MULTIPLEX BIOASSAYS USING A SUSPENSION ARRAY PLATFORM; TOWARDS THE HIGH THROUGHPUT SCREENING OF DRUGS TARGETING CANCER STEM CELLS

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ABSTRACT

This paper summarizes our work to-date in the development of a new encoded microparticle suspension array and its use for the multiplex analysis of biological samples. The system, which has a very large encoding capacity, is designed to be highly automated, with the combination of a microfluidic reading device and decoding/fluorescence analysis software. Particles have been modified for a range of different end-use requirements, with the goal of developing an assay for the high throughput screening and tailoring of therapies specifically targeted against cancer stem cells.

KEYWORDS: encoded-microparticle, suspension-array, multiplex, bioassay, SU-8.

INTRODUCTION

For many diagnostic and drug discovery applications, the simultaneous analysis of large numbers of biomolecules in high throughput and small sample volumes is desirable and such multiplexed analysis has traditionally been accomplished using surface-based microarray technologies. Bead-based suspension arrays have been shown to have a number of advantages over microarrays including smaller sample volumes and improved binding kinetics [1]. The particles can also be used as a support for the synthesis of large numbers of different molecules (probes/drugs etc) on the surface (split-and-mix synthesis) [2]. In a suspension array, microparticles coated with different probe molecules are used as platforms for the analysis of biological samples. Each particle carries a unique code which serves to identify the probe on its surface with biological responses often being detected by observing changes in particle fluorescence. Many coding methods and readout platforms for bead arrays have been developed [3] [4].

THEORY

We are developing a new integrated platform for readout of specially designed coded microparticles which offer very high (up to 5×10^5) multiplexing capability. The technology is part of a large project titled Integrated Chemical Synthesis and Cell-screening (ICSC), combining encoded combinatorial drug synthesis with on-particle cell monitoring. The vision is for a high-throughput and low-cost assay system for use in both the discovery of novel anti-cancer drugs and for the tailoring of individuals' treatment regimes (personalized medicine). The technology is versatile and has been adapted for the multiplex analysis of oligonucleotide sequences (genotyping) and antibody mixtures (immunoassays).

EXPERIMENTAL

Microparticles are fabricated by single-step photolithography in SU8 and are flat discs 250 μm in diameter. The coding element uses an arrangement of holes similar to that used in an on-the-fly particle encoding system reported recently [5]. The pattern of holes forms a binary code with incorporated error detection bits, increasing the robustness of code-reading. Code reading has been automated using custom image analysis software.

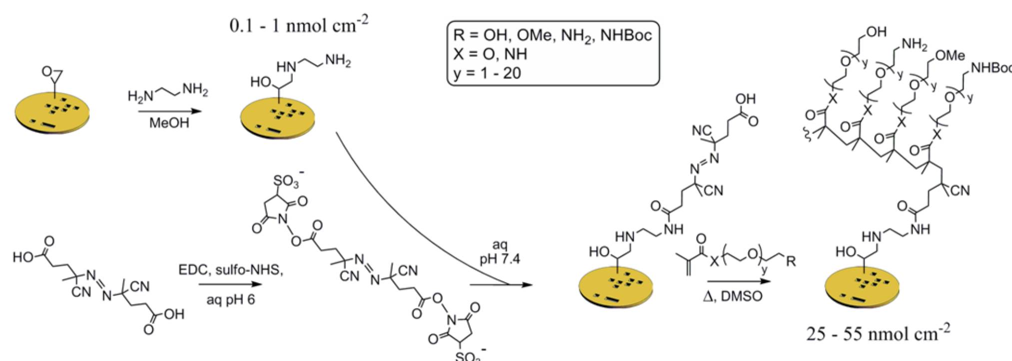

Figure 1; Synthesis on SU-8, enabling control of various functional group densities.

After fabrication, SU-8 particles retain surface epoxide functionality which can be used as a handle for further chemical functionalisations [6]. One example of the scope of this surface chemistry is the use of free radical polymerization to graft functionalised polymer brushes from the surface, allowing the loading density, spacing and type of functionality to be controlled (Figure 1). Epoxide groups are first ring-opened by the nucleophilic addition of bis-amine to yield an amine-modified surface. An *N*-Hydroxysuccinimide (NHS) activated carboxyl radical initiator can then be immobilised

using amide coupling chemistry. This initiator-functionalised surface yields immobilized free radicals under thermal decomposition resulting in the graft polymerization of suitable monomer molecules. We have studied the graft polymerization of PEG methacrylate and methacrylamide monomers incorporating a variety of terminal functionalities, aiming to control particle surface properties. PEG was chosen as a linker in the monomers due to its widely reported ability to reduce surface fouling with biomolecules [7].

By using mixtures of different monomers, co-polymers can be grafted from the surface of particles allowing a mixture of different functionalities to be achieved in a controlled ratio. The resulting surface can be a mixture of blocking or spacer groups (R = methoxy), reactive groups (R = amine or hydroxyl) and protected groups (R = Boc-protected amine). Biomolecular probes can then be covalently immobilized to these functionalised particles for use in multiplexed assays.

RESULTS AND DISCUSSION

At present 1.2×10^5 uniquely encoded particles can be fabricated with each photolithographic exposure, and using automated manufacturing up to 3×10^6 such particles can be manufactured in a single run. A microfluidic system has been developed that spreads 1000 particles at a time into an array for imaging (Figure 2). The design of this device eliminates particle clumping and stacking, allowing the coding element of each to be easily imaged and decoded, the fidelity of the code reading software has been tested and found to be correct for 99.8 % of reads. Incorporated into the analysis software are tools for automatically quantifying particle fluorescence, used for hit detection in multiplex bioassays. Reversing the channel flow allows particles to be recovered from the device if necessary, e.g. to continue a synthetic sequence.

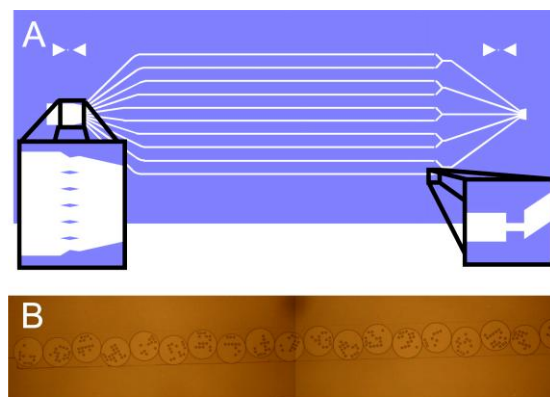

Figure 2; A) Layout of the microfluidic device for arraying particles. Insets show filtering elements and channel constriction which prevents particles exiting. B) Encoded microparticles aligned in a section of the device designed for particle imaging and decoding.

Graft polymerization of methacrylate and methacrylamide monomers from the particle surface have achieved up to a 400-fold increase in the functional group loading density, for example the polymerization of amino-PEG-methacrylamide monomer increased particle amine loading to ~ 45 nmol cm^{-2}, up from an initial loading of 0.1 nmol cm^{-2}. Using mixtures of blocking-group and reactive-group modified monomers to form well defined co-polymers has been shown to work equally well. Hindering the formation of very long polymer brushes are termination reactions between pairs of growing (free radical) polymer chains in close proximity on the surface, which appears to be mostly independent of the initial surface density (always resulting in a loading of 40 ± 15 nmol cm^{-2}) [8].

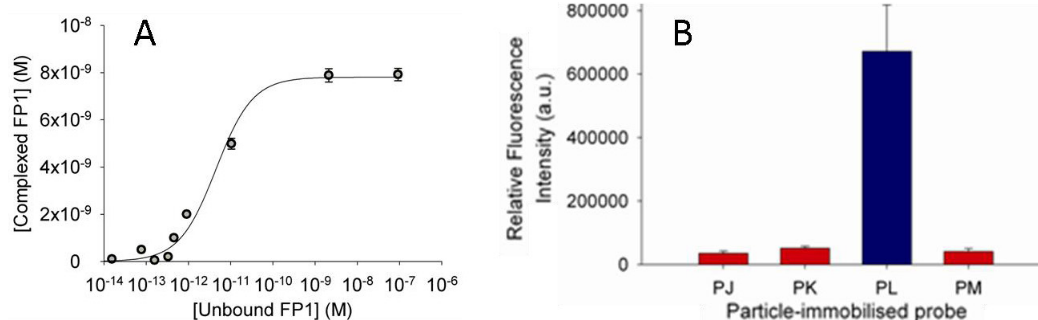

Figure 3; A) Affinity binding curve between particle-immobilized avidin and oligonucletide-linked biotin (FP1). B) Multiplex DNA hybridization assay, encoded microparticles functionalised with different oligonucleotide probes (~15 bases long, PJ – PM) were mixed and exposed to a labeled target which was complementary to PL. PJ and PM were completely non-complementary to target whilst PK represented a single-base mismatch.

Particles have been covalently functionalised with biomolecular probes and used in biological assays, a number of which are described herein. Avidin-functionalised particles have been used to quantify biotin by titration with a fluorescently labelled and biotinylated oligonucleotide probe (FP1) (Figure 3A), the system was also used to characterise the kinetic parameters of biotin-probe binding to particle-immobilised avidin. Mixtures of encoded particles, each functionalised with differing oligonucleotide sequences, have been used for the multiplex analysis of DNA samples, achieving clear discrimination between sequences differing by a single base (Figure 3B). Likewise, human, mouse and rabbit IgG antibodies have been immobilised (and encoded) and the system developed as a multiplexed immunoassay, correctly identifying mixtures of target antibodies (Figure 4).

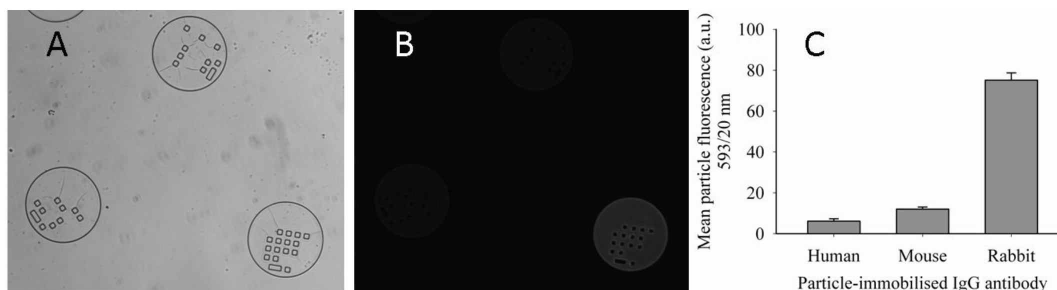

Figure 4; Multiplexed immunoassay analyzing a mixture of IgG-functionalised particles exposed to labeled target IgG. A) white light, B) fluorescence imaging, C) summary of the relative fluorescence intensities of each particle population.

A key target application for the work is as a cell screening array to assay the effects of potential drug molecules on cancer cells (in particular cancer stem cells). As a typical patient biopsy only contains approximately 5,000 cancer stem cells, encoded microparticles with their small form factor make an ideal assay platform for high throughput drug screening, whereas more traditional arrays such as 384 well plates are unsuitable due to a relatively high cell requirement. The assay format requires both the immobilization of anti-cancer-stem-cell antibodies (to capture cells) and a photo-cleavable handle used for the synthesis of cancer therapeutics (Figure 5). With cells immobilized, the drug molecules can be released from the particle resulting in a high concentration of drug in the vicinity of the cells, and by monitoring cell growth any biological effect of the drugs can be assayed. This would allow the parallel screening of hundreds of (potential) drug molecules and would be useful for *in vitro* screening during the drug discovery process. The platform is also designed to be used to help tailor cancer therapy to the patient (personalized medicine), cells from a patient will be screened against established medications to determine the most suitable combination of treatments for the individual.

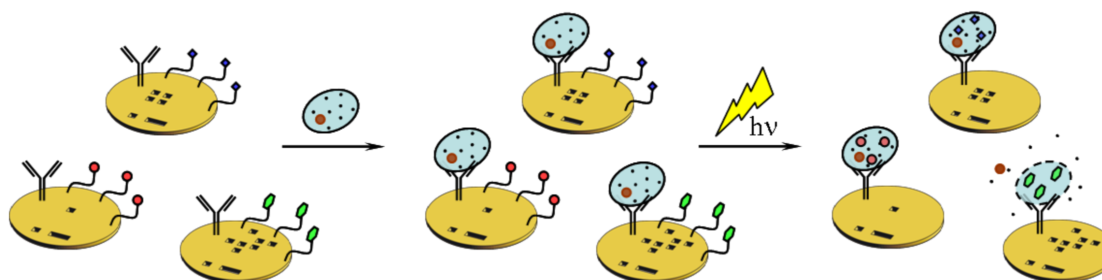

Figure 5; An overview of the cell/drug screening array being developed.

CONCLUSION

We have developed a new, very high capacity encoded microparticle suspension array and demonstrated the versatility for its adaption to use in different biological assays. Research is ongoing and has a particular focus on the development of cell screening assays for anti-cancer therapeutics.

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