Polymer Microarrays for Biomaterial Development

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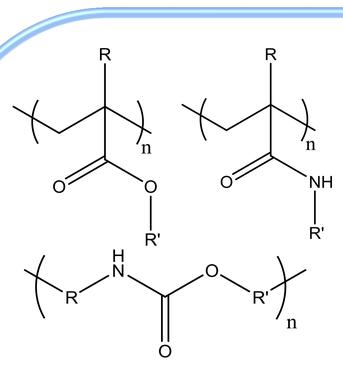


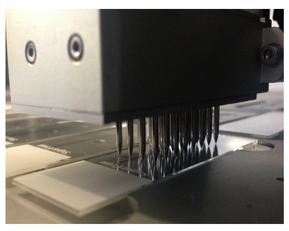
Polymer Library

Polymer microarrays

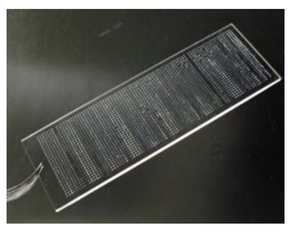
High-throughput techniques that allow for any number of investigations to occur simultaneously have greatly advanced the speed in which new interactions between biological materials and biocompatible surfaces can be discovered. Polymers are a prime example of such surfaces, which have found use in a diverse range of applications within the biomedical arena. From the basic (blood bags and culture ware) to the complex (stimuli-dependent hydrogels and leukocyte filters), the diversity of polymers, combined with their ability to be precisely tailored, make them ideal candidates for future biomaterials. As such, the ***polymer microarray*** was created to stream-line their discovery. We report here the fabrication of a ***polyacrylate/amide and polyurethane microarray***, ideal for biomaterial investigation based on existing applications of these polymers.

380 pre-synthesised polymers from a Bradley group library were contact printed in n-methyl pyrrolidinone onto glass slides, dip-coated in cytophobic agarose coating, using the Genetix Qarrayer^{mini}. Each polymer was printed in replicates, along with solvent as an internalised negative control. Printing parameters were optimised to produce features of 300-400 µm diameter.

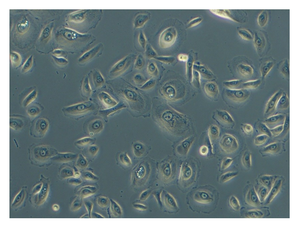
Prior to incubation, polymer microarrays were UV sterilised for 30 min and washed. Biological material was interrogated, followed by fixation and staining steps in preparation for high-throughput imaging. Following the identification of polymers with desirable interactions, they are scaled-up for further investigation on spin-coated coverslips [1].



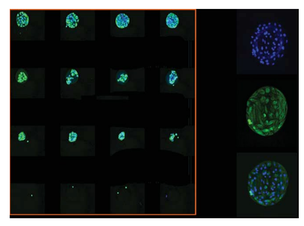
Contact Printing



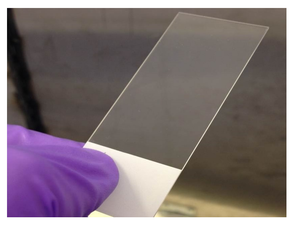
Polymer Microarray



Biological Interrogation



Imaging and Analysis [2]



Agarose-Coated Slide

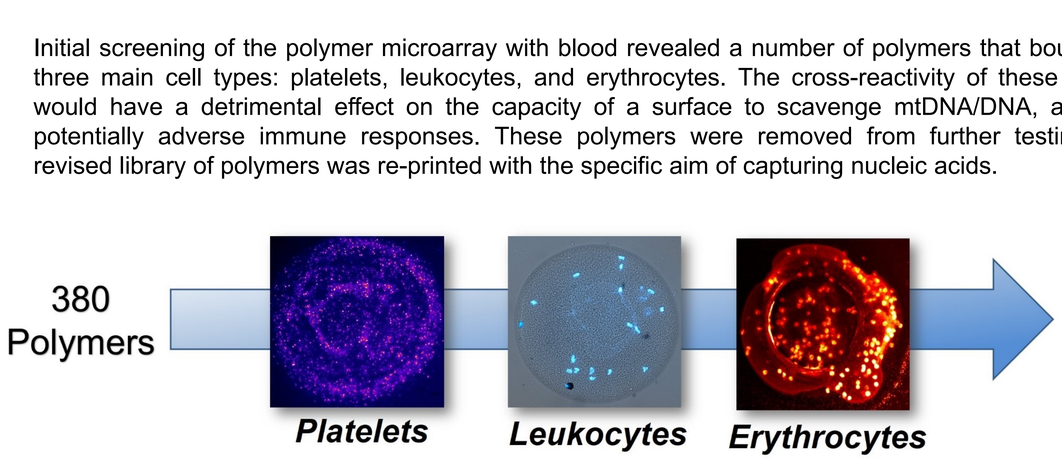
This protocol can be easily adapted to proteins, carbohydrates, and other substrates, for the creation of microarrays with several thousands of features. The main benefits of the polymer microarray approach are that it is cheap (micrograms of material), can be made in batches, and requires little prior knowledge about specific cell-surface interactions, vastly reducing the time required to identify promising candidates. Additionally, this is applicable to all cell types with previous examples including human embryonic stem cells, oligodendrocytes, platelets, skeletal progenitor cells, and a variety of cell lines.

Progress in the use of this polymer microarray approach in two contrasting studies is detailed below, aimed at both discovering novel biomaterials, as well as broadening the application of polymer microarrays.

[1] Hansen, *et al*, Biomaterials 2011;**32**:7034-41
[2] Tourniaire, *et al*, ChemComm 2006;**20**;2118-20

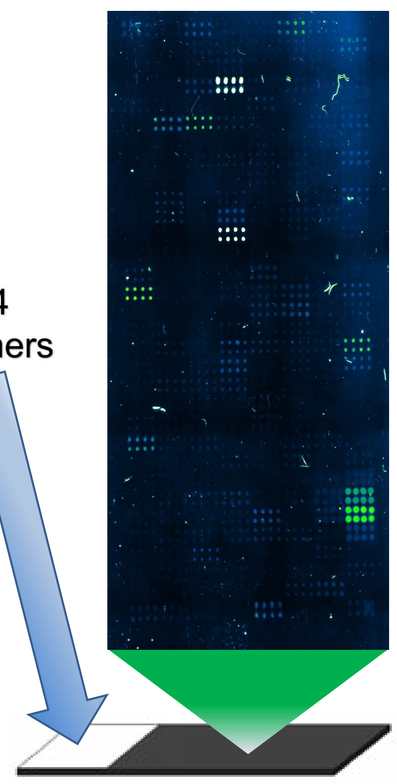
Polymer-based blood filter for scavenging immunogenic nucleic acids During tissue damage, inflammatory molecules, known as damage-associated molecular patterns (DAMPs), are released from disrupted cells. Mitochondrial DNA (mtDNA) and DNA-protein-complexes are two such DAMPs that trigger an innate immune response. By removing these molecules from blood, an improved outcome for systemic inflammatory syndromes is expected [3]. Herein, we describe progress towards the development of a polymer filter for mtDNA/DNA.

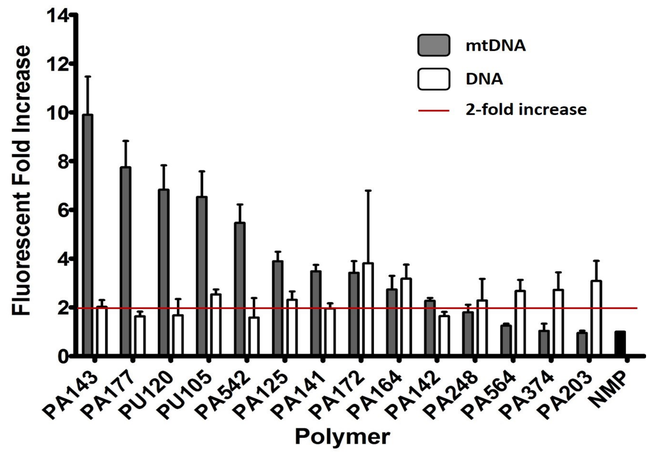
Initial screening of the polymer microarray with blood revealed a number of polymers that bound to the three main cell types: platelets, leukocytes, and erythrocytes. The cross-reactivity of these polymers would have a detrimental effect on the capacity of a surface to scavenge mtDNA/DNA, as well as potentially adverse immune responses. These polymers were removed from further testing and a revised library of polymers was re-printed with the specific aim of capturing nucleic acids.



Non-reactive polymers were interrogated with mtDNA/DNA, extracted from HepG2 cells. The fluorescent dye PicoGreen was used to evaluate mtDNA/DNA-polymer interactions. Normalised to internal solvent controls, the fluorescent fold increase was used to determine nucleic acid-binding polymers. Using a two-fold increase as a benchmark of binding ability, a short list of candidate polymers were chosen for scale-up.

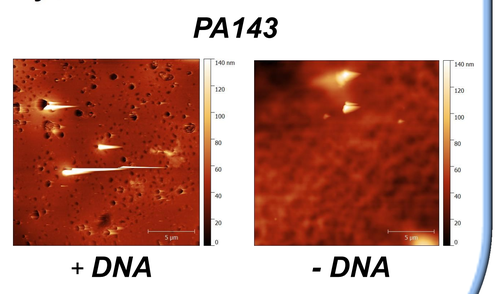
Current work is aimed at confirming and evaluating this binding ability under the pressures of venous blood flow. It is intended that such a polymer could be introduced into an extracorporeal filter to help treat trauma and shock, as well as a number of other inflammatory diseases.





Preliminary AFM work provides evidence of nucleic acid-polymer interactions. After 2 minutes incubation, a 20-30 nm layer of mtDNA/DNA is present, suggesting scavenging during clinically relevant concentrations (2 µg/ml).

PA143



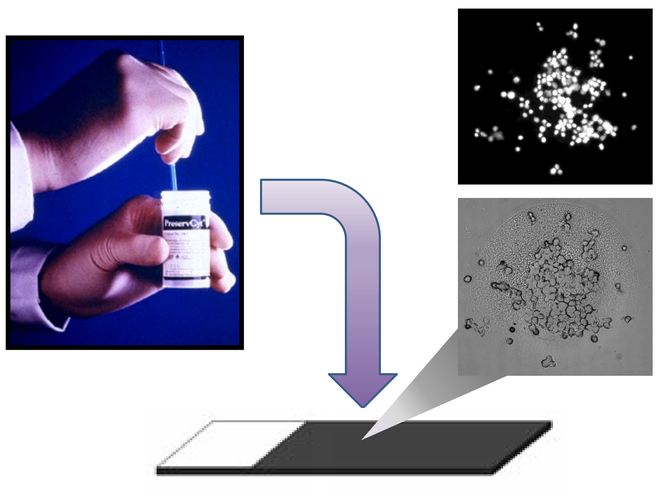
+ DNA ***- DNA***

[3] Jaewoo *et al*. PNAS 2011;**108**(34):14055-60

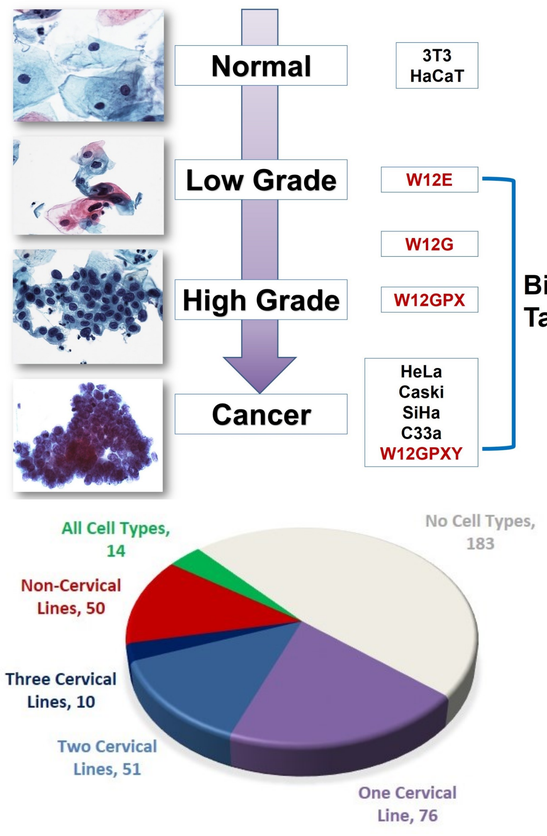
Acknowledgements to Dr Vasileios Koutsos for AFM images.

Improving cervical cytology using a polymer substrate Cervical cancer diagnosis is based on the presence of high-risk HPV and cytological evaluation of cells exfoliated from the cervix. Cytology is a cheap and effective diagnostic, but time-consuming and suffers from subjectivity between analysts. Therefore, we aim to improve reproducibility, and the specificity/sensitivity of this method by screening polymers for selective attachment to abnormal cells over healthy cells.

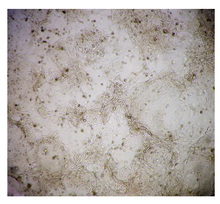
Common liquid-based cytology practices require that samples are stored in the methanol-based fixative PreservCyt. To allow for an easier transition from cell lines to patient samples, cell cultures were pre-treated with PreservCyt before microarray interrogation.



Polymers were interrogated with cancerous cervical cell lines (**HeLa**, **CaSki**, **SiHa**, **C33a**) as well as non-cervical lines (**3T3**, **HaCaT)** and categorised based on their specificity. This revealed an efficient population of polymers that could encourage the enrichment of abnormal cervical cell types.

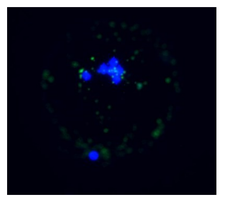


The **W12E** line is derived from a low grade lesion with episomal HPV16 genome [4]. The genomic W12 lines (**G**, **GPX**, **GPXY**) were derived sequentially from W12E and represent a progression in lesion severity. A polymer that preferentially binds these cells, over healthy cells, would be an ideal candidate in a disease that demands earlier detection. Preliminary microarray data has identified the presence of polymers that bind to these precancerous cells.



W12E and G (pictured) require 3T3 feeder layer. GPX and GPXY can be cultured without.

Microarray



Precancerous cell binding - W12G on polymer spot with DAPI (blue) and connexin 43 (green), lost in more advanced stages of neoplasm.

Candidates are currently being identified for scale up, which will take advantage of patient samples of known composition. Successful implementation of these polymer substrates could leave only abnormal cells attached, potentially leading to automatable detection.

[4] Aasen *et al*. Oncogene 2003;**22**:7969-80

Acknowledgements to Dr Tim Palmer for Pap staining images.