Function-Spacer-Lipid (FSL) constructs enable inkjet printing of blood group antigens "biological invisible ink"

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Background

Limitations of inkjet printing antigens to solid surfaces are that the molecule to be printed must be dispersible in solution, retain antigenicity and remain on the printed surface when exposed to biological solutions. FSL (function-spacer-lipid) constructs were designed to be dispersible in water and attach a range of synthetic molecules to cell membranes. FSL constructs currently include carbohydrates, peptides, fluorophores, ligands and biotin and are being used in the manufacture of modified cells called "kodecytes" for use in blood group typing laboratories and for research purposes[1-4].

FSL constructs are analogous in structure to a flower and consist of three components; a **F**unctional head group (like the flower head), a **S**pacer (like a stalk) and a diacyl **L**ipid tail (the anchoring roots). All FSLs are biocompatible and allow users to introduce novel **F**unctional moieties to membrane surfaces including those of living cells.

Materials & Methods

Printer: Epson Stylus T21 piezoelectric printer (figure 1).
Printing solution: FSL 1mg/ml in 1X PBS with 0.05% bromophenol blue as a visualisation-dye loaded into a volume (reduced) modified standard refillable cartridge. A variety of FSLs including those with carbohydrate F groups and FSL-biotin were successfully printed. Results are only shown for FSL-A(GALNa3[Fa2]GALb)-SA1-L1 #421604

Figure 1. Standard inkjet printer used to print FSL constructs. The cartridge was only modified to hold a smaller volume of 1ml

Print surfaces: silica TLC plates (silica 60), nitrocellulose membrane and a variety of different papers.
Printing: A standard computer program is used to print words or images directly onto a membrane loaded in the printer. Cut out, or glue on microwell templates. Wash away visualisation-dye by soaking in water for approx 15mins , then air dry. Membrane will now be blank.
Immunostaining: All reactions are at RT. Block membrane with 2% BSA in PBS for 30 mins. Flood or fill well with monoclonal or polyclonal antibody diluted 1 in 4 in 2% BSA in PBS. Incubate 60 mins, wash and add species-appropriate anti-Ig alkaline phosphatase enzyme conjugate diluted 1:400 in 2%BSA in PBS. Incubate 30 mins then wash. Add chromogenic substrate NBT/BCIP (Roche) and incubate for 15-30 mins. Stop reaction by washing in water. Image is stable and can be laminated when dry.

Results

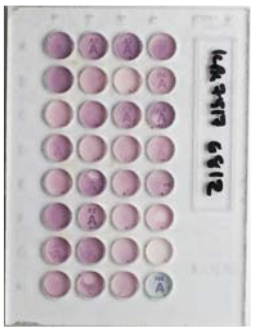

Figure 2. FSL-A printed onto silica and overlaid with a perspex microwell template. The letter A and microwell co-ordinates when visible indicate the presence of mouse serum anti-A. Blank wells indicate an absence of anti-A. Unprinted areas indicate background staining. Results 100% correlated with salivary A antigen immunised mice and were able to determine antibody status from a tail vein bleed of 0.01ml. Control reaction in microwell H4 is due to monoclonal anti-A .

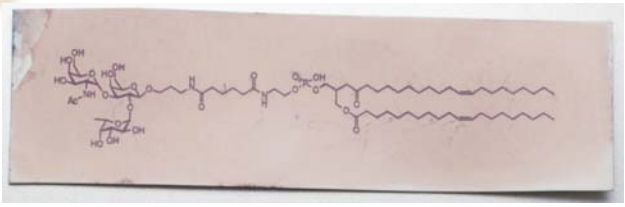

Figure 3. Schematic image of the FSL-A blood group A construct printed with FSL-A on paper and immunostained via monoclonal anti-A.

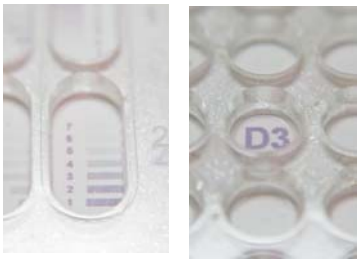

Figure 4. FSL-A printed onto silica in two different formats and overlaid with perspex microwell templates. Reactions are visualised via monoclonal anti-A

Conclusions

FSL constructs have now been used to create prototype diagnostic assay kits using standard desktop inkjet printers, by simply replacing the ink in a cartridge with a solution of FSL construct. The FSL constructs can be printed onto a substrate in much the same way that one might print a letter or drawing on a sheet of paper. Blood group FSLs have been printed onto silica, nitrocellulose and paper surfaces and used to identify blood group antibodies in diagnostic and research samples. Combining FSL technology with existing inkjet printing technology provides a cost effective alternative to existing methods of manufacturing diagnostic test kits, including microarrays which for the most part use robotics. In addition to cost savings the versatility of this manufacturing method provides additional benefits for research laboratories and micro-analyses.

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