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High throughput screening for biomaterials discovery

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ABSTRACT

Using microarray technologies thousands of biomedical materials can be screened in a rapid, parallel and cost effective fashion to identify the optimum candidate that fulfils a specific biomedical application. High throughput surface characterization (HTSC) of printed microarrays has played a key role in the discovery and development of biomedical materials. This review focuses on the production and HTSC of microarrays, their application in specific biomedical fields and a future perspective on the development of this technology.

1. Introduction

There are unprecedented challenges facing the research and development of pharmaceuticals. The cost to bring a new drug or biomedical device to the market continues to soar with a parallel decrease in the number of approved products [1,2]. The limited sources of materials that are suitable for biomedical applications has severely limited product development and increased the demand to find effective and rapid solutions with limited time and cost. To this end, the development of high throughput methodologies offers a potential solution to the biomaterial discovery logjam. Indeed, microarray technology has revolutionized genomic and drug discovery by enabling parallel, rapid and cost effective screening of thousands of microscopic spots attached to solid substrates [3]. High throughput methodologies are based on the parallel screening of large libraries of molecules or materials against specific biological responses, resulting in the rapid identification of lead candidates [4]. Microarrays can be fabricated by depositing 'probes' onto a substrate surface using a variety of different techniques including photolithography, electron beam lithography, dip pen lithography, soft lithography and most frequently contact or ink-jet printing [5,6].

The advances in this technology rely on the concomitant development of suitable analytical techniques that are not only sensitive and selective but also amenable to acquiring data from spatially defined locations of the order of a hundred microns dimensions in an automated fashion. The material surface region defines the physical, chemical and biological interaction between the material and the surrounding biological milieu. In order to develop a structure-function relationship, there is a need to understand the influence of the surface chemistry and topography on the material-biological function. To address this need, a collection of high throughput surface characterization (HTSC) techniques have been developed and applied to microarrays [7]. With the help of chemometric analytical techniques, HTSC approaches are capable of transforming HT screening from the random screening of a polymer library to a more predictive method of screening. This is achieved by correlating the major variances between the material properties with the observed biological performance, which can then direct the design of future candidate materials [8,9]. HTSC is amenable to any HT platform that presents a library of molecules or materials on a single substrate.

This review will initially focus on the common methods for microarray production, including an outline of the HTSC and statistical methodologies that may be used to investigate the collected data and aid in the identification of the "lead" materials. The utilization of polymer microarray technology for the screening of new materials for specific biomedical applications will then be described. Finally, future opportunities in the field of HT biomaterials discovery will be discussed.

2. Material microarrays

A microarray is a platform where hundreds to thousands of unique 'probes' are presented at unique, discrete and addressable locations. The key advantage of microarrays is that each probe represents a unique experiment run in parallel with each other probe. This results in hundreds to thousands of measurements on a system being conducted in a single screen, which can lead to a rapid advance in understanding and concomitant development of a system. In order to achieve this it

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must be possible to rapidly assess the interaction of the probe with the sample in a spatially defined manner such that the location of a measurement can reliably be linked back to a specific probe. For example, in the case of DNA microarrays thousands of unique nucleotide sequences are presented at specific locations. Typically a DNA microarray experiment screens for the hybridisation of target DNA strands with probe DNA attached to the microarray surface. This is often detected using a fluorescence scanner or fluorescence microscopy. In order for this readout to be successful the fluorescence must be discrete: bright on a dark background and separate from other spots. All these aspects must be considered for the production of an effective microarray. The various design elements of a microarray will now be presented.

2.1. Substrate modification

Substrate chemistry and topography play vital roles in the success of all microarray technology, ranging from small molecules to cellular microarrays. Microarray formation is typically achieved by the transfer of a liquid onto a solid substrate to form spots, whereby the spot shape and size is controlled by liquid-solid interfacial interactions and the evaporation rate [10]. This, in turn, will have a direct effect on the microarray performance. Irregular spot size or a distorted shape complicates the automated readout from a microarray, which is reliant on homogeneous spot morphology. Furthermore, the substrate plays a key role in both the immobilization of printed material and the minimization of background signal in the subsequent bioassay. When producing material microarrays the substrate materials must be judiciously chosen to:

1. minimize defects within the printed materials [11]
2. ensure good adhesion/immobilization of printed material
3. prevent non-specific adsorption during the bioassay of choice [12].

It has long been recognised that the immobilization strategies for biomolecules employed for DNA and protein arrays are fundamental to their use in DNA sequencing and gene expression studies as it directly influences the interaction between the probe and the analyte [13]. Similarly, in the case of materials discovery, the use of physical adsorption and chemical cross-linking are common techniques used for immobilization where the substrate chemistry can strongly influence the immobilization process. Poly(hydroxyethyl methacrylate) (pHEMA) is a common slide coating material that is commercially available [7]. The pHEMA coating provides a polymer mesh that allows physical entrapment of the printed material whilst also inhibiting cell growth [7,14]. Agarose gel has also been used as a substrate coating as they possess antifouling properties known to suppress cellular adhesion [15]. Similar to pHEMA, agarose coatings rely on the physical entrapment to immobilize printed material. Both agarose and pHEMA create a hydrated compliant surface suitable for biomaterial microarrays.

Another approach to create soft polymer substrates was developed by Gupta et al. [16]. This strategy was achieved in four steps as outlined schematically in Fig. 1. Poly(ethylene glycol) (PEG) was selected as the main component of the gel substrate as it inhibits protein and cell adhesion and maintains a hydrated soft environment. The use of thiol-ene allowed the covalent immobilization of a large selection of molecules at the surface of the hydrogel.

The "coffee ring" or "donut shape" is a well-known defect observed that occurs when a droplet containing a solute dries with a pinned droplet-surface interface [17,18]. One strategy to combat these defect was proposed by Ressine et al., who demonstrated a relationship between the surface wetting and the formation of "coffee rings" [19]. Wetting is defined as the interfacial interaction of a liquid with a solid surface to minimize the overall free energy [20]. Using a macro/nano-structured silicon substrate wetting could be minimized, confining the droplet to smaller area and, thus, minimizing the formation of "coffee ring" defects (Fig. 2).

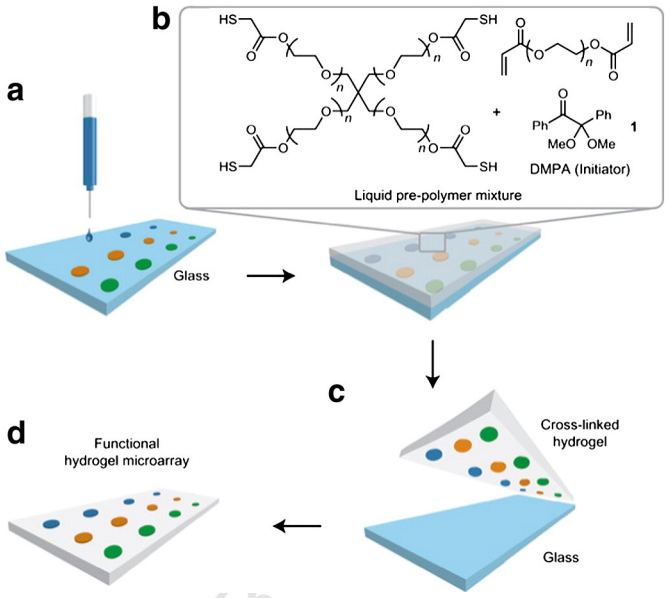

Fig. 1. Illustration shows the strategy to produce functional hydrogel microarray. (a) Thiol- or alkene- functional biomolecules microarray printed on plain glass slide using ink-jet printing. (b) Pouring a liquid thiol-ene pre-polymer mixture solution. (c) And treated for 2 minutes under 365 nm light. (d) Peeling of the gel from the glass slide to expose the functional microarray [16].

2.2. Microarray production

Microarray production has been achieved using different patterning methods, such as photolithography, soft-lithography and electron beam lithography [5], but are most commonly fabricated by direct writing techniques in the form of contact and non-contact printing (Fig. 3) [21,22]. Direct writing technique use the robotic movement of the printing head to achieve surface patterning and are most suitable for microarray formation as the resultant patterns can readily include multiple components and pattern designs can be altered in a facile manner [5].

2.2.1. Direct contact printing

Direct contact printing is based upon loading a pin with an "ink" that is then transferred to a substrate by direct contact, or close-to-contact. The pins are attached to a high-precision robotic arm that facilitates the precise location of the microarray spotting. The pin is either a solid or grooved pin, similar to an ink quill, and is typically made from stainless steel. A solid pin is easy to clean and suitable for printing proteins and other sticky samples whilst a grooved pin reduces the time of printing by increasing the number of spots printed from a single "inking" [23]. The spot size is primarily determined by the size and the shape of the printing pin. This method has many advantages, including microarray spot reproducibility, adaptability to a wide range of solutions, relatively facile maintenance and an easy to clean system. On the other hand, the volume deposited by a pin is not easily tuneable and the direct contact of the pin with the substrate can result in surface damage, for example on 3-D gel coated substrates [6].

The first use of contact printing in genetic analysis was by Brown et al. at Stanford in 1995 [24]. Brown and co-workers developed microarrays for quantitative monitoring of gene expression of a large library of complimentary DNA simultaneously that were printed onto glass slides. This technology was adapted to proteins by MacBeath and Schreiber [25], and later Anderson et al. used contact printing to produce a microarray of polymers [7]. This platform was used to study human embryonic stem (hES) cell growth and has also been applied to maturation and phagocytosis of dendritic cells [26], materials resistant to bacteria [27–29], switchable materials [30], platlet activation [31], cell sorting [32–34], hepatocytes and toxicity models [35,36],

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