[Frontiers in Bioscience 12, 3956-3964, May 1, 2007]

Surface chemistries for antibody microarrays

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1. ABSTRACT

Enzyme-linked immunosorbent assay (ELISA) microarrays promise to be a powerful tool for the detection of disease biomarkers. The original technology for printing ELISA microarray chips and capturing antibodies on slides was derived from the DNA microarray field. However, due to the need to maintain antibody structure and function when immobilized, surface chemistries used for DNA microarrays are not always appropriate for ELISA microarrays. In order to identify better surface chemistries for antibody capture, a number of commercial companies and academic research groups have developed new slide types that could improve antibody function in microarray applications. In this review we compare and contrast the commercially available slide chemistries, as well as highlight some promising recent advances in the field.

2. INTRODUCTION

It has recently been shown that protein profiling can be used for the diagnosis of complex diseases, such as cancer (1, 2). A properly designed assay could allow for the early detection of cancer, ultimately leading to an increase in survival rate. Enzyme-linked immunosorbent assays (ELISA) performed in a 96-well plate capture the target protein via a surface immobilized antibody and use a second labeled antibody, specific for a different epitope, for protein detection. While these sandwich assays are capable of high throughput and reproducible detection of proteins or analytes, they are not able to efficiently screen large numbers of proteins. In addition, large sample volumes are required when there are multiple proteins of interest. Clearly a high throughput, reproducible, and sensitive multiplexed assay is needed for the efficient diagnosis of complex diseases.

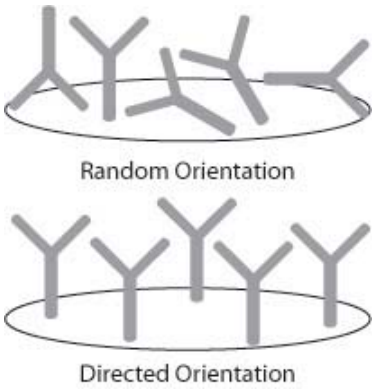

Figure 1. Schematic representation of random and oriented antibody immobilization.

ELISA microarrays have the potential to fill this need (3). ELISA microarrays are capable of simultaneously screening 10 to 50 proteins within a single sample, and only require a small sample volume (20-50 µL of diluted sample per chip). In addition, the theoretical detection limit for microarrays is significantly lower than that for a 96-well plate assay (4). There has been a great deal of research focused on the development of ELISA microarrays, and while they have yet to achieve the low sensitivities calculated theoretically, they have been shown to be useful for rapidly screening many proteins using only small sample volumes (3, 5-9). One reason that ELISA microarrays have not been able to match theoretical predictions is that current experimental protocols are a compilation of DNA microarray and 96-well plate ELISA protocols, and have not been fully optimized for antibody microarrays (3). Some general areas of concern include the development of assay conditions and sample preparation for use with multiple assays (10), the length of incubation required to reach equilibrium (11-13), reducing the amount of cross-reactivity and non-specific binding (14), and attachment of antibodies to a solid substrate (15-18).

In this review we discuss the immobilization of antibodies on a solid substrate without loss of activity for ELISA microarrays. Proteins are structurally more complex molecules than DNA, and can unfold and lose activity when immobilized on a solid substrate due to hydrophobic or ionic interactions with the surface. There is also potential for proteins to denature during the drying process. Unlike a 96-well plate ELISA, the capture antibody for a microarray ELISA is printed at low volume (0.3 to 1 nL). The capture antibody spots dry quite rapidly due to the low print volume, and long-term storage conditions typically require the chip to be dry. While antibodies are more stable than most proteins, there is still potential for a loss of activity upon drying and storage.

Even if the natural conformation of the antibody is retained, it seems likely that a portion of the antigen binding sites are not accessible after immobilization. Many slide chemistries allow antibodies to randomly immobilize on the surface, leaving the antigen binding regions of some antibodies directly attached to the glass surface and inaccessible (Figure 1). In addition, steric hindrance caused by tight packing of immobilized antibodies can lead to a decrease in activity. Therefore, alternative slide chemistries have been developed that allow for the directed orientation of immobilized antibodies via a unique attachment site, as well as regular spacing to reduce steric hindrance. Furthermore, it is possible to engineer recombinant antibodies and antibody fragments to include a unique tag for oriented immobilization on the slide. Although conceptually these modified surfaces should prove superior to simpler binding chemistries, studies by us and others have indicated these more complex surfaces may have problems of their own (discussed below).

The ideal surface for antibody immobilization must be optimized based on many parameters associated with good performance of ELISA microarrays. That is, the suitability of the surface needs to be assessed in terms of spot size and morphology, and total antibody binding as well as by a comprehensive assay evaluation, including background signal, lower limit of detection, dynamic range and reproducibility. Thus, the criteria for a good solid support for antibodies include: (i) high binding capacity, (ii) an ability to retain activity, (iii) low variability between slides, (iv) high signal-to-noise ratios, and (v) a long shelf-life. There are a number of slides with a variety of surface modifications and chemistries available commercially. Unfortunately, it is not possible to compare studies from different laboratories due to differences in experimental protocols and antibodies. This review includes a detailed discussion of the different slide types commercially available, recent advances in antibody surfaces that appear promising, and insights from our own systematic testing of a variety of different slide types.

3. ANTIBODY IMMOBILIZATION

There are three general categories of immobilization chemistries whereby antibodies are attached to glass slides: (i) physical adsorption, (ii) covalent attachment via reactive groups, and (iii) affinity-based interactions between functional groups on the slide and the antibody. Specific surface chemistries and reaction sites associated with each of these categories are listed in Table 1. Following is a thorough discussion of each of these categories.

3.1. Physical Adsorption

Physical adsorption of proteins occurs via hydrophobic or ionic interactions between the protein and the slide surface. While this is the simplest immobilization technique, it is not easily controlled and may result in high variability, both between spots on the same slide and between slides. Some commonly used slide coatings that can be used to physically adsorb antibodies include agarose (19), polyacrylamide (20), nitrocellulose (21), poly-L-lysine (22), or aminosilane (16) (Table 1). Since the antibodies are not permanently attached to the surface, they are susceptible to loss or exchange with other proteins during slide processing. Exchange of reagents can potentially lead to irreproducible results, higher background levels, and lower assay sensitivities.

Table 1. Slide chemistries available for antibody immobilization

Immobilization Chemistry	Surface Chemistry	Attachment Site	Advantages[1]	Disadvantages[1]
Adsorption	Agarose	Electrostatic Interactions Hydrogen Binding Hydrophobic Interactions	Simple immobilization	Random orientation. High background.
	Polyacrylamide			
	Nitrocellulose			
	Poly-L-lysine			
	Amino			
Covalent Binding	Maleimide	Thiol	Simple immobilization Stable binding of antibody	Possible pretreatment of antibody required. Potentially random orientation.
	Hydrazine	Carbohydrate		
	Succinimidyl ester	Primary Amine		
	Epoxide			
	Aldehyde			
Affinity Based	Protein A or G	Fc region	Directed orientation	Specific for certain antibody classes and species. Antibody migration. Low specificity.
	Streptavidin	Biotin		
	Cellulose	Carbohydrate binding molecule		
	Nickel	Histidine tag		
	Nickel-nitrilotriacetic acid			
	Copper			
	Glutathione	GST tag		
	Tag-specific antibody	His, HSV, Myc, and others		

[1] The major advantages and disadvantages may vary depending on specific application.

3.2. Covalent Binding

In order to prevent loss of antibody and protein exchange during processing, antibodies can be permanently attached to the surface through covalent bonds with functional groups on the antibody. Some common functional groups include primary amines in lysines or arginines (16, 18, 23, 24), reactive thiols in the cysteines in the hinge region (25, 26), or carbohydrates linked to the H2 domains of the constant (Fc) region (27) (see Table 1 for more detail). Although attachment through thiols or carbohydrates allows for directed orientation of antibodies, the protocol for attachment is more complex. Specifically, the disulfide bonds must be reduced or the carbohydrate groups must be oxidized prior to attachment to the surfaces reactive towards these groups. These redox reactions can destabilize the antibody structure and decrease activity and may require additional purification steps. Our experiences with these redox binding chemistries suggest that they should be avoided.

The most commonly used chemistries for covalent immobilization of antibodies are epoxides (16, 18), aldehydes (24), and *N*-hydroxy succinimidyl esters (NHS esters) (23, 28), all of which are reactive towards primary amines on the protein surface. Figures 2a and 2b show schematics of epoxide and NHS ester chemistries. While epoxides and aldehydes are relatively stable under standard storage conditions, NHS esters are extremely susceptible to hydrolysis and have short shelf lives unless stored under dry, cool conditions. Slides with NHS ester functionality can be made by treating an amine-terminated slide with a homobifunctional linker, such as disuccinimidyl suberate or bis(sulfosuccinimidyl)suberate (BS^3), or treating a carboxy-terminated slide with NHS and 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide. In our experience, aminosilanated slides treated with BS^3 are relatively stable for about 12 hours (in our hands this is long enough to print 24+ slides with 16 chips per slide).

While attachment through primary amines overcomes the issues of antibody loss and protein exchange, there are still problems to be addressed. Since most proteins have a large number of lysines and/or arginines on the surface, multiple attachment sites are available. Attachment to the slide at multiple sites is more likely to lead to a loss of activity due to inactivation of antigen binding site. In addition, primary amines are randomly located on the surface of the protein and possibly in the antigen-binding region, so the final immobilized orientation of the antibody is unknown (Figure 1). This can result in decreased antibody activity since not all antigen-binding regions are accessible by antigen due to steric hindrance.

3.3 Affinity-Based Immobilization

The immobilization of antibodies through affinity-based interactions typically utilizes a unique functional group or protein sequence on the antibody, resulting in orientation of the antigen-binding sites (see Figure 2c for schematic). Some current techniques used for affinity-based immobilization of antibodies are (i) protein A or G coated slides, which have a high affinity for the Fc region of antibodies (17, 29) or (ii) affinity slides that are specific for a unique tag in the antibody (30-36) (see Table 1 for more detail). Immobilization via an Fc specific antibody is attractive because commercially available monoclonal antibodies can be used without any further processing. However, proteins A and G are specific for only certain IgG subclasses and can not be used universally with all monoclonal antibodies. In addition, the affinity of protein A or G varies with respect to antibody species as well as with buffer conditions. Therefore, it is not possible to use protein A or G to immobilize all antibodies under all conditions; each must be individually tested for the stability of the interaction under the proper experimental conditions. For sandwich ELISA, another concern is that the detection antibody will bind to the protein A or G-coated slide, potentially leading to high background.

In the case of recombinant antibodies or antibody fragments, it is relatively simple to add specific tags such as biotin, 6X histidine (His), myc epitope, glutathione-S-transferase (GST), carbohydrate binding molecule (CBM), and many others. These tags can be bound to the slide with

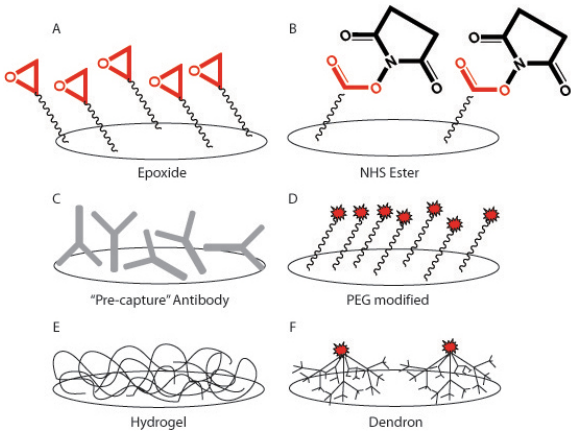

Figure 2. Schematic representation of select slide coatings. A, epoxide chemistry; B, NHS-ester chemistry; C, pre-capture antibody surface; D, PEG-modified surface; E, hydrogel; F, dendron surface. Red areas represent sites for covalent attachment to the slide.

a "pre-capture" molecule that binds to the recombinant antibody (which is usually referred to as the "capture" agent). The whole slide can be coated with the pre-capture agent, but we have found that it is also possible to print the pre-capture agent first and then overlay the capture antibody. This process conserves valuable antibodies and other reagents. The use of a non-contact printer for overlay printing is preferable because there is no risk of sample cross-contamination or marring of the pre-capture spot. Although using pre-capture reagents can be more complicated, there are several advantages to this approach. First, the capture antibody is properly oriented. In addition, immobilization via an affinity tag also allows for the spotting of antibodies without any prior purification since only proteins containing the affinity tag will be captured. Finally, a pre-capture agent such as a protein can serve as a barrier between the capture antibody and the glass surface. We have found that scFv that had virtually no activity when bound directly to a glass slide performed as well as commercial monoclonal antibodies when pre-captured by an anti-epitope antibody (Seurynck-Servoss, Zangar, Rodland and Baird, unpublished).

Similarly to the capture antibody, it is important that the binding region of the pre-capture molecule is completely available for interaction with the capture antibody. This is crucial when the pre-capture molecule is a large protein such as an antibody and the binding region can easily be hidden from potential interactions. A decrease in binding efficiency of the capture antibody has previously been observed when a pre-capture molecule has been utilized (37). However, this issue is circumvented when small molecules are used for the affinity interactions since there are a proportionately large number of pre-capture molecules on the slide in the space that would be occupied by antibody. Some examples include Ni- (38), Ni-NTA- (39), or copper-coated slides (30) for the capture of His-tagged antibodies, cellulose-coated slides for the immobilization of CBM-containing antibodies (34), or glutathione-coated slides that are specific for GST-tagged proteins (32).

The streptavidin-biotin interaction has a very high affinity, and studies have shown that immobilization of antibodies via the streptavidin- or avidin-biotin interaction can result in highly sensitive assays (31). However, it is necessary to use biotinylated antibodies for capture on streptavidin- or avidin-coated slides. While biotin can be chemically added or included in recombinant antibodies, these processes can be inefficient, greatly increasing the cost of reagents. In addition, many detection systems include the use of biotinylated antibodies, which would also be captured on the slide. This could lead to higher background levels and low sensitivity and specificity. Another similar immobilization strategy utilizes CBM-tagged proteins and cellulose-coated slides (34), which resulted in higher signal intensity and better spot morphology than slides that rely on covalent binding or physical adsorption, and lower background signal than nitrocellulose slides.

Immobilization of antibodies containing a His tag can be achieved using nickel (Ni)-coated slides (38), Ni-nitrilotriacetic acid (NTA)-coated slides (39), copper-coated slides (30), or using an antibody specific for the His tag (36). However, these interactions are not particularly strong and can result in low levels of antibody binding. By engineering a single-chain antibody (scFv) to contain two His tags, Steinhauer *et al.* (35) nearly doubled the binding efficiency on Ni-NTA-coated slides. However, since antibody activity assays were not performed on a microarray, it is not clear what the overall affect of the double His tag is on assay parameters such as sensitivity and dynamic range. In other studies, the immobilization of scFv using antibodies specific for either the His or Myc tags resulted in picomolar sensitivities (36). Our own tests with scFv that contained a single His tag found that pre-capture antibodies against the His tag were much less useful than pre-capture antibodies against an HSV epitope. Specifically, the spots had an irregular shape and the binding efficiency of scFv was low.

A disadvantage to affinity-based immobilization is the potential for migration of antibody on the slide or between spots, especially during the long sample incubations required for high sensitivity (greater than 12 hours) (11, 13). This can lead to high background signal and false positives, particularly when antibodies migrate to spots intended to have a different specificity. The targeted affinity interactions are generally weak, leading to relatively quick detachment from the slide. There is also a higher potential for nonspecific binding, especially when the slide is coated with the pre-capture molecule rather than pre-arrayed.

4. OPTIMIZATIONS FOR ANTIBODY MICROARRAYS

The above section describes different chemistries used to immobilize antibodies on a solid surface. Some issues that arise when considering slide chemistries

Table 2. Summary of general surface and slide types and some examples of surface coatings and modifications.

Surface Type	Slide Type	Surface Coating	Advantages[1]	Disadvantages[1]
One-Dimensional	Erie HD	Hydrophobic surface	Small spot size	Potential denaturation of protein. High background.
	Erie ES	Enhanced surface		
Two-Dimensional	PEGylated	PEG	Stable immobilization Protein in natural conformation	
Three-Dimensional	Agarose	Agarose	High binding capacity Protein in natural conformation	Random orientation. Potential for high background.
	Perkin Elmer hydrogel	Polyacrylamide		
	FAST	Nitrocellulose		
	Full Moon Protein	Proprietary polymer		
	Amine Reactive Hydrogel[2]	DMA, NAS, MAPS copolymer		
	Sugar Hydrogel[2]	Galactoside polyacrylate		
	Dendrimer	Dendrimer		
	NSB Dedron	Dendron		
	Macroporous slide[2]	None or nitrocellulose		

[1] The major advantages and disadvantages may vary depending on specific application. [2] Slides not available commercially.

are antibody orientation, binding density, and binding affinity. In some cases the optimization of these properties will result in good antibody activity. However it is also possible for the antibody activity to decrease as these properties are optimized. A great deal of research has focused on further optimization of slide surfaces for antibody microarrays taking into account both the immobilization strategy as well as the final antibody conformation. In particular, the coating material and three-dimensional architecture of the slide surface has been altered with the goal of optimizing key microarray properties including spot size and morphology, antibody structure and activity, and binding surface area. There are a number of slides commercially available and under current investigation that aim to address these issues. Table 2 provides a list of surface types and examples of slide types and surface coatings. A more detailed discussion of a subset of these slide chemistries is included below.

4.1. Spot Size and Morphology

Erie Scientific offers several slide types designed to decrease the spot size, and thereby increase the number of spots per slide, as well as improving the spot morphology. One slide type, Erie "HD", has a highly hydrophobic surface. However, we have found that these slides are not optimal for antibody immobilization, possibly since spotting on hydrophobic surfaces results in a loss of antibody structure and activity as well as higher nonspecific protein binding (17). An alternate slide modification, Erie "ES", utilizes a roughened surface to decrease spot size and maintain uniform shape, while retaining the hydrophilic nature of the slide coating. ES-epoxysilane slides have shown good assay sensitivity, however the background signal tends to be high (18). In our lab, the ES slides were found to result in smaller spot size than the same binding chemistry with no surface modification, but also increased background in many cases. Both HD and ES slide modifications are available with multiple surface coatings, including poly-L-lysine, aminosilane, epoxysilane, and aldehyde.

4.2. Antibody Structure and Activity

The secondary structure of the immobilized antibody must remain intact in order to retain full activity. However, proteins tend to unfold and lose activity on plain glass surfaces. Therefore, a great deal of research has focused on the use of surface coatings that act as a buffer between the immobilized antibody and the glass to maintain antibody structure and activity. The use of a slide coating such as poly(ethylene glycol) (PEG), self-assembling monolayers, or other polymers has shown promising results.

Because of its antifouling properties, PEG is an ideal coating for microarray surfaces. See Figure 2d for a schematic of a PEG coating. The ability of PEG to repel proteins leads to low nonspecific binding (40), but still allows for immobilization of antibodies without disruption of the native conformation, resulting in antibody activity similar to that observed in solution-phase. PEG can also be modified to include reactive groups for covalent binding of antibodies, such as NHS esters or epoxides. The use of PEG-modified slides has resulted in higher signal intensities and lower overall background (30, 41, 42). In addition, Kusnezow *et al.* (17) found that PEG-coated slides resulted in more efficient binding of large analytes.

Zhou *et al.* (43) recently reported the use of slides with a thin-film polymer coating for the immobilization of antibodies. This slide coating is based on the self-assembly of polyelectrolyte multilayered thin films. These films allow the antibody to retain its natural conformation, while also increasing the surface area available for binding. When compared to aldehyde and poly-L-lysine slides, polyelectrolyte-coated slides showed a 5- to 10-fold improvement in the lower limit of detection, as well as a 5-fold wider dynamic range.

4.3. Binding Surface Area

In addition to retaining antibody structure, a coating can also serve to increase the surface area available for antibody binding. This may increase total antibody binding and provide a structural support that reduces protein denaturation when the slides are dried. A number of hydrogel surfaces have been developed that produce higher maximal signal intensities, presumably due to higher antibody binding (see Figure 2e for a schematic of a hydrogel coating). Hydrogels are a three-dimensional slide coating designed to protect proteins from structural changes during the drying process and increase the binding capacity. These surfaces contain pores that are large enough to accommodate an antibody, but may also restrict diffusion. The slow diffusion of reagents through the hydrogel can result in poor assay reproducibility, more

difficulty detecting low levels of protein, and higher background signal (17). Some commercially available three-dimensional surfaces include Perkin Elmer hydrogels, FAST slides, Full Moon protein slides, and a variety of dendrimer or dendron coated slides. While the first two slide types rely on physical adsorption of spotted antibody, Full Moon slides are coated with a proprietary polymer containing covalent binding sites (aldehyde, epoxide, amine, and others). These slides take advantage of the higher binding surface area, but include reactive sites to prevent the loss or exchange of immobilized antibody. Based on our experience, hydrogels that rely on physical adsorption perform poorly in ELISA microarrays analyses.

In addition to the commercially available hydrogel slides, many researchers have been working on alternate polymer coatings. Cretich *et al.* (44) have investigated a surface coating comprising a copolymer of *N*,*N*-dimethylacrylamide, *N*,*N*-acryloyloxysuccinimide, and [3-(methacryloyl-oxy)propyl]trimethoxysilyl. Reactive NHS esters present in the polymer chain are available for covalent binding, and are spaced such that each antibody is attached at only one or two residues. Slides coated with this copolymer resulted in assay sensitivities similar to commercially available polymer coated slides, but with decreased background signal. Goldman *et al.* (45) have used a hydrogel composed of galactoside polyacrylate, a hydrophilic polymer with ordered sugar repeats. Due to larger pore sizes, these galactoside-modified hydrogels should have fewer diffusional limitations than polyacrylamide hydrogels. This will facilitate migration of reagents in and out of the gel, resulting in low background and high assay sensitivity. In addition, proteins are covalently immobilized to the slide surface via NHS esters. Comparison with a commercially available hydrogel showed higher protein binding and better reproducibility. Unfortunately, assays to assess antibody function were not performed.

An alternate to polymer coatings is dendrimers, which are highly branched polymers that form spherical structures that are similar in size to proteins. Unlike standard polymers, dendrimers do not entangle and have a large number of chain ends available that can be functionalized with various reactive groups for immobilization of antibodies. Much like PEG-coated slides or hydrogels, dendrimers provide a protective layer that should help maintain the native conformation and functionality of the immobilized antibody, as well as increasing the binding surface area. Dendrimers functionalized with NHS esters show a high binding capacity for streptavidin and good spot reproducibility (46). Antibody microarrays spotted on slides coated with epoxy-functionalized dendrimers show promise for the detection of low abundance proteins, however the dynamic range was low due to signal saturation at a lower antigen concentration (16).

Another promising surface chemistry is dendrons, which are essentially half of a dendrimer. Dendrons can be synthesized with only one reactive group per molecule, which allows for regular spacing of the reactive groups across the glass surface (Figure 2f). Since the size of the dendron is controllable, an antibody can potentially be cross-linked at only one or two residues (47). While these slides have been shown to work well for DNA microarray assays (48-51), we know of no reports on the utility of dendrons for antibody microarrays.

Finally, it is also possible to induce higher antibody binding by altering the three-dimensional structure of the glass slide to include pores large enough to accommodate antibodies. Silicon macroporous slides used to perform an immunoassay showed reproducible results and promise to be useful for the detection of cancer biomarkers (52, 53). Steinhauer *et al.* (54) repeated similar studies, but compared varying pore sizes as well as differing amounts of a nitrocellulose coating. In this study, a macroporous surface with a nitrocellulose coating was shown to have superior activity in terms of spot morphology, binding capacity, and assay sensitivity as compared to commercially available silane, nitrocellulose, and hydrogel slides.

4.4. Experimental Design

A number of different research groups are working towards the optimization of slide chemistry for the immobilization of antibodies. Still, it remains unclear which chemistry is best for protein immobilization. It is clear, however, that experimental comparisons of different slide chemistries must be carefully designed. Specifically, a large number of slide types should be tested in a single experiment rather than over several days. In addition, it is important to compare many different assays. Not all capture reagents are affected in a similar manner by slide chemistry. As shown in Figure 3, the standard curve for the soluble form of the Her2 receptor was clearly influenced by different slide chemistries but the curves for hepatocyte growth factor (HGF) varied only slightly. Therefore, it is clear that studies conducted with a single assay can lead to conclusions that are not generally applicable. Future studies should be planned taking these points into consideration.

5. PERSPECTIVE

There are a number of criteria that need to be investigated in order to optimize the output from antibody microarrays, including (i) tight antibody binding to slide, (ii) proper orientation of antibody, (iii) retention of natural conformation and antigen binding activity, (iv) assay sensitivity/background signal, (v) antibody stability, (vi) accessibility to antigen. To date, there is no one slide chemistry that is able to successfully address all of these issues. For example, covalent binding chemistries can be used to attain tight antibody binding to the slide, but result in random orientation of the antibodies and inaccessibility of some antigen-binding sites due to steric hindrances. Affinity-based immobilization results in properly oriented antibody and accessibility to the antigen binding region, however assay sensitivity is not good. Hydrogel coatings allow for the immobilization of antibodies in their natural conformation, but result in high background levels and poor assay sensitivity. It is also important to note that the

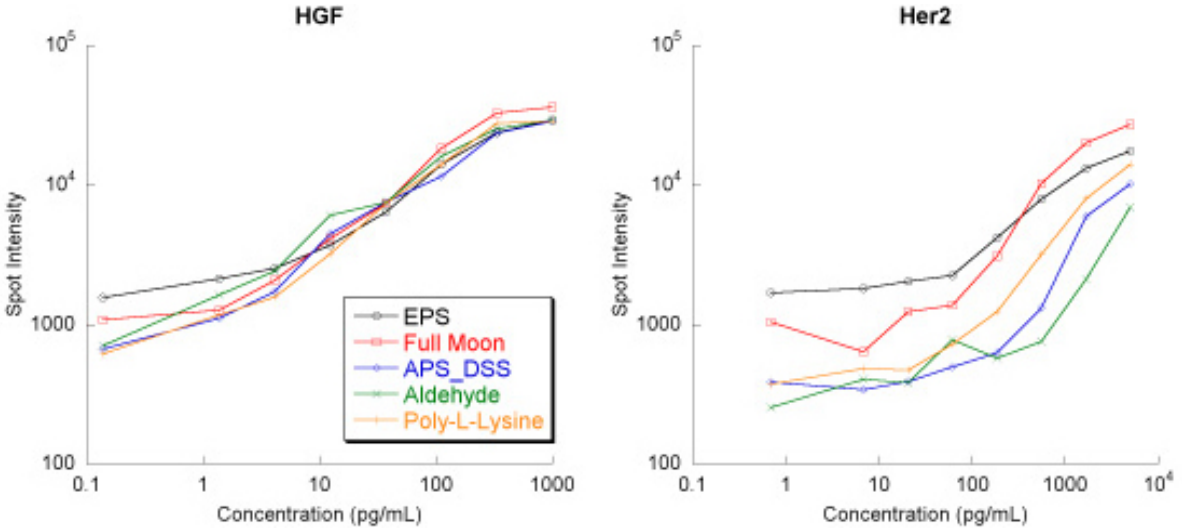

Figure 3. Differential effects of slide chemistry on two different assays. Multiplexed ELISA microarray analyses were performed using 5 different slide types. Abbreviations: EPS, epoxysilane; APS_DSS, aminosilane treated with DSS; HGF, hepatocyte growth factor; Her2, soluble form the Her2 receptor.

usefulness of particular slide chemistries will vary depending on the specific application. For example, the low abundance of some proteins requires that good assay sensitivity must be achieved, while for other assays sensitivity may not matter as much. Whenever using a new assay, slide chemistry should be considered.

Based on the available literature, it is unclear which of the available surface chemistries will result in optimal performance of ELISA microarrays. The ideal surface must have a large surface area available for binding, allow for the stable attachment of antibodies, retain antibody structure and activity, and prevent nonspecific protein binding. Surface chemistries that include reactive sites or affinity-based immobilization sites appear to improve assay sensitivity. It is also advantageous to increase the surface area available for binding through the use of a three-dimensional coating or roughening of the surface. However, it is impossible to choose the best slide chemistry based on the available data. One drawback is that these data were collected in multiple laboratories and therefore do not use the same experimental protocols or reagents. In addition, not all slide types work universally for all capture antibodies. Even though it is essential to test a variety of antibodies to truly evaluate slide chemistries, most reports only focus on one or a few assays. While ELISA microarrays show great promise for the diagnosis of complex diseases, it is still unclear which slide chemistry will produce the optimal assay sensitivity and specificity needed to reach the full diagnostic potential of this technology.

6. ACKNOWLEDGEMENT

This work was funded by the Early Detection Research Network of the National Cancer Institute by contract Y1-CN-5014 and grant U01 CA117378.

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Abbreviations: CBM – carbohydrate binding molecule; ELISA – Enzyme-linked immunosorbent assay; GST – glutathione-S-transferase; His – histidine; NHS esters – succinimidyl esters; Ni – Nickel; NTA – nitrilotriacetic acid; PET – polyelectrolyte; PEG – poly(ethylene glycol); scFv – single-chain antibody; EPS – epoxysilane; APS_DSS – aminosilane treated with disuccinimidyl suberate; HGF – hepatocyte growth factor; Her2 – soluble form the Her2 receptor

Key Words: ELISA, Antibody, Microarray, Solid Support, Review

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