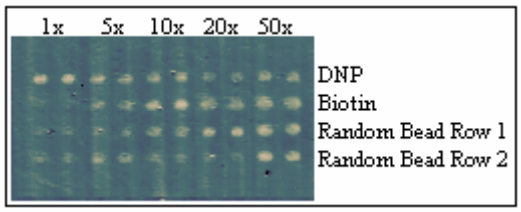


FIG 6: Schematic of disulfide bonds forming after cleaving.

To verify if the free biotin is due to disulfide bonds, we reacted a microarray from the same printing batch as before with TCEP first, then with anti-biotin. As seen in Figure 7(a), the TCEP did not cleave any of the presumable disulfide bonds. When 0.07 μM anti-biotin was flowed in, all of the spots reacted as see in Figure 7(b). This result nullifies our previous hypothesis concerning disulfide bonds.



(a)



(b)

FIG 7: Reaction of TCEP then of Anti-Biotin. TCEP does not react with the microarray.

The binding of the backbones contained in the scaffolding is another scenario in which free biotin would occur. Figure 8 shows a schematic of this.

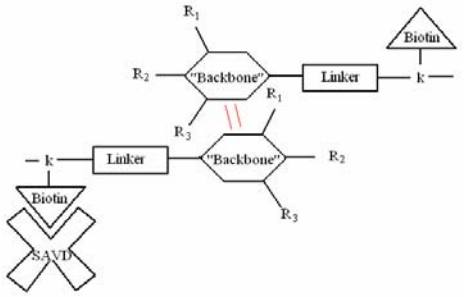


FIG 8: Free biotin due to backbone binding. The double red line signifies a type of binding, although unknown under which mechanisms.

Besides backbone binding and disulfide bonds, there is a third case wherein free biotin is formed, particularly, compounds binding to each other. Figure 9 shows only one example of this case, for there are many different permutations.

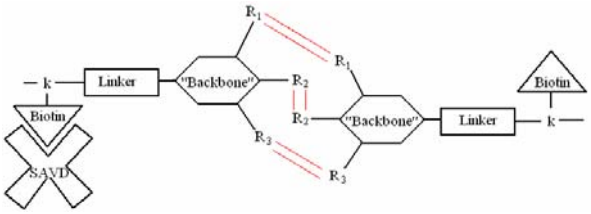


FIG 9: Free biotin due to compound binding. This only shows one permutation.

Although the mechanism which forms the free biotin is unknown, we know that the free biotin is not detrimental to our experiments; it will, in actuality, aid us in detecting the small molecules. Because the small molecules are so minuscule, it is hard to determine if they have successfully printed. By utilizing the free biotin, we will be able to react a slide with anti-biotin antibody, therefore, enabling us to see if the small molecules, conjugated to biotin, are present.

VI. Future Work

To understand the mechanism behind the free biotin, we will take ten known beads whose compounds do not bind to one another. Experiments with these ten known beads will dismiss the hypothesis concerning compound binding. In addition to the ten beads, five known beads with an extra linker will also be examined. The additional linker will be used for mass spectrometry measurements. These experiments will ultimately lead us to scanning the six thousand sample OBOC combinatorial library.

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Referneces:

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