A cell interaction microarray for blood phenotyping.

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Supplementary Information

Surface Choice

For antibody arrays, it is particularly important that the antibody is attached to the surface in a robust manner and that it retains sufficient structural integrity to fulfil its molecular recognition role. Array surfaces such as poly-l-lysine which are commonly used in DNA microarray technology to adsorb DNA on the basis of charge complementarity were used in some early protein array developments and we have also used these.[1] Recently, several other surface types have become available for protein array fabrication. The advantages of such surfaces range from the ability to covalently attach proteins to their stabilisation through the formation of a three dimensional scaffold which helps maintain structural integrity. Other researchers have demonstrated the importance of such microarray surfaces in the function of an assay[2,3] and we have considered this an important place to start in optimizing array performance.

We used a variety of microarray substrates to produce arrays for blood typing; all were printed with a collection of antibodies from the same source plate, and blocked at the same time. To compare these surfaces we used type A Rhesus D positive (ARhD) erythrocytes since it has both carbohydrate based antigens (ABO) and peptide antigens (Rhesus). This means that we could simultaneously measure the response of our anti-A antibodies (IgMs) and a commercial anti-D antibody (IgG) while having anti-B antibodies (IgMs), phosphate buffer spots and a

non-specific control protein (JLDM3) as negative controls where we expect no cell binding. All antibodies were spotted in a dilution series (i.e. Neat, 1:2, 1:4, 1:8). In all cases, the best signal was from the most concentrated antibody spots and the others have been removed from the graph for clarity.

It can be seen from Figure 1 that gold is the best surface in terms of S/N performance for both carbohydrate and peptide antigens. The worst performance is from the Hydrogel II surface (P Value for comparison of gold and Hydrogel II is 0.0005). Although it cannot be inferred from the graph, we noted that poly-l-lysine gave poorer spot morphology than any other surface; this is probably due to the relatively high hydrophilicity of this surface giving rise to lower contact angles. Epoxy-silane coated glass slides gave good performance compared with poly-l-lysine (P Value 0.037), and this reflects the difference between covalent and non-covalent attachment methods. Interestingly, of the two hydrogel surfaces measured, there is a significant difference in performance (P Value = 0.0004), with Hydrogel I slides from Full Moon outperforming Hydrogel II from Schott by more than 100%. We do not expect that the three-dimensional nature of the surface should offer significant benefits for this assay since the target cells are too big to penetrate the pores of such a material. As a result, we attribute the performance to the surface characteristics in terms of antibody attachment and presentation.

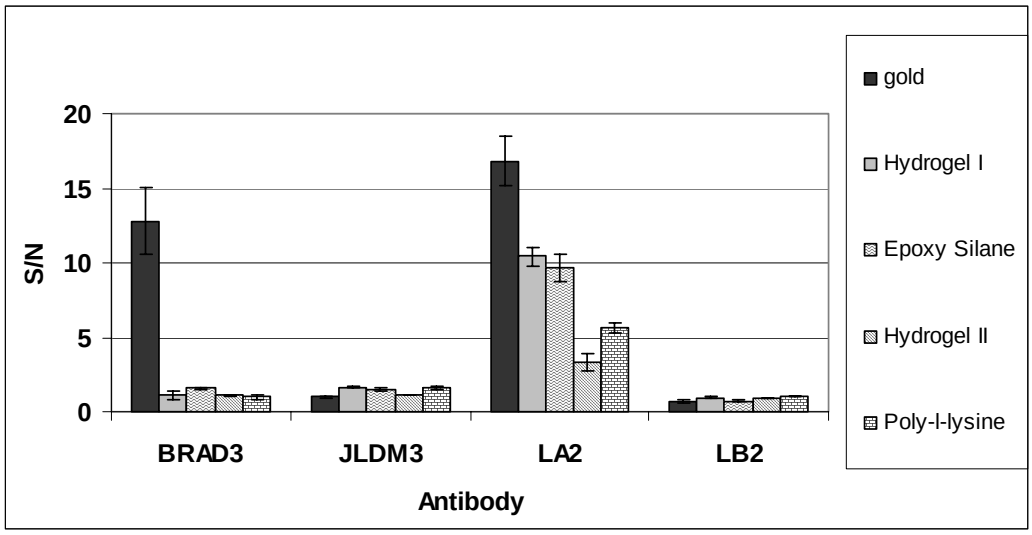

Figure S1

Figure S1
Comparison of surfaces for blood typing array using ARhD blood. Values are the median of five slides and bars represent standard errors. P Values from t-tests can be seen in Table S1 in supporting information.

	gold LA2 neat	Full Moon LA2 Neat	Epoxy LA2 Neat	PLL LA2 Neat	SchottHydrogel LA2 Neat
gold LA2 neat	1	0.045168116	0.010213983	0.001869453	0.000560105
Full Moon LA2 Neat	0.045168116	1	0.098300147	0.001510829	0.000368565
Epoxy LA2 Neat	0.010213983	0.098300147	1	0.037159163	0.002861806
PLL LA2 Neat	0.001869453	0.001510829	0.037159163	1	0.021390335
SchottHydrogel LA2 Neat	0.000560105	0.000368565	0.002861806	0.021390335	1

Table S1
P Values relating to figure 1 from t-tests comparing antibody activity on different surfaces (n=5).

References

[1] Haab, B.B.; Dunham, M.J.; Brown, P.O. *Genome Biology*, **2001**, *2*, 1-13

[2] Angenendt, P.; Glökler, J.; Murphy, D.; Lehrach, H.; Cahill, D.J. *Anal. Biochem*., **2002**, *309*, 252-260

[3] Angenendt, P.; Glökler, J.; Sobek, J.; Lehrach, H.; Cahill, D.*J . Chromatogr . A,* **2003** *100,* 997–104