Polymer microarrays for cellular adhesion†

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Microarray screening of polymer libraries for cellular adhesion was developed utilising a thin film of agarose to allow unsurpassed localisation of cell binding onto the array substrate and the discovery of cell specific polymers.

The profound impact of arrays in the biological arena cannot be overlooked, taking into consideration the tremendous multiplexing ability an array can offer to a specific application. The most common examples are DNA "arrays" or chips, which are widely used for mRNA profiling, touted for diagnostic applications, used for SNP analysis and potentially have a role to play in DNA sequencing,[1] but the multiplexing power of arrays has been exploited in an increasing number of arenas such as the high-throughput (HT) characterisation of gene function with, for example, cell-based screens developed in a microarray type format.[2]

Polymers are essential in the area of biomaterials and have been used in a myriad of applications.[3] The mechanism of cell attachment onto polymer surfaces in cell culture has been extensively studied[4] and it is broadly accepted that the first steps in this process are the adsorption of extracellular matrix proteins onto the surface of the polymer. Cells then indirectly interact with the polymer through the adsorbed proteins which control a variety of cellular processes such as adhesion, growth and differentiation.[5] As a result of such complex and imperfectly understood interactions, it is still impossible to predict, from the chemical structures of a polymer, how such materials will perform when in contact with cells, blood or body fluids. As a consequence, the use of an HT approach to allow the rapid synthesis of chemically diverse polymers offers an important tool to find correlations between the design and performance of such materials.[6] Traditional methods of synthesis, identification and testing of new polymers are slow and thus over recent years, the field of automated and parallel synthesis of polymers has grown enormously[7] but, as is usually the case in any HT process, the development of high-throughput characterisation and screening methods are often the rate limiting steps. The use of polymer arrays for cellular screening was recently reported[8] where human embryonic stem cells were successfully differentiated following attachment and growth onto a poly(acrylate) array. However, in this case the uncharacterised polymers were prepared using a nanoliter-scale synthetic approach which was complicated by the very rapid evaporation of the "spotted" monomers meaning the exact composition of the final polymer was hard to define.

The poly(urethane)[9] library used in our studies (see Fig. 1 for the monomers used) was prepared by parallel synthesis and all individual members were fully purified and characterised by gel permeation chromatography, differential scanning calorimetry and contact angle measurements prior to use.[9] Before printing in a microarray type format each library member (for details see ESI†) was dissolved in a common solvent and transferred into a 384 well plate prior to contact printing. A number of parameters, such as the nature of solvent and substrate, inking and printing time had to be optimised in this process to ensure uniformity of the polymer spots within the array.

To obtain uniform printing, the polymer library needed to be printed from a common, non-volatile solvent. 1-Methyl-2-pyrrolidinone (NMP) was selected on the basis that the majority (> 95%) of the polymer library was soluble in this solvent and that it allowed uniform spots to be printed. The formation of so called "rings"[10] during solvent evaporation was minimised by a

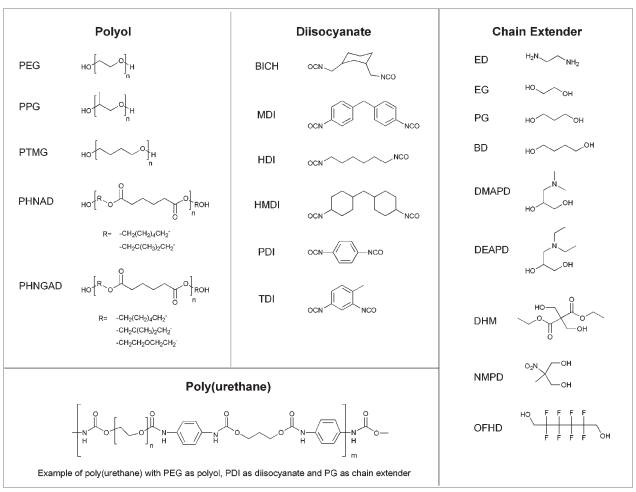

Fig. 1 Structures of the different monomers used in the polymer library synthesis. One example of poly(urethane) structure is given. The monomers, molecular weights, abbreviations and proportions used during the synthesis can be found in the ESI.

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† Electronic supplementary information (ESI) available: List of monomer abbreviations and molecular weights, experimental details of substrate preparation and cell culture. See DOI: 10.1039/b602009g

combination of the high boiling point solvent and successive layering of polymer solutions (5 stamps per spot).

In order to develop a cell compatible assay in a microarray format, the substrate had to comply with the following requirements. Firstly, the substrate had to be unaltered by the contact printing of polymer solution in NMP, which ruled out the use of polymer coatings such as poly(hydroxyethyl methacrylate), p(HEMA)[11] which would be dissolved locally and give rise to polymer mixtures. Secondly, a substrate with low levels of background cell binding had to be developed to facilitate data analysis (the majority of work published to date with cells is the result of dramatic data manipulation to remove data/cells that surround the spots) and thirdly, the substrate had to be stable under UV-irradiation to allow sterilisation prior to the plating of the cells.

A number of substrates were prepared: C18 functionalised Silane-Prep™ slides, perfluoroalkylthiol monolayers on gold coated slides and Silane-Prep™ slides dip-coated with a layer of agarose gel and the antifouling properties of the different substrates were tested with several mammalian cell lines (HEK293, HeLa, ND7 and B16F10). The C18 functionalised slides, as expected, were highly hydrophobic and were able to reduce non-specific binding, but not all cell lines could be blocked in this manner. The use of perfluoroalkylthiol-modified slides inhibited cellular adhesion, however it was impossible to use UV-irradiation for sterilisation as this degraded the surface. The best results were obtained by dip-coating aminoalkylsilane slides (Silane-Prep™; Sigma) with a thin film of agarose (Fig. 2).[12] Although agarose has been used to amplify loading on DNA arrays[13] and is known to inhibit cellular adhesion in a number of different formats,[14] agarose has not been used as coating material for cell based microarray assays.

The polymer arrays were fabricated by contact printing using polymer solutions in NMP with each polymer printed in quadruplicate. Once printed, the slides were dried overnight under vacuum at 45 °C and sterilised by exposure to UV irradiation for 15 minutes prior to cell plating.

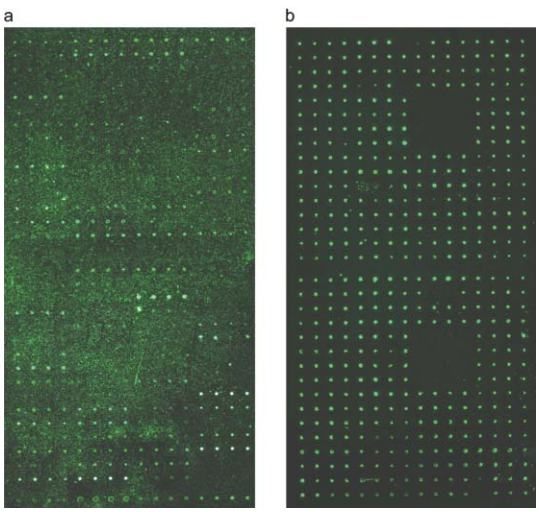

Fig. 2 Non-specific cell binding reduction using an agarose-coated substrate. Non-processed images obtained with Stro-1 + cells stained with CellTracker Green on two arrays with different substrate; (a) unmodified glass slide, (b) agarose-coated slide.

To illustrate the potential of the array, screening was carried out with primary cells using antibody staining as a means of detection. This was undertaken using human renal tubular epithelial cells. The cells were plated at 10^5 cells per slide and incubated for 5 days. Following fixation and permeabilisation, the cells were incubated with CAM5-2 anti-cytokeratin monoclonal antibody and visualised using Alexa Fluor® 488 labelled IgG antibody. Finally Hoechst 33342 was used to stain the nuclei. Analysis was carried out using the HCS platform and the Pathfinder™ software (IMSTAR S.A., France). This platform, based on a fluorescent microscope with an X-Y-Z stage, allows the automated capture of single images (0.46 mm^2) for each polymer spot with a resolution of 0.58 μm (Fig. 3).

Cell compatibility was evaluated in terms of the total number of cells immobilised onto each polymer spot which was identified using the DAPI channel and the Pathfinder™ software. Several poly(urethanes) were shown to provide significant attachment with an average over the 4 identical polymer spots of up to 153 human renal tubular epithelial cells (for details see ESI†). The 6 poly(urethanes) showing the highest number of bound cells (more than 140 cells per spot) all contained 4,4′-methylenebis(phenylisocyanate) (MDI) (PU-18; 161; 165; 182; 195; 217), while the diol PTMG (650 Da or 1000 Da) was present in four of these top six polymers, thus allowing the rapid and direct correlation of polymer structure with cell binding.

Overall, the microarray platform allows the identification of new polymers for the attachment of various cell types, including primary cells which are of significant interest within the medical community. Using this approach, a whole library of biocompatible polymers presenting a wide range of properties can be screened in a single experiment, in a self-consistent manner allowing the microarray platform to provide a rapid correlation of polymer structure with cell binding ability. Furthermore, since each library member was synthesised on a scale that allowed characterisation prior to array fabrication there is full confidence

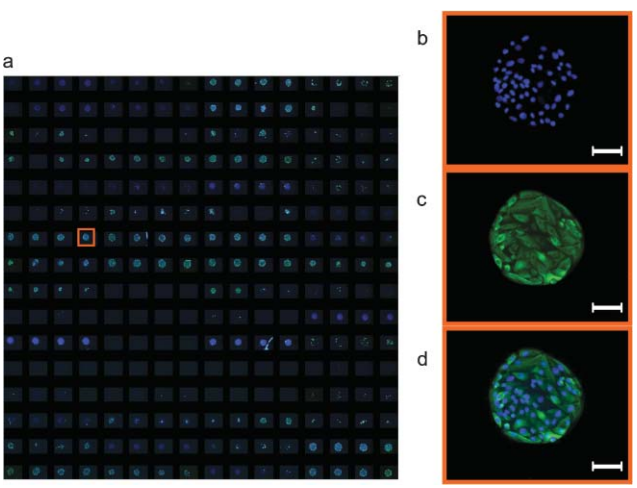

Fig. 3 Primary renal tubular epithelial cells on polymer array. (a) Cells on an array containing 60 polymers each printed as 4 replicate spots; one polymer spot with no background subtraction. (b) Nuclei stained with Hoechst 33342. (c) Cam5-2 antibody staining with Alexa Fluor® 488 secondary antibody. (d) Composite image of (b) and (c) (the bar represents 100 μm). Note: the boundaries of the cells are a function of the polymer spot and are not software processed.

in any structure–activity relationship generated while allowing immediate scale-up following polymer identification. The agarose substrate proved to be very reliable in preventing unwanted cellular adhesion, with the potential to be developed for use with a large variety of cell-specific applications, such as global RNAi cell based phenotypic screens.[15]

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