High Throughput Screening of Biocompatible Polymers

Guilhem A. Tourniaire; Jérôme Sallette and Mark Bradley
Department of Chemistry, University of Edinburgh, EH9 3JJ, UK
E-mail: G.A.Tourniaire@sms.ed.ac.uk

- AIMS OF THE PROJECT -

The aim of this project was to develop **high throughput screening methods using an IMSTAR Pathfinder™ High-content imaging platform** to support the analyses of polymeric materials synthesised using a parallel approach. This was achieved by developing **arrays of polymeric materials**[1,2].

- PRINTING -

Microarray printing required extensive optimisation to ensure uniform polymer spot and shape.

Stage 1- Solutions

Polymers were dissolved at 1% w/v in a high viscosity and high boiling point solvent such as N-methyl pyrrolidinone. The solutions were placed into a 384-well polypropylene microplate prior to printing.

Stage 2- Printing

The robot used was the *Qarray mini*® (Genetix Ltd; UK).The solutions were deposited by contact printing using 150 μm solid pins. Each polymer solution was printed as 4 identical spots and each spot was formed by a minimum of 5 contact printings in order to reduce the effect of the ring stain formation[3]. The nature of the substrates were adapted to the different screenings in order to obtain the lowest background and most uniform spots. The typical spot diameter was 300 μm (±20 μm) with a volume about 7 nL which is equivalent to about 70 pg of polymer.

Stage 3- Drying

Once printed, the solvent was removed by drying under vacuum at 45°C overnight, to give a microarray with up to 2048 spots per slide.

- APPARATUS -

Arrayer:

***Qarray mini* (Genetix Ltd, UK)**

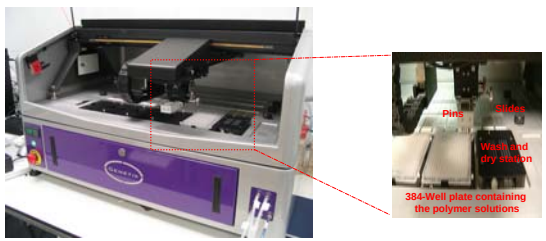
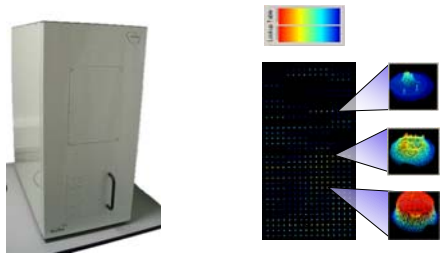


Fig. 1. *Qarray mini*

High Throughput Scanner:

***Bioanalyser 4F/4S scanner* (LaVision BioTech GmbH, D)**

White light source scanner with CCD camera allowing the rapid determination of fluorescence intensities.



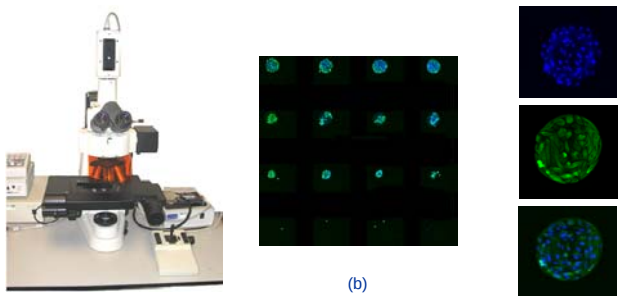
(a) (b)

Fig. 2. (a) High throughput scanner; (b) Scan of an array of 512 spots after incubation with a protein labelled using AlexaFluor® 647. (Cy5 filter; 300 ms exposure time, spot integrated over an area of 0.080 mm^2).

High Content screening platform:

Pathfinder™ / OSA Reader™ (IMSTAR S.A., F, www.imstar.fr)

High content, high resolution (0.3 μm) image cytometry platform allowing the determination of cell numbers, cellular coverage in the spot, all individual cell fluorescence intensities, spots fluorescence ratio & morphology characteristics.



(a) (b) (c)

Fig. 3. (a) High content imaging platform; (b) Sub-array of 16 spots using composite of Fluorescein and DAPI scan after incubation with primary human epithelial tubular cells; (c) Example of scan (20X objective) obtained for each polymer spot showing from top to the bottom: the nuclei stained with Hoechst 33342 (DAPI filter), the cytoplasm stained using secondary antibody detection (Fluorescein Filter) and merged image.

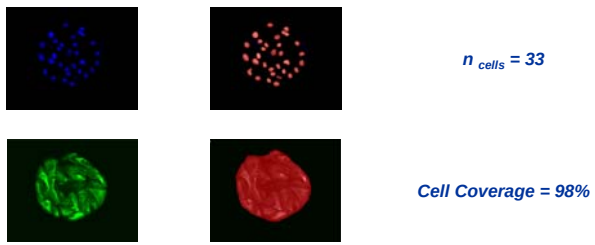
- SCREENING -

Protein binding:

Proteins were labelled using fluorescent dyes such as AlexaFluor® (Molecular Probes, NL). Labelled protein solutions (300μL/slide) were then placed onto the polymer arrays inside a Gene Frame (ABgene, UK) in order to obtain a uniform layer of solution. After a given incubation time, the protein solution was washed away and the slide was scanned to give data such as that shown in Fig. 2b.

Cell compatibility:

Cells were grown on the polymeric array at 37°C under 5% CO_2. After cell staining, the array was washed and the cells fixed (3.7% w/v p-formaldehyde and 4.0% w/v sucrose in H_2O).



(a) (b) (c)

Fig. 4. (a) Raw images; (b) Masks generated by automatic detection protocols; (c) Results obtained

- CONCLUSION -

It has been shown that polymer arrays are a **versatile format allowing the development of very high throughput methods for the evaluation of cell and protein binding**. Moreover, the quantities of both the analytes and reagent used being minimal, these methods are **particularly attractive on economical and environmental grounds**.

- ACKNOWLEDGMENTS -

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- REFERENCES -

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