Chapter 4
Polymer Microarrays for High Throughput Biomaterials Discovery

Andrew L. Hook

Abstract High throughput screening has emerged as a powerful technique for discovering novel medical tools and therapies. This is particularly true for biomaterials that are applied to poorly understood biological–material systems. The polymer microarray format has become a key enabling tool for high throughput materials discovery, whereby hundreds to thousands of unique polymers can be presented on a single glass slide and screened in parallel for biological interactions of interest. This approach has successfully been utilized to develop the surface chemistry, topography, bioactivity, and mechanical properties of biomaterials as well as allowing the development of 3D culture systems. In order to optimize a polymer microarray for a given application the substrate used, the coating on the substrate, and the material library screened must be carefully selected. Furthermore, development of suitable biological assays with high throughput readouts is imperative for expanding the applications of polymer microarrays. The biological systems screened on this format include supporting cell attachment and outgrowth, maturation and phagocytosis of dendritic cells, materials resistant to microbes, switchable materials, platelet activation, cell sorting, hepatocytes and toxicity models, and cell transfection. Further to the discovery and development of biomaterials, the large datasets when coupled with modelling techniques can establish structure–function relationships that help elucidate the underlying biological–material interactions. Continued development of microarray designs and high throughput biological assays compatible with the format will lead to the discovery of new biomaterials that exhibit unprecedented control over the biological systems they are designed to function in.

A.L. Hook (✉)
Laboratory of Biophysics and Surface Analysis, University of Nottingham, Nottingham NG7 2RD, UK
e-mail: Andrew.Hook@nottingham.ac.uk

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Abbreviations

2D/3D	2/3 Dimensions
AFM	Atomic force microscopy
BMDC	Bone marrow dendritic cells
ECM	Extracellular matrix
FBS	Fetal bovine serum
hEB	Human embryoid bodies
hES	Human embryonic stem cell
hMSC	Human mesenchymal stem cell
mES	Mouse embryonic stem cell
PEG	Poly(ethylene glycol)
pHEMA	Poly(hydroxyethyl methacrylate)
PLS	Partial least square
SEE	Standard error of estimation
SPR	Surface plasmon resonance
ToF-SIMS	Time-of-flight secondary ion mass spectrometry
WCA	Water contact angle
XPS	X-ray photoelectron spectroscopy

4.1 Introduction

Hypothesis led research has been a foundation of scientific endeavour since the seventeenth century. In some cases the level of understanding is not at a sufficient level to enable the formulation of pertinent hypotheses to effectively promote a field of science. In these cases a complementary data-driven approach has emerged, where the starting point is the accumulation of data that will, in turn, lead to new ideas [1, 2]. This approach has been applied to drug discovery projects that have used high throughput methods to screen for novel drugs [3–5]. High throughput screening has also been applied successfully to genomics [6–11], which has driven the emergence of the microarray format as a screening tool. On a microarray hundreds to thousands of unique analytes can be displayed on a sample slide in addressable locations and assessed in parallel. More recently, cell-based microarrays, where cells themselves are arrayed onto a slide or cells are allowed to interact with the arrayed analyte, have been developed to explore gene expression in systems where all the cell machinery is present to ensure correct protein function [12–16]. A number of studies have applied microarrays to materials, with a focus on the discovery of novel materials that are ideally suited to a given application. It is this body of work that this chapter will cover.

4.2 The Development of a Material Microarray

The concept of a materials array was first reported in 1995 [17], whereupon an array of solid-state materials was produced by vacuum deposition onto areas selected by the use of a series of binary masks. A 128-member array was produced from seven precursor materials and two novel superconducting films, BiSrCaCuO and YBaCuO, were identified. Typical sample size was 2×2 mm, however, samples as small as 200×200 μm were also generated.

Soon after in 1998 the concept of screening a combinatorial library of polymeric materials was demonstrated [18]. A library of 112 degradable polyarylates was prepared by copolymerizing 14 different diphenols with 8 different aliphatic diacids, and the water contact angle (WCA), glass transition temperature, mechanical properties, and fibroblast attachment and proliferation were assessed for each material. This study demonstrated how screening a combinatorial library of materials could be used to develop structure–property relationships. Generally cell proliferation decreased with increasing WCA; however, cell proliferation was unaltered by changes in surface hydrophobicity for materials where methylene groups in the polymer backbone were substituted with an oxygen atom. This result suggested that fibroblast proliferation was sensitive to subtle chemical changes that are not associated with changes in WCA. The throughput of this study was limited by the sample format: polymers in the library were spin coated onto glass cover slips and assessed individually. To circumvent this constraint, the polymer microarray was developed, allowing hundreds to thousands of unique polymers to be presented on a single glass slide [19–21]. Typically materials are presented as 300–500 μm diameter spots, allowing for approximately 2000 materials to be presented simultaneously. Allowing for replicate measurements, over 600 unique polymer compositions can be screened in a single assay. A table listing the key advances in the use of material microarrays for biomaterials discovery are presented in Table 4.1.

4.2.1 Substrate Preparation

A number of factors must be considered when designing a polymer microarray and applying it to the high throughput discovery of biomaterials [22], which are summarized in Fig. 4.1. This includes the identity of the substrate, the coating on the substrate, the members of the polymer library, and the biological assay which can couple with the microarray.

4.2.1.1 Substrate Material

The key substrate requirement is the ease with which it can be applied to the biological assay and associated high throughput readout. The substrate of choice is a glass microscope slide due to the range of stage holders, scanners, and microscopes

Table 4.1 Description of key advances in biomaterial microarray technology, in chronological order

Advancement	Description of study	Reference
Demonstrate a microarray of materials	Investigation of the formation of a combinatorial library of ceramic materials in an array format	Xiang et al. (1995) [17]
Demonstrate screening a combinatorial library of polymers for biological performance as an approach to biomaterials design	Study of the proliferation of fibroblasts on a combinatorial library of polymers.	Brocchini et al., (1998) [47]
Produce a microarray of polymers for screening cell response. Demonstrate in situ polymerization	Study of stem cell attachment and differentiation on a polymeric library	Anderson et al. (2004) [19]
Combinatorial screen of biological polymers	5 ECM proteins were printed as 32 different combinations to assess the attachment of rat hepatocytes and mouse embryonic stem cells (mES)	Flaim et al. (2005) [38]
Demonstration of printing pre-synthesized polymers for formation of a materials microarray	Study the attachment of stem cells with polymer materials	Anderson et al. (2005) [20]
	Study attachment of human renal tubular cells	Tourniaire et al. (2006) [21]
Development of the high throughput surface characterization of polymer microarrays	Study chemical and wettability properties of a polymer microarray	Urquhart et al. (2007) [78]
	Assessment of the binding of 3 different proteins to 21 polymer spots using SPRi	Hook et al. (2009) [64]
	AFM screen of a 576 member polymer microarray, identifying materials with switchable topographies	Hook et al. (2011) [81]
Establish PLS as a method for modelling a univariate dataset with multivariate chemical information	Study comparing wettability with the chemical functionality of a polymer spot as measured by ToF-SIMS	Urquhart et al. (2008) [79]
Production of a polymer microarray using ink-jet printing	Formation of a hydrogel microarray	Zhang et al. (2008) [29]
Development of a high throughput 3D culture system	hMSCs were fixed into microwells within a PEGDA matrix. The combined influence of the cell adhesive peptides, RGDSP and IKVAV, was assessed	Jongpaiboonkit et al. (2008) [76, 77]
Production of a topographical array	2,176 unique topographical units derived from circles, isosceles triangles, and rectangles were produced and used to study the influence of topography on the bioactivity of hMSCs	Unadkat et al. (2011) [32]
Application of polymer microarrays to discover materials that resist bacterial attachment	Screen of 370 polyurethanes with two bacterial strains	Pernagallo et al. (2011) [45]
	Assessment of the attachment of three bacterial strains to >700 polymers using a multi-generation-screening methodology	Hook et al. (2012) [33]
Model biological properties of polymer library from calculated molecular descriptors	hEB adhesion to a 496-member polymer library was modelled using a nonlinear Bayesian neural network model	Epa et al. (2012) [85]

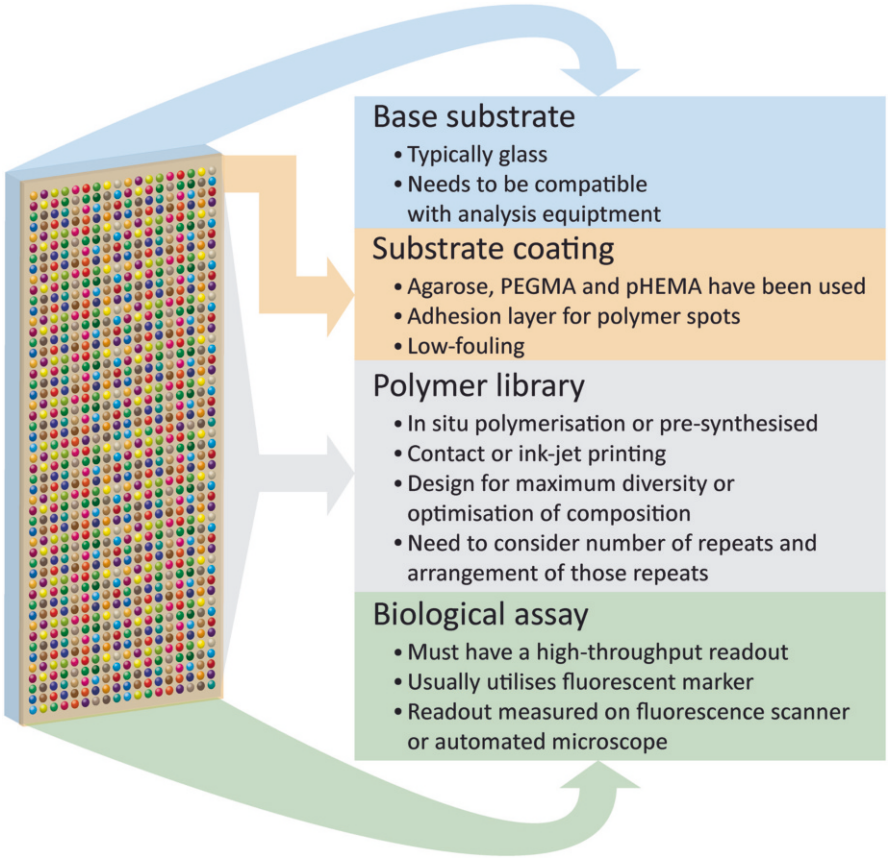

Fig. 4.1 The key aspects of polymer microarray design. Schematic depiction of a polymer microarray where each colored dot represents a unique polymer. The various aspects of the polymer microarray that should be considered when designing an array are highlighted

that have been adapted to this format. Furthermore, the slide is transparent, which allows cells growing on the slide to be easily assessed using light microscopy. The glass slide is also widely compatible with most cell culture methods, enabling a diverse range of biological assays to be applied. However, polymer microarrays can equally be applied to other materials, for example directly onto polystyrene-based tissue cultureware, which may enable the polymer microarray format to be accessible to a greater number of biological assays.

4.2.1.2 Substrate Coating

The surface chemistry of the underlying substrate plays an important role in the formation of a microarray as well as the success of subsequent bioassays.

The substrate coating must be both adherent to the materials printed onto it and resistant to the attachment of biomolecules and living cells (non-fouling) in order to optimize the signal-to-noise ratio of any biological assay and prevent cross-talk from one spot to another. A number of different surface coatings have been developed with these needs in mind, with emphasis generally placed on using cheap and robust-coating methodologies. One such example is the dip-coating of a commercially available epoxide functionalized slide into a poly(hydroxyethyl methacrylate) (pHEMA) solution [19, 20]. Dip-coating is a simple technique that can easily be achieved in any laboratory, making this modification approach accessible. pHEMA is an attractive coating as it is able to resist cell attachment as well as providing a matrix into which printed material can penetrate and physically entangle to improve the stability of the spots [19]. Agarose is an alternative coating that may be applied by dip-coating using commercial aminoalkylsilanated slides [21]. Agarose and pHEMA are intended to prevent cell attachment whilst being nontoxic. Poly(ethylene glycol) (PEG)-based coatings have also been widely used for producing low-fouling coatings and are widely used to effectively inhibit biomolecular adsorption [23]. In order to produce a PEG-modified surface, a methodology has been proposed by the groups of Griesser, Thissen, and Voelcker in which a PEG layer is reacted with an amine plasma polymer-coated slide [24–26]. Key to the success of a PEG coating is the production of a dense, brush-like layer. Polymers arrayed onto this surface can be covalently attached by modifying the polymer with a cross-linker or incorporating epoxy groups into the PEG layer. For example, a grafting-to approach was accomplished by producing a multifunctional coating with both PEG groups, which provided a low-fouling background, and epoxy groups, which enabled subsequently spotted biomolecules, synthetic and natural polymers to be covalently linked to the surface in a suitable manner for subsequent cell attachment assays using HeLa cells [26]. This is advantageous because the structure of the arrayed material is unaltered; however, the substrate chemistry becomes limited. In this particular study a plasma polymer slide-coating approach was used, which is advantageous as it can be applied to almost any base substrate.

4.2.2 Material Microarray Production

The creation of material arrays requires a combinatorial library of materials to be generated and positioned at addressable locations on a substrate surface. The library can be generated prior to the synthesis of the array, for example by printing pre-synthesized polymers, or by printing monomer solutions that are polymerized in situ. Once a material has been deposited onto the substrate it needs to adhere to the surface with sufficient strength and durability that it can resist the biological evaluation process. This may be achieved by physical entanglement, non-covalent or covalent interactions. A schematic depicting the printing of a polymer microarray is shown in Fig. 4.2.

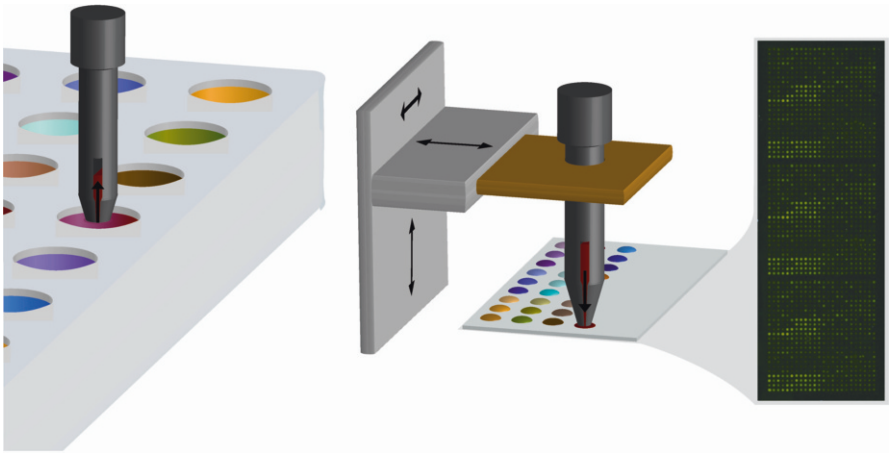

Fig. 4.2 Schematic of the formation of a polymer microarray using contact printing with a quilled pin. Initially the pin is dipped into a well-containing monomer or polymer solution and then moved using a robot to the substrate to transfer the solution. The pin can be replaced with an ink-jet nozzle. On the right is a fluorescence image of a polymer microarray formed by this method. The fluorescence seen is due to autofluorescence of the resultant polymer spots

4.2.2.1 Microarray Formation

Polymer microarrays are typically formed by either contact or ink-jet printing. Contact printing involves the use of a robot moving a metallic pin, which is dipped into a solution and then spotted onto the substrate surface by making contact. The pin may either be solid or contain a groove analogous to an ink quill, such that the solution is drawn up into the pin and the spotted material is taken from this reservoir. The ink quill design has the advantage that more spots can be printed from a single dip in the solution. Contact printing is attractive for ease of transfer and the absence of small apertures in the system that can become blocked. The size and shape of the pin used is the determining factor in the resultant spot size. Contact printing was first used to produce an acrylate microarray on a pHEMA-coated slide by Anderson et al. using in situ polymerization [19]. This was achieved in five steps; mixing monomers at various ratios in a source plate, printing acrylate monomers with an initiator, activation of initiator upon UV irradiation, polymerization of the monomer, and finally removal of the solvent. This resulted in stable, covalently cross-linked polymer spots. By premixing the various monomers at a set ratio, a large polymer library of 576 materials was readily achieved. This approach decreases the time required for polymer library synthesis and microarray formation by combining these processes and also allows materials that cannot be printed, such as cross-linked polymers, to be included in the polymer library. However, the polymer synthesis conditions are likely to differ when materials are produced on a larger scale.

An alternative approach is to print a library of pre-synthesized materials [20, 21], which allows standard polymer characterization techniques such as gel permeation chromatography, nuclear magnetic resonance, and differential scanning calorimetry to be utilized without having to extract minute amounts of polymer from the slide. However, this method is restricted to polymers that can be solvated, and is considerably more laborious for the initial creation of the polymer library.

An alternative to contact-printing is ink-jet printing. In this approach a nozzle is used to draw up, then eject polymer solution onto the substrate at a defined droplet volume. The volume of the droplet and the surface energy of the substrate material and the printed solution determine the resulting spot size. This technique enables the precise control of the amount of material deposited and avoids contact with the surface. However, solutions of different viscosities and surface energies cannot easily be printed under similar conditions, limiting the number of different materials that can be included in a single printing run. In addition, the small orifices are susceptible to blockage by dust particles or aggregates of material [27]. This method can be cost-effective and readily accessible to most laboratories by using modified, commercially available ink-jet printers [28].

Ink-jet printing was first used to prepare a polymer array from individually deposited monomer for water soluble acrylamide monomers to form hydrogels [29]. Three monomers were deposited sequentially onto the same position, with a solution containing a catalyst to initiate the reaction being printed subsequently. This *drop-in-drop* mixing approach required that the solvent, water in this case, did not evaporate before the mixing was complete. The turbulence induced by the printing procedure resulted in the complete mixing of the monomers within the drops after 1.5 min. This approach was used to create an array comprising 36 different materials from six monomers. The polymers produced using the on-slide mixing methodology must be carefully assessed as they are likely to differ for each monomer, polymerization, and printing system employed.

4.2.2.2 The Design of the Polymer Library

When designing the components of a polymer microarray, careful consideration must be given to the objective of the experiment. In cases where the aim of an experiment is to *generate new data* for a poorly understood system the polymer library should be designed to *maximize the diversity* of chemical and/or physical properties represented. This can be achieved by selecting a large number of unique base components [30], but can equally be achieved by producing a combinatorial library of polymers by mixing a smaller number of base components at various ratios [31, 32]. In some cases sufficient understanding of the biological–material interaction can exist to allow the formulation of a hypothesis that can be tested by the judicious design of a polymer library [30, 33, 34]. This may include the *optimization* of a “hit” formulation identified from a previous screen, or testing a structure–function relationship. Typically these material libraries include subsets of polymers formed from

a pair of base components where the composition of each component is varied systematically and sequentially. This results in material gradients that are formed from discrete units rather than a continuously changing material. It is prudent to randomize the position of compositionally similar materials (including replicates) on an array to avoid the occurrence of pseudo-trends that result from experimental conditions such as the inhomogeneous distribution of cells within a cell culture well. Gradients are highly useful tools for the optimization of materials, but have limited applicability to material discovery applications. Hansen et al. [35] combined gradients and microarrays, forming an array of polymer gradients. This enabled both the combinatorial screen of polymers and also compositional optimization. Successful formation of the arrays was assessed by X-ray photoelectron spectroscopy (XPS) and also through the introduction of a fluorescently tagged monomer, allowing the gradient to be tracked by fluorescence microscopy. Onto a single glass slide, 24 monomers were printed as 84 pairs to produce 84 polymer gradients. The array of gradients was used to assess the adhesion of HeLa and K562 cells.

Both design strategies, *maximizing diversity* or *optimizing a composition*, are complementary and can be utilized to employ a multi-generation approach to materials discovery whereby the first-generation array is designed to screen a large diversity of chemical and/or physical properties and subsequent generations seek to optimize hit formulations [33, 34].

4.3 Biological Application of Material Microarrays

The final consideration for applying polymer microarrays to the discovery of biomaterials is the design of a suitable bioassay that is compatible with the high throughput format. To achieve this, the key requirement is the inclusion of a suitable readout that can be assessed in an automated fashion. Typically, a fluorescence marker is used that can be measured using an automated fluorescence microscope or a fluorescence scanner. Utilizing this general strategy polymer microarrays have been successfully used to probe a diverse range of biological systems including supporting stem cell outgrowth [19, 31, 32, 36–43], maturation and phagocytosis of dendritic cells [44], materials resistant to bacteria [30, 33, 45], switchable materials [34, 43, 46], platelet activation [47], cell sorting [39, 48, 49], hepatocytes and toxicity models [38, 50], human skeletal cell attachment [49, 51], endothelization [52], *giardia lamblia* material interactions [53], cell transfection [13, 14, 54], and *Cryptosporidium parvum* material interactions [55].

The development of novel biomaterials has focussed on a number of key aspects, which are depicted schematically in Fig. 4.3. This includes probing cell–material interactions, including biological recognition of surface-bound biomolecules, the response of cells to surface chemistry and topography, as well as mechanical properties of the biomaterial and whether the cell culture system is 2D/3D (2/3 dimensions). The key studies that have explored these aspects are discussed below.

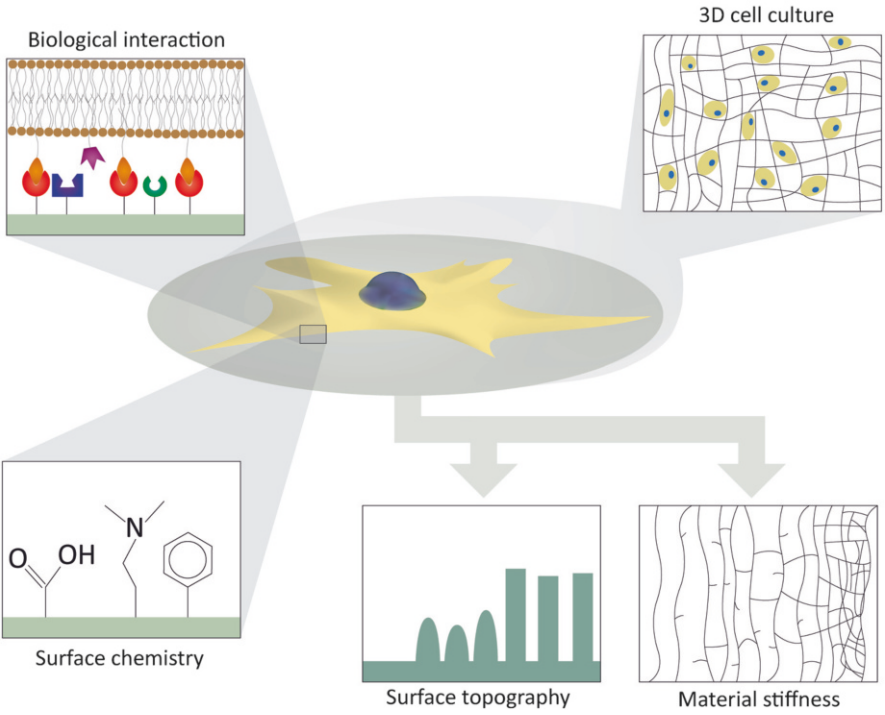

Fig. 4.3 Key aspects of biomaterials development explored using polymer microarrays. The use of polymer microarrays to discover novel biomaterials focusses on exploring cell–material interactions. The various aspects of these interactions that have been investigated are highlighted

4.3.1 Synthetic Polymers

In the pioneering study by Anderson et al. [19], a polymer microarray containing 496 unique polymers was produced from 24 monomers to screen for materials that support human embryonic stem cell (hES) attachment. This work demonstrated the large number of biological–material interactions that could be investigated in parallel. A number of different responses of hES cells derived from human embryoid bodies (hEB) were observed, including cell attachment, no attachment, and directed differentiation. Subsequent work targeted new materials that were able to support the clonal growth of hES in a xeno-free environment [31]. Stem cells hold enormous potential for regenerative medicine and tissue-engineering applications as they are able to differentiate into any cell within the body. However, methods to culture hES rely on animal products, typically using matrigel as a cell adhesive matrix or a layer of "feeder" mouse embryonic fibroblast cells. This limits the ability to expand these cells in vitro for clinical use. To identify xeno-free materials able to support stem cell attachment and expansion hES were cultured with an array of 496 materials produced

by mixing 22 monomers at various ratios. Protein readily adsorbs to the surfaces of materials, thus, in most cell culture conditions the attachment of cells is regulated by the intermediate adsorbed protein layer [31]. Thus, the microarray was pre-adsorbed with laminin, fibronectin, fetal bovine serum, and bovine serum albumin to study how different proteins alter cell attachment. Fetal bovine serum (FBS)-coated materials resulted in the highest level of attachment without initiating cell differentiation, as determined by the highest number of cells expressing Oct4 (a marker of pluripotent cells) [56]. The "hit" materials identified typically contained a large number of acrylate groups suggesting the degree of cross-linking plays a role on cell attachment although a direct correlation with material hardness was not observed. For subsequent scaled-out experiments the FBS pre-adsorption was successfully replaced with a vitronectin pre-adsorption step and culture was conducted in mTeSR1, a fully defined medium, resulting in a xeno-free substrate for cell culture. A long-term cell culture was supported under these conditions for a month (five passages).

Zhang et al. [43] sought to develop a material that would allow stem cells to detach from a surface upon a reduction in temperature rather than using chemical, enzymatic or mechanical methods of removal. To achieve this, an array of 609 unique acrylate and acrylamide polymers was prepared composed of various mixtures of 18 monomer base units. Initially hits were selected that could support hES culture up to 7 days. The top 25 polymers were then scaled-up and assessed for their ability to release attached cells upon a reduction in temperature. The top three polymers that could support hES culture and achieved a thermoresponsive release all contained the monomers 2-(acryloyloxyethyl) trimethylammonium chloride and 2-(diethylamino)ethyl acrylate. On a copolymer of these two monomers hES were passaged 20 times utilizing a temperature reduction to release attached cells. Cell growth was typically slower on this polymer than compared to Matrigel. Cells grown on the thermoresponsive polymer were positive for Nanog and Oct3/4, which are markers for pluripotent stem cells.

Polymer microarrays have been applied to a number of other eukaryotic cells, for example dendritic cells [44]. These cells play a key role in regulating the immune response, and are targets for vaccine design. These cells are usually sourced as bone marrow dendritic cells (BMDCs), immature dendritic cells that are highly phagocytic, however, the cells are highly susceptible to maturation, preventing them from capturing antigens by phagocytosis, and are difficult to immobilize as this event can also cause their maturation. Effective immobilization of BMDCs whilst maintaining their immature state would enable the presentation of these cells for phenotypic studies, or allow cell-based assays that would lead to vaccine development. Mant et al. [44] used polymer microarrays to screen 120 polyurethanes to identify materials for the immobilization of BMDCs labelled with flurorescein, enabling a rapid fluorescent read-out of cell numbers. Hit polymers, all of which contained poly(tetramethylene glycol), were spin coated onto glass slides and attached cells were assessed for their ability to prevent maturation of the BMDCs by antibody staining for CD11c, a marker for this cell line. The initial BMDC culture was 90 % pure, and once attached the majority (approximately 70 %) of cells were maintained as BMDCs. Phagocytosis was also assessed of attached cells and compared with

cells attached to poly-L-lysine, the traditional cell adhesive polymer coating. Cells were incubated with 3 μm latex microspheres and the degree of phagocytosis was assessed by counting the number of microspheres internalized by the cells. The maximum number of internalized microspheres was 3.6 per cell, compared with 1.3 for cells cultured on poly-L-lysine-coated samples. The authors noted improved cells attachment correlated with a decreased number of phagocytized microspheres, suggesting that the two processes share similar signalling pathways and are, thus, directly in competition.

In another example, Hansen et al. [47] used polymer microarrays to search for a synthetic polymer that would activate platelets to cause hemostasis to replace costly naturally derived products including materials based upon fibrin, chitosan, or cellulose. This is towards the goal of improving wound treatment by providing materials that cause the rapid and effective hemostasis of injuries. In this study 291 unique polyacrylates were incubated with platelet rich plasma for 30 min, after which the attached platelets were stained with antibodies CD41-FITC, which recognizes GPIIb a protein present on all platelets [57], and CD62P-PE, which is a marker specific to activated platelets [58]. In this experiment the binding of platelets to a material was synonymous with platelet activation. The "hit" polymer showed a fourfold higher activation compared with collagen. All "hit" polymers contained a sterically non-hindered tertiary amine, suggesting that surface charge may play a role in successfully activating platelets.

In addition to studies investigating the attachment and interaction of eukaryotic cells with polymers, material microarrays have also been used to discover novel polymers that resist the attachment of prokaryotic cells [30, 33, 45]. Bacterial attachment to biodevices can lead to the formation of biofilms, within which bacteria are 1,000 times more resistant to the host immune system and antibiotics [59]. Biofilms are estimated to be associated with 80 % of hospital acquired infections [60]. It is, therefore, of interest to discover new materials that can be used as biodevices to prevent bacterial attachment, subsequent biofilm formation and hence reduce the incidence of device-associated infections. Hook et al. [33] screened a library of 496 unique polymers to identify materials that could resist the attachment of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. Each of these strains was transfected with a plasmid encoding for a green fluorescing protein in order to provide a rapid fluorescent readout of bacterial numbers using a fluorescence scanner. This study used a multiple generation approach, whereby bacterial attachment was initially assessed on the first-generation array designed to screen a large chemical variance, whilst the second-generation array sought to optimize the composition of "hit" polymers. Using this approach, a new class of materials resistant to bacteria attachment was discovered. This underlines the importance of high throughput screening studies, where new material–biological interactions can be uncovered that would not be predicted from the current understanding. The new class of materials consisted of a hydrophilic ester group combined with cyclic hydrocarbon pendant groups to create weak amphiphiles. This is significantly different from the alternative anti-adhesive materials (PEG and zwitterions) that are highly hydrophilic and are able to resist bacteria on the basis of exclusion caused by a highly organized

water layer [61–63]. The weakly amphiphilic polymer was able to reduce coverage of bacteria by up to 96 % compared with silicone, and significantly, when scaled up and applied as a coating the anti-adhesive polymer was able to reduce bacteria numbers by an order of magnitude in vivo in a mouse model. An exhaustive search of the polyacrylate combinatorial space was conducted in a subsequent study that again utilized a multiple generation approach [30]. One hundred and sixteen monomers were used to produce 1,273 unique materials and in over 10,000 separate assays an optimal antibacterial polymer was identified with up to 99 % reduction in bacterial coverage compared with silicone. This study highlighted the efficacy of the new class of weakly amphiphilic polymers at resisting bacterial attachment.

4.3.2 Biological Polymers

High throughput screens of material–cell interactions are not limited to synthetic polymers but can equally be applied to biological polymers, and can offer greater control of cells due to the inherent biological activity of the polymers. These studies have focussed on cell adhesive molecules such as extracellular matrix (ECM) proteins and cell adhesive-derived biomolecules. Pioneering studies were performed by Flaim et al. [38]. In this study, collagen I, collagen III, collagen IV, laminin, and fibronectin were printed as 32 different combinations onto a hydrogel-coated glass slide to assess the attachment of rat hepatocytes and mouse embryonic stem cells (mES). After hepatocyte attachment cells were immunostained for albumin as a marker of liver-specific function. The highest albumin signals were associated with ECM combinations that contained collagen IV. However, albumin signal did not correlate with the amount of collagen when hepatocytes were cultured on serially diluted collagen IV without other ECM components. This result showed that two ECM components could act synergistically to produce an effect that would not be predicted if the components were assessed separately [38]. As another example, Fn and collagen III individually induced a negative effect on hepatocyte function compared to the average response to other ECM components present on the array, however, when combined with collagen I a positive effect on hepatocyte function was observed. The occurrence of synergistic effects is a key justification for the use of combinatorial libraries of polymers for high throughput screens. When applied to mES, the combination of collagen I and Fn was best able to instigate the differentiation of ES cells to an early hepatic fate, as observed by increased levels of B-galactosidase. This demonstrated the possibility of screening ES cells with a materials microarray to identify surfaces that trigger differentiation pathways in a controlled manner. The stem cell niche was probed further by Gobaa et al. [40] by printing biomolecules directly onto silicon pillars that were subsequently used as a microstamp to transfer the biomolecule pattern onto a PEG hydrogel. Simultaneously the hydrogel was embossed to create microwells that were 35 μm deep and 450 μm in diameter. Biomolecules were tethered to the bottom of the wells using either a nonspecific cross-linker or through the interaction of an Fc tag with protein A or G.

The stiffness of the gel was easily altered by changing the amount of cross-linker, allowing for both biomolecule composition and material stiffness to be assessed in parallel. This platform was used to screen an array of 23 candidate proteins with mouse neural stem cells. This study identified Jagged 1 and DLL4 as the main inducers of neurosphere growth, consistent with the previous literature.

A further aim of high throughput material screens has been to identify specific cell–material interactions that can allow certain cell types to be selected from mixed populations. One specific area of investigation has been the isolation of spermatogonia stem cells from primary sources [26, 39, 48, 64]. These cells are pluripotent [65] and can be used for germ cell transfer technologies, however, little is known of their cell surface markers that could be used for their isolation from primary sources and subsequent culture. A microarray was formed from 27 biological factors that represented a range of potential germ cell-recognition properties. This included ECM proteins, cell-surface active growth factors, lectins, antibodies against cell surface proteins, as well as amine-functional synthetic polymers. The microarrays were incubated with mixed cell populations derived from freshly isolated bovine testicular tissue. Cells positive for Plzf (a nuclear transcription factor present in type A spermatogonia [66]) were detected by immunohistochemistry. A vimentin antibody was used to identify Sertoli and myoid cell populations [67]. The lectin *Dolichos biflorus* was identified to successfully enrich spermatogonia stem cells from sertoli and myoid cells, whereas the lectins *Pisum sativum* and Concanavalin A preferentially attached sertoli and myoid cells over the spermatogonia stem cells, thus, may be useful to enrich spermatogonia stem cells through negative selection.

4.3.3 Topography

The topography of a surface plays a key role in the attachment and behavior of cells; contact guidance of cells along grooves has been observed down to nanoscale features [68, 69], altering the roughness of prosthetic implants has been a key parameter to improve bone-implant contact [70–72], and it has also been demonstrated that surface roughness can influence the differentiation of preosteoblast cells [73]. Thus, surface topography is a key parameter to be included in biomaterial development programs and, as such, high throughput tools for screening surface topography will be invaluable for furthering the understanding of cell–material interactions.

Unadkat et al. [32] reported on a high throughput platform for screening the response of cells to various topographic features. Initially, 2,176 unique topographical units derived from circles, isosceles triangles, and rectangles were designed using mathematical algorithms that arranged these three base shapes within an imaginary square. The three base shapes were selected because they allow the inclusion of large smooth areas (circles), angles (triangles), and stretched elements (rectangles) within the resultant topographical features. A silicon mould of these patterns was produced by photolithography and used to emboss poly(lactic acid) films. These films were used to study the influence of topography on the bioactivity of

human mesenchymal stem cells (hMSCs). Varying levels of alkaline phosphatase, which is a marker for early osteogenic differentiation [74], were observed for cells grown on the varied topographical patterns demonstrating that various topographies can induce cellular differentiation. Increased cell spreading was accompanied with osteogenic differentiation.

In another strategy to investigate material topography, Adler et al. [54] produced an array of pits, either square or circular, using chemical etching through a resist. Patterns were made with poly(dimethyl siloxane) patterned from a metallic master. Ten patterns of pits were produced with 16 variations in pit spacing and size, resulting in 160 variations in surface topography. This materials array was used to assess the interaction of topography on the transfection of fibroblast cells. The improved efficiency of nonviral transfection is an important development to advance gene medicines. Cells were attached to the patterned substrate and transfection efficiencies were assessed after 24 h. Up to 25 % enhancement in transfection efficiency was observed for cells grown on pitted topographies compared with a flat surface. Large pits close together offered the greatest improvement, but also produced the least spread cells.

4.3.4 3D Cell Culture

A key challenge for tissue-engineering applications is translating the 2D in vitro culture methods into 3D devices in vivo. Towards this goal 3D in vitro culture methods have been developed [75] that attempt to bridge the gap between in vitro and in vivo cell culture. Concurrently, 3D arrays have been developed that enable multiple experimental factors to be explored within 3D matrices in parallel.

Jongpaiboonkit et al. [76] developed an array of 3D culture matrices based upon a PEG diacrylate background that contained microwells. A liquid-handling system filled these wells with a hydrogel precursor solution that also contained cells of varied density. The hydrogels were then UV cured to fix the cells within the 3D matrix. The entire array could then be placed into a larger multi-well plate for culture. This system was used to study the interaction of the cell adhesive peptide RGDSP and growth factor (FGF2) on hMSCs [42]. The viability of cells grown in the wells was maintained at 82.7 % after 7 days growth with RGDSP and FGF2 compared with 45.5 % without these added components. The array format allowed for the optimal combination of these biological factors to be determined. The combined influence of the cell adhesive peptides, RGDSP and IKVAV on hMSCs, was also assessed [77]. After 7 days culture within the 3D matrices, the viability was improved from 45 % to up to 70 % with the addition of cell adhesive peptides. However, viability was improved further up to 80 % when both peptides were used.

In a different approach, Fernandes et al. [41] printed mouse EBs in an alginate gel to produce a 3D cell culture array. Cells remained viable, undifferentiated, and expanded within the 3D matrix. As a proof-of-concept of the high throughput utility of this system, a small molecule array containing FGF-4 or reionoic acid was

stamped onto the 3D cell culture and the small molecules diffused into the cultured cells. These molecules induced cellular differentiation as evidenced by a drop in Oct-4 and Nanog expression, markers of pluripotency.

4.3.5 Development of Structure–Function Relationships

A key advantage of high throughput screening methodologies is the discovery of new materials optimal for a given biological application. Additionally, the large number of biological–material interactions assessed using polymer microarrays can be used to develop structure–function relationships that, in turn, allow the design of biologically functional materials. However, this requires extensive surface characterization of the materials within a library. High throughput measurements of polymer microarrays have been achieved by XPS, time-of-flight secondary ion mass spectrometry (ToF-SIMS) [78, 79], WCA [80], atomic force microscopy (AFM) [81], surface plasmon resonance (SPR) [64], and force measurements [82]. The application of AFM and force measurements to a polymer microarray allows the mechanical properties of the materials to be included in subsequent structure–function relationships [31]. "High throughput surface characterization" refers to the automation of measurements, allowing an instrument to be set up to take measurements from every member of a library with little to no user input. Once material properties have been measured the challenge remains to correlate these properties with a biological response. This is particularly difficult because the systems typically being studied are complex and poorly understood. Simple correlations with properties that are commonly implicated in cell–material interactions, such as WCA or roughness, typically do not work. The key step in achieving a correlation between biological response and a measured surface property has been the use of partial least square (PLS) regression to correlate a univariate property, such as cell numbers, with a multivariate dataset, such as the hundreds of secondary ions produced in ToF-SIMS spectra [83]. This method was initially validated by linking ToF-SIMS spectra with WCA [83], and has been successfully applied to predict the frequency of colony formation of stem cells from the chemical information represented in ToF-SIMS spectra [31, 37]. Similarly bacterial attachment was predicted for *P. aeruginosa* and *S. aureus* [33]. The successful construction of these models likely results from the diverse material properties that are represented within ToF-SIMS spectra. Secondary ions emitted from a surface can be assigned to chemical functionalities that are associated with surface charge, hydrophobicity, aromatic and cyclic groups, and material stiffness [31, 33, 34, 79, 83]. However, the PLS models produced have thus far been restricted to interpolative predictions. A powerful application of this approach would enable the design of hit materials based upon the models produced. Limiting this is the extensive surface analysis required to gather the relevant chemical/physical properties of the materials being studied. Moreover, the number of materials that can be included is limited to the number of different samples that can

feasibly be screened. Although this is significantly expanded by the use of polymer microarrays to the order of hundreds to thousands of materials, this is still small compared with the infinite number of varied polymer chemistries that are possible.

To overcome this constraint, modelling based upon chemical descriptors rather than a measured property has been explored [84]. Using this method, materials do not need to be produced and analyzed, but rather a virtual library of materials can be created where the size of the library is limited by computational power rather than experimental requirements. Hook et al. used this approach to predict defects within polymers from their molecular descriptors using a PLS regression model [84]. Materials were initially screened by ToF-SIMS imaging and light microscopy to identify defects such as chemical heterogeneity or spreading. A PLS model based upon the molecular descriptor was successfully able to predict whether a pair of monomers would produce a defective polymer in 85 % of cases. This study demonstrated that the molecular descriptors of a polymer and their material properties are linked, and as such other material properties may be predicted.

This work was expanded by Epa et al. [85], whereby nonlinear Bayesian neural network models were developed that could predict the adhesion of hEB using only calculated molecular descriptors. In particular, this work demonstrated substantially higher predictive power of nonlinear models compared with linear models. An r^2 value from a plot of measured versus predicted values was reported as 0.68 for a linear model with a standard error estimation (SEE) of 0.163log EB whereas for a nonlinear model the r^2 value was 0.81 with an SEE of 0.108log EB. This suggests that there is some nonlinearity in the relationship between hEB adhesion and polymer structure [85]. Combining such modelling methods with polymer microarray screens, and specifically the design of polymer libraries, enables exciting opportunities to assess a larger material property space than could be accessed by experimental methods alone.

4.4 Closing Remarks

Polymer microarrays are a key enabling technology for biomaterials discovery. They have been successfully used to develop the surface chemistry, topography, bioactivity, and mechanical properties of biomaterials as well as allowing the high throughput study of 3D matrices. Polymer microarrays have been applied to numerous biological applications including supporting cell attachment and outgrowth, maturation and phagocytosis of dendritic cells, materials resistant to microbes, switchable materials, platelet activation, cell sorting, hepatocytes and toxicity models, and cell transfection. Additional to the discovery and development of new biomaterials, the large datasets generated by polymer microarrays can also be used to develop structure–function relationships and help elucidate underlying biological–material interactions. In particular, the modelling of the biological response of polymers using calculated molecular descriptors enables the screening of virtual libraries of polymers. This will allow

a larger set of material properties to be accessed than by experimental methods alone. Future insight into material–cell interactions and expanded knowledge of currently *ideas-poor* fields of material science will be greatly aided by polymer microarrays and their application to increasingly ambitious biological assays and methods for presenting materials.

References

1. Kell DB, Oliver SG (2003) Here is the evidence, now what is the hypothesis? The complementary roles of inductive and hypothesis-driven science in the post-genomic era. Bioessays 26:99–105. doi:10.1002/bies.10385
2. Derda R, Musah S, Orner BP, Klim JR, Li LY, Kiessling LL (2010) High-throughput discovery of synthetic surfaces that support proliferation of pluripotent cells. J Am Chem Soc 132:1289–1295. doi:10.1021/ja906089g
3. Bleicher KH, Bohm HJ, Muller K, Alanine AI (2003) Hit and lead generation: beyond high-throughput screening. Nat Rev Drug Discov 2:369–378. doi:10.1038/nrd1086
4. Gallop MA, Barrett RW, Dower WJ, Fodor SPA, Gordon EM (1994) Application of combinatorial technologies to drug discovery. 1. Background and peptide combinatorial libraries. J Med Chem 37:1233–1251. doi:10.1021/jm00035a001
5. Gordon EM, Barrett RW, Dower WJ, Fodor SPA, Gallop MA (1994) Applications of combinatorial technologies to drug discovery. 2. Combinatorial organic-synthesis, library screening strategies, and future-directions. J Med Chem 37:1385–1401. doi:10.1021/jm00036a001
6. Heller MJ (2002) DNA microarray technology: devices, systems, and applications. Annu Rev Biomed Eng 4:129–153. doi:10.1146/annurev.bioeng.4.020702.153438
7. Kumar A, Goel G, Fehrenbach E, Puniya AK, Singh K (2005) Microarrays: the technology, analysis and application. Eng Life Sci 5:215–222. doi:10.1002/elsc.200420075
8. Leung YF, Cavalieri D (2003) Fundamentals of cDNA microarray data analysis. Trends Genet 19:649–659. doi:10.1016/j.tig.2003.09.015
9. Stoll D, Templin MF, Bachmann J, Joos TO (2005) Protein microarrays: applications and future challenges. Curr Opin Drug Dis Dev 8:239–252
10. Templin MF, Stoll D, Schrenk M, Traub PC, Vohringer CF, Joos TO (2002) Protein microarray technology. Trends Biotechnol 20:160–166. doi:10.1016/S0167-7799(01)01910-2
11. Wilson DS, Nock S (2003) Recent developments in protein microarray technology. Angew Chem Int Edit 42:494–500. doi:10.1002/anie.200390150
12. Angres B (2005) Cell microarrays. Expert Rev Mol Diagn 5:769–779. doi:10.1586/14737159.5-5.769
13. Hook AL, Creasey R, Thissen H, Hayes JP, Voelcker NH (2009) Laser-based patterning for transfected cell microarrays. Biofabrication 1:045003. doi:10.1088/1758-5082/1/4/045003
14. Hook AL, Thissen H, Voelcker NH (2009) Advanced substrate fabrication for cell microarrays. Biomacromolecules 10:573–579. doi:10.1021/bm801217n
15. Hook AL, Voelcker N, Thissen H (2006) Surface manipulation of biomolecules for cell microarray applications. Trends Biotech 24:471–477. doi:10.1016/j.tibtech.2006.08.001
16. Ziauddin J, Sabatini DM (2001) Microarrays of cells expressing defined cDNAs. Nature 411:107–110. doi:10.1038/35075114
17. Xiang XD, Sun XD, Briceno G, Lou YL, Wang KA, Chang HY, Wallacefreedman WG, Chen SW, Schultz PG (1995) A combinatorial approach to materials discovery. Science 268:1738–1740. doi:10.1126/science.268.5218.1738
18. Brocchini S, James K, Tangpasuthadol V, Kohn J (1998) Structure–property correlations in a combinatorial library of degradable biomaterials. J Biomed Mater Res 42:66–75. doi:10.1002/(SICI)1097-4636(199810)42:1<66::AID-JBM9>3.0.CO;2-M

19. Anderson DG, Levenberg S, Langer R (2004) Nanoliter-scale synthesis of arrayed biomaterials and application to human embryonic stem cells. Nat Biotech 22:863–866. doi:10.1038/nbt981
20. Anderson DG, Putnam D, Lavik EB, Mahmood TA, Langer R (2005) Biomaterial microarrays: rapid, microscale screening of polymer-cell interaction. Biomaterials 26:4892–4897. doi:10.1016/j.biomaterials.2004.11.052
21. Tourniaire G, Collins J, Campbell S, Mizomoto H, Ogawa S, Thaburet JF, Bradley M (2006) Polymer microarrays for cellular adhesion. Chem Comm 28:2118–2120. doi:10.1039/b602009g
22. Kohn J, Welsh WJ, Knight D (2007) A new approach to the rationale discovery of polymeric biomaterials. Biomaterials 28:4171–4177. doi:10.1016/j.biomaterials.2007.06.022
23. Kingshott P, Thissen H, Griesser HJ (2002) Effects of cloud-point grafting, chain length, and density of PEG layers on competitive adsorption of ocular proteins. Biomaterials 23:2043–2056
24. Hook AL, Thissen H, Hayes JP, Voelcker NH (2005) Spatially controlled electro-stimulated DNA adsorption and desorption for biochip applications. Biosens Bioelectron 21:2137–2145. doi:10.1016/j.bios.2005.10.008
25. Yasuda H (1985) Plasma polymerization. Academic, Orlando
26. Kurkuri MD, Driever C, Johnson G, McFarland G, Thissen H, Voelcker NH (2009) Multifunctional polymer coatings for cell microarray applications. Biomacromolecules 10:1163–1172. doi:10.1021/bm801417s
27. Tekin E, de Gans BJ, Schubert US (2004) Ink-jet printing of polymers—from single dots to thin film libraries. J Mater Chem 14:2627–2632. doi:10.1039/b407478e
28. Pardo L, Wilson WC, Boland TJ (2003) Characterization of patterned self-assembled monolayers and protein arrays generated by the ink-jet method. Langmuir 19:1462–1466. doi:10.1021/la026171u
29. Zhang R, Liberski A, Khan F, Diaz-Mochon JJ, Bradley M (2008) Inkjet fabrication of hydrogel microarrays using in situ nanolitre-scale polymerisation. Chem Comm 11:1317–1319. doi:10.1039/b717932d
30. Hook AL, Chang C, Yang J, Atkinson S, Langer R, Anderson DG, Davies MC, Williams P, Alexander MR (2013) Discovery of novel materials with broad resistance to bacterial attachment using combinatorial polymer microarrays. Adv Mater 25:2542–2547. doi:10.1002/adma.201204936
31. Mei Y, Saha K, Bogatyrev SR, Yang J, Hook AL, Kalcioglu ZI, Cho SW, Mitalipova M, Pyzocha N, Rojas F, Van Vliet KJ, Davies MC, Alexander MR, Langer R, Jaenisch R, Anderson DG (2010) Combinatorial development of biomaterials for clonal growth of human pluripotent stem cells. Nat Mater 9:768–778. doi:10.1038/nmat2812
32. Unadkat HV, Hulsman M, Cornelissen K, Papenburg BJ, Truckenmuller RK, Carpenter AE, Wessling M, Post GF, Uetz M, Reinders MJT, Stamatialis D, van Blitterswijk CA, de Boer J (2011) An algorithm-based topographical biomaterials library to instruct cell fate. Proc Natl Acad Sci U S A 108:16565–16570. doi:10.1073/pnas.1109861108
33. Hook AL, Chang C, Yang J, Luckett J, Cockrayne A, Atkinson S, Mei Y, Bayston R, Irvine DJ, Langer R, Anderson DG, Williams P, Davies MC, Alexander MR (2012) Combinatorial discovery of polymers resistant to bacterial attachment Nat Biotech 30:868–875. doi:10.1038/nbt.2316
34. Hook AL, Scurr DJ, Anderson DG, Langer R, Williams P, Davies M, Alexander MR (2013) High throughput discovery of thermo-responsive materials using water contact angle measurements and time-of-flight secondary ion mass spectrometry. Surf Interface Anal 45:181–184. doi:10.1002/sia.4910
35. Hansen A, Zhang R, Bradley M (2012) fabrication of arrays of polymer gradients using inkjet printing. Macromol Rapid Comm 33:1114–1118. doi:10.1002/marc.201200193
36. Mei Y, Gerecht S, Taylor M, Urquhart A, Bogatyrev SR, Cho SW, Davies MC, Alexander MR, Langer RS, Anderson DG (2009) Mapping the Interactions among biomaterials, adsorbed proteins, and human embryonic stem cells. Adv Mater 21:2781–2786. doi:10.1002/adma.200803184
37. Yang J, Mei Y, Hook AL, Taylor M, Urquhart AJ, Bogatyrev SR, Langer R, Anderson DG, Davies MC, Alexander MR (2010) Polymer surface functionalities that control human embryoid body cell adhesion revealed by high throughput surface characterization of combinatorial material microarrays. Biomaterials 31:8827–8838. doi:10.1016/j.biomaterials.2010.08.028

38. Flaim CJ, Chien S, Bhatia SN (2005) An extracellular matrix microarray for probing cellular differentiation. Nat Method 2:119–125. doi:10.1038/NMETH736
39. Anglin E, Davey R, Herrid M, Hope S, Kurkuri M, Pasic P, Hor M, Fenech M, Thissen H, Voelcker NH (2010) Cell microarrays for the screening of factors that allow the enrichment of bovine testicular cells. Cytom Part A 77A:881–889. doi:10.1002/cyto.a.20913
40. Gobaa S, Hoehnel S, Roccio M, Negro A, Kobel S, Lutolf MP (2011) Artificial niche microarrays for probing single stem cell fate in high throughput. Nat Method 8:949–955. doi:10.1038/nmeth.1732
41. Fernandes TG, Kwon SJ, Bale SS, Lee MY, Diogo MM, Clark DS, Cabral JMS, Dordick JS (2010) Three-dimensional cell culture microarray for high-throughput studies of stem cell fate. Biotech Bioeng 106:106–118. doi:10.1002/bit.22661
42. King WJ, Jongpaiboonkit L, Murphy WL (2010) Influence of FGF2 and PEG hydrogel matrix properties on hMSC viability and spreading. J Biomed Mater Res Part A 93A:1110–1123. doi:10.1002/jbm.a.32601
43. Zhang R, Mjoseng HK, Hoeve MA, Bauer NG, Pells S, Besseling R, Velugotla S, Tourniaire G, Kishen REB, Tsenkina Y, Armit C, Duffy CRE, Helfen M, Edenhofer F, de Sousa PA, Bradley M (2013) A thermoresponsive and chemically defined hydrogel for long-term culture of human embryonic, stem cells. Nat Comun 4:1335. doi:10.1038/ncomms2341
44. Mant A, Tourniaire G, Diaz-Mochon JJ, Elliott TJ, Williams AP, Bradley M (2006) Polymer microarrays: identification of substrates for phagocytosis assays. Biomaterials 27:5299–5306. doi:10.1016/j.biomaterials.2006.04.040
45. Pernagallo S, Wu M, Gallagher MP, Bradley M (2011) Colonising new frontiers-microarrays reveal biofilm modulating polymers. J Mater Chem 21:96–101. doi:10.1039/c0jm01987a
46. Zhang R, Liberski A, Sanchez-Martin R, Bradley M (2009) Microarrays of over 2000 hydrogels—identification of substrates for cellular trapping and thermally triggered release. Biomaterials 30:6193–6201. doi:10.1016/j.biomaterials.2009.07.055
47. Hansen A, McMillan L, Morrison A, Petrik J, Bradley M (2011) Polymers for the rapid and effective activation and aggregation of platelets. Biomaterials 32:7034–7041. doi:10.1016/j.biomaterials.2011.06.001
48. Anglin EJ, Salisbury C, Bailey S, Hor M, Macardle P, Fenech M, Thissen H, Voelcker NH (2010) Sorted cell microarrays as platforms for high-content informational bioassays. Lab Chip 10:3413–3421. doi:10.1039/c0lc00185f
49. Tare RS, Khan F, Tourniaire G, Morgan SM, Bradley M, Oreffo ROC (2009) A microarray approach to the identification of polyurethanes for the isolation of human skeletal progenitor cells and augmentation of skeletal cell growth. Biomaterials 30:1045–1055. doi:10.1016/j.biomaterials.2008.10.038
50. Hay DC, Pernagallo S, Diaz-Mochon JJ, Medine CN, Greenhough S, Hannoun Z, Schrader J, Black JR, Fletcher J, Dalgetty D, Thompson AI, Newsome PN, Forbes SJ, Ross JA, Bradley M, Iredale JP (2011) Unbiased screening of polymer libraries to define novel substrates for functional hepatocytes with inducible drug metabolism. Stem Cell Res 6:92–102. doi:10.1016/j.scr.2010.12.002
51. Khan F, Tare RS, Kanczler JM, Oreffo ROC, Bradley M (2010) Strategies for cell manipulation and skeletal tissue engineering using high-throughput polymer blend formulation and microarray techniques. Biomaterials 31:2216–2228. doi:10.1016/j.biomaterials.2009.11.101
52. Pernagallo S, Tura O, Wu M, Samuel K, Diaz-Mochon JJ, Hansen A, Zhang R, Jackson M, Padfield GJ, Hadoke PWF, Mills NL, Turner ML, Iredale JP, Hay DC, Bradley M (2012) Novel biopolymers to enhance endothelialisation of intra-vascular devices. Adv Healthc Mater 1:646–656. doi:10.1002/adhm.201200130
53. Pickering H, Wu M, Bradley M, Bridle H (2012) Analysis of Giardia lamblia interactions with polymer surfaces using a microarray approach. Environ Sci Technol 46:2179–2186. doi:10.1021/es203637e
54. Adler AF, Speidel AT, Christoforou N, Kolind K, Foss M, Leong KW (2011) High-throughput screening of microscale pitted substrate topographies for enhanced nonviral transfection efficiency in primary human fibroblasts. Biomaterials 32:3611–3619. doi:10.1016/j.biomaterials.2011.01.040

55. Wu M, Bridle H, Bradley M (2012) Targeting Cryptosporidium parvum capture. Water Res 46:1715–1722. doi:10.1016/j.watres.2011.12.041
56. Nichols J, Zevnik B, Anastassiadis K, Niwa H, Klewe-Nebenius D, Chambers I, Scholer H, Smith A (1998) Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor. Cell 95:379–391. doi:10.1016/s0092-8674(00)81769-9
57. McMichael AJ (1987) Leucocyte typing III: white cell differentiation antigens. Oxford University Press, Oxford
58. Hsulin SC, Berman CL, Furie BC, August D, Furie B (1984) A platlet membrane-protein expressed during platlet activation and secretion—studies using monoclonal-antibody specific for thrombin-activated platelets. J Bio Chem 259:9121–9126
59. Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284:1318–1322. doi:10.1126/science.284.5418.1318
60. Davies D (2003) Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discov 2:114–122. doi:10.1038/nrd1008
61. Kingshott P, Wei J, Bagge-Ravn D, Gadegaard N, Gram L (2003) Covalent attachment of poly(ethylene glycol) to surfaces, critical for reducing bacterial adhesion. Langmuir 19: 6912–6921. doi:10.1021/la034032m
62. Cheng G, Li GZ, Xue H, Chen SF, Bryers JD, Jiang SY (2009) Zwitterionic carboxybetaine polymer surfaces and their resistance to long-term biofilm formation. Biomaterials 30: 5234–5240. doi:10.1016/j.biomaterials.2009.05.058
63. Jiang SY, Cao ZQ (2010) Ultralow-fouling, functionalizable, and hydrolyzable zwitterionic materials and their derivatives for biological applications. Adv Mater 22:920–932. doi:10.1002/adma.200901407
64. Hook AL, Thissen H, Voelcker NH (2009) Surface plasmon resonance imaging of polymer microarrays to study protein-polymer interactions in high throughput. Langmuir 25:9173–9181. doi:10.1021/la900735n
65. Guan K, Nayernia K, Maier LS, Wagner S, Dressel R, Lee JH, Nolte J, Wolf F, Li MY, Engel W, Hasenfuss G (2006) Pluripotency of spermatogonial stem cells from adult mouse testis. Nature 440:1199–1203. doi:10.1038/nature04697
66. Costoya JA, Hobbs RM, Barna M, Cattoretti G, Manova K, Sukhwani M, Orwig KE, Wolgemuth DJ, Pandolfi PP (2004) Essential role of Plzf in maintenance of spermatogonial stem cells. Nat Genet 36:653–659. doi:10.1038/ng1367
67. Steger K, Schimmel M, Wrobel KH (1994) Immunocytochemical demonstration of cytoskeletal proteins in seminiferous tubules of adult rams and bulls. Arch Histol Cytol 57:17–28. doi:10.1679/aohc.57.17
68. Johansson F, Carlberg P, Danielsen N, Montelius L, Kanje M (2006) Axonal outgrowth on nano-imprinted patterns. Biomaterials 27:1251–1258. doi:10.1016/j.niomaterials.2005.07.047
69. Tessier-Lavigne M, Goodman CS (1996) The molecular biology of axon guidance. Science 274:1123–1133. doi:10.1126/science.274.5290.1123
70. Cochran DL, Buser D, ten Bruggenkate CM, Weingart D, Taylor TM, Bernard JP, Peters F, Simpson JP (2002) The use of reduced healing times on ITI (R) implants with a sandblasted and acid-etched (SLA) surface: early results from clinical trials on ITI (R) SLA implants. Clin Oral Implant Res 13:144–153. doi:10.1034/j.1600-0501.2002.130204.x
71. Martin JY, Schwartz Z, Hummert TW, Schraub DM, Simpson J, Lankford J, Dean DD, Cochran DL, Boyan BD (1995) Effect of titanium surface-roughness on proliferation, differentiation, and protein-synthesis of human osteoblast-like cells (MG63). J Biomed Mater Res 29:389–401. doi:10.1002/jbm.820290314
72. Price RL, Ellison K, Haberstroh KM, Webster TJ (2004) Nanometer surface roughness increases select osteoblast adhesion on carbon nanofiber compacts. J Biomed Mat Res Part A 70A:129–138. doi:10.1002/jbm.a.30073
73. Schneider GB, Zaharias R, Seabold D, Keller J, Stanford C (2004) Differentiation of preosteoblasts is affected by implant surface microtopographies. J Biomed Mater Res Part A 69A:462–468. doi:10.1002/jbm.a.30016

74. Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP (1997) Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. J Cell Biochem 64:295–312. doi:10.1002/(sici)1097-4644(199702)64:2<295::aid-jcb12>3.0.co;2-i
75. Cukierman E, Pankov R, Stevens DR, Yamada KM (2001) Taking cell-matrix adhesions to the third dimension. Science 294:1708–1712. doi:10.1126/science.1064829
76. Jongpaiboonkit L, King WJ, Lyons GE, Paguirigan AL, Warrick JW, Beebe DJ, Murphy WL (2008) An adaptable hydrogel array format for 3-dimensional cell culture and analysis. Biomaterials 29:3346–3356. doi:10.1016/j.biomaterials.2008.04.040
77. Jongpaiboonkit L, King WJ, Murphy WL (2009) Screening for 3D environments that support human mesenchymal stem cell viability using hydrogel arrays. Tissue Eng Part A 15:343–353. doi:10.1089/ten.tea.2008.0096
78. Urquhart AJ, Anderson DG, Taylor M, Alexander MR, Langer R, Davies MC (2007) High throughput surface characterisation of a combinatorial material library. Adv Mater 19:2486–2491. doi:10.1002/adma.200700949
79. Urquhart AJ, Taylor M, Anderson DG, Langer R, Davies MC, Alexander MR (2008) TOF-SIMS analysis of a 576 micropatterned copolymer array to reveal surface moieties that control wettability. Anal Chem 80:135–142. doi:10.1021/ac071560k
80. Taylor M, Urquhart AJ, Zelzer M, Davies MC, Alexander MR (2007) Picoliter water contact angle measurement on polymers. Langmuir 23:6875–6878. doi:10.1021/la070100j
81. Hook AL, Yang J, Chen X, Roberts CJ, Mei Y, Anderson DG, Langer R, Alexander MR, Davies MC (2011) Polymers with hydro-responsive topography identified using high throughput AFM of an acrylate microarray. Soft Matter 7:7194–7197. doi:10.1039/c1sm06063e
82. Tweedie CA, Anderson DG, Langer R, Van Vliet KJ (2005) Combinatorial material mechanics: high-throughput polymer synthesis and nanomechanical screening. Adv Mater 17:2599–2604. doi:10.1002/adma.200501142
83. Taylor M, Urquhart AJ, Anderson DG, Langer R, Davies MC, Alexander MR (2009) Partial least squares regression as a powerful tool for investigating large combinatorial polymer libraries. Surf Interface Anal 41:127–135. doi:10.1002/sia.2969
84. Hook AL, Scurr DJ, Burley JC, Langer R, Anderson DG, Davies MC, Alexander MR (2013) Analysis and prediction of defects in UV photo-initiated polymer microarrays. J Mater Chem B 1:1035–1043. doi:10.1039/c2tb00379a
85. Epa VC, Yang J, Mei Y, Hook AL, Langer R, Anderson DG, Davies MC, Alexander MR, Winkler DA (2012) Modelling human embryoid body cell adhesion to a combinatorial library of polymer surfaces. J Mater Chem 22:20902–20906. doi:10.1039/c2jm34782b