A novel solution for diagnostic printing

The image in this title was printed on A4 paper with FSL-A constructs, reacted with anti-A, and visualised with anti-Ig alkaline phosphatase conjugate + BCIP chromogenic substrate - see Fig 5.

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Background

Inkjet printing is a promising method for delivering biological molecules onto solid surfaces. Recently a new construct has been developed and used to modify the biosurfaces of cells and virions. As these constructs, known as FSLs (function-spacer-lipid) are dispersible in water, it makes them ideal candidates for use as 'bio-ink'. The FSL constructs are analogous in structure to a flower and consist of three components: a **F**unctional head group, a **S**pacer and a diacyl **L**ipid. Current FSL functional groups include carbohydrates, peptides, and cell labels (fluorophores, biotin, tyrosine-^{125}I) and the repertoire is continuously being expanded. The lipid tail anchors the FSL construct to the membrane while the spacer is designed to allow both dispersion in biological compatible solutions and spacing away from the membrane. If the functional group is not in the form of an FSL construct it does not attach to membranes/surfaces. The FSL construct *per se* is biologically inert, and the only biological activity observed is that introduced by the F group.

Materials & Methods

Printer: Epson Stylus T21 piezoelectric printer (Fig 1).
Printing solution: FSL 1mg/ml in PBS with 0.05% bromophenol blue as a visualisation-dye loaded into a volume-reduced standard refillable inkjet cartridge. A variety of different FSLs have been successfully printed. Representative results are shown for FSL-A(GALNa3[Fa2]GALb)-SA1-L1

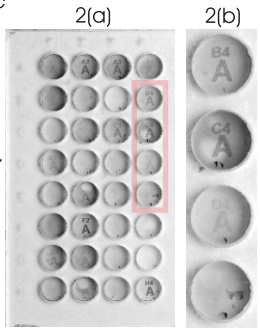
Fig 1. Standard inkjet printer used to print FSL constructs. The cartridge was modified to hold a smaller volume of 1ml

Print surfaces: Paper including photocopier paper, silica TLC plates, nitrocellulose membrane, cotton, silk.
Printing: Print image or words using any standard computer program directly onto the membrane loaded in the printer (Fig 1). Use as is, or cut out and glue on acrylic microwell templates to create reaction wells. Wash away visualisation-dye by soaking in water for approx 15 mins, then air dry. Membrane will now be blank.
Immunostaining: All reactions are at room temperature. Treat membrane with 2% BSA in PBS for 30 mins. Flood surface or fill well with 10 μL of serum or biological fluid diluted 1 in 4 in 2% BSA in PBS. Incubate 60 mins, wash and add anti-Ig alkaline phosphatase enzyme conjugate diluted 1:400 in 2% BSA in PBS. Incubate 30 mins then wash. Add chromogenic substrate NBT/BCIP and incubate for 15-30 mins. Stop reaction by washing in water. Pre-printed surfaces and final developed results are stable for years.

Representative Results

Fig 2. FSL-A printed and overlaid with an acrylic microwell template to create 6mm wells. The letter A and microwell co-ordinates when visible indicate the presence of anti-A. Blank wells indicate an absence of anti-A. Unprinted areas indicate background staining. Results 100% correlate with validated method. Fig 2(a) is a magnification of wells B4 (++), C4 (+++), D4(+) and E4 (-) highlighted in 2(a).

2(a) 2(b)



3(a)

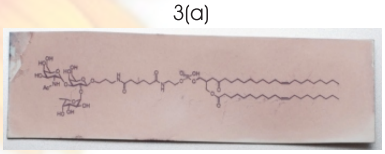
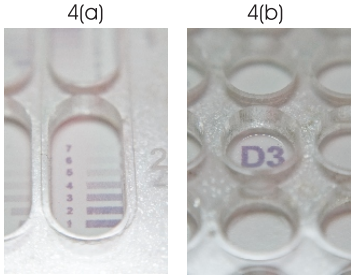


Fig 3. Printing on paper. 3(a) A schematic image of the FSL-A blood group A construct printed with FSL-A and immunostained with monoclonal anti-A. 3(b) FSL-A and FSL-B printed in a 6mm microwell and immunostained with anti-A+B present in blood group O serum

3(b)

Fig 4. Examples of flexibility. Two different microplate formats. 4(a) ladder format (shades of grey) with control as numbers 4(b) microwell coordinate format

4(a) 4(b)



Conclusions

By simply replacing the ink in a desktop printer with FSL constructs, novel informative diagnostic systems can be created. The FSL constructs when printed are colourless, and only appear as words or images if they react with a diagnostic marker (following development). The flexibility of the system allows informative words (in any language), images, barcodes, etc, to be printed allowing for machine and/or human readable results. As this technology uses stable reagents and works at room temperature, it is very suitable for third-world and field diagnostic applications. The recent development of FSLs for infectious disease now has the potential to extend printing into low cost infectious disease assays.

The inkjet printing of FSL constructs onto paper is proving to be a novel solution to create robust diagnostics assays.

Fig 5. Original result used to create the title banner

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