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Arrays for the Combinatorial Exploration of Cell Adhesion

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The surfaces with which cells interact are important for maintaining cellular viability and localization. The features of these surfaces can act as signals that influence cellular behavior. Dissecting the critical features of physiological surfaces is difficult given their complexity and heterogeneity. Synthetic, homogeneous, surfaces presenting specific ligands for cell surface receptors can be used to unravel key features of physiological surfaces responsible for eliciting a specific cellular response.[1,2] Here we present a method to screen diverse surfaces to identify those with illuminating cell adhesive or signaling properties.

Combinatorial chemistry and diversity-oriented synthesis can be used to generate ligands that bind a specific receptor. Parameters beyond binding specificity must be considered when synthesizing surfaces that interact with cells. Cell binding to another cell or matrix is influenced not only by the presence of a specific ligand but also by its density on the surface. Moreover, cell surface ligands can act synergistically. Thus, it would be valuable to display combinations of ligands at defined densities. To explore how surface presentation of ligands influences cell viability, adhesion, and response, we developed a method to generate patterned surfaces that present arrays of ligands (Figure 1).

Useful arrays for testing cell attachment exist;[2] however, none can be used to present many different ligands and/or ligand combinations at defined densities. To design a strategy to accommodate our needs, we chose to employ self-assembled monolayers (SAMs). SAMs are well-characterized surfaces[3] that have been used for cell attachment and patterning.[4] SAMs are attractive because they are well-packed, are homogeneous across the assembly, and can be reproducibly assembled and patterned. With SAMs, differences in cell binding can be attributed to the structure and density of the ligands presented.

We sought to develop a method to pattern SAMs for the combinatorial study of cell adhesion (Figure 1A). Compounds that bind cell surface receptors include proteins, peptides, carbohydrates, and small molecules; the solubilities of these ligands vary. Thus, we required a method to pattern the SAMs that would resist cross-contamination of spots containing ligands of different solutes; therefore, the alkane thiol (AT) that is used between array elements (background) must create a "solvophobic" surface (i.e., one that resists spreading of solvents) (Figure 1B). The background SAM also must be cytophobic; it serves as a barrier to cell attachment and growth (Figure 1A). With these criteria in mind, we reasoned that a SAM formed from a perfluoro AT would serve as an excellent background. Such surfaces are known to be cytophobic[4a,4c,5,6] and solvophobic.[7,8] By taking advantage of the features of the fluorous SAM, we could employ an "assembly after conjugation" strategy to fabricate the target arrays. Thus, we envisioned assembling SAMs by spotting ATs with preattached ligands on bare gold "holes" in fluorous SAMs (Figure 1C).

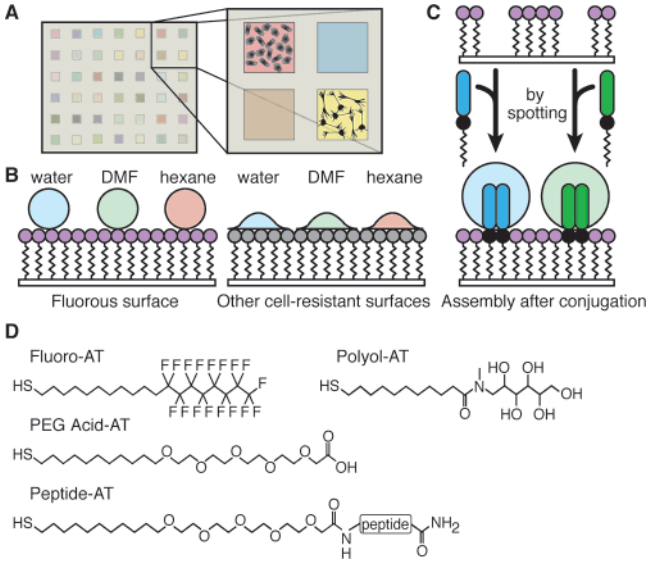

Figure 1. (A) Arrays for the combinatorial evaluation of cell adhesion and signaling. Each square represents a different surface element. The magnified section illustrates that elements may afford cell adhesion and/or differentiation. (B) Illustration of the solvophobicity of a fluorous SAM (the colored shapes represent different solvents). (C) SAM composed of fluoro-AT patterned to reveal bare gold holes. ATs containing ligands are then spotted to create the array. The blue and green ovals represent solutions of different ATs. (D) ATs employed.

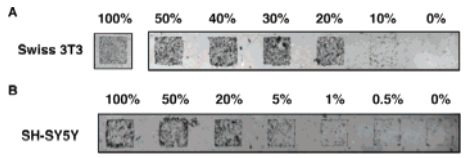

Figure 2. Two cell lines plated on arrays formed by spotting various percentages of PEG acid-AT and polyol-AT on bare gold squares surrounded by a fluoro-AT SAM (percentage refers to the amount of PEG acid-AT). (A) Fixed (2% paraformaldehyde) and stained (Coomassie) Swiss 3T3 fibroblast cells (the 100% spot was spatially separated but in the same array). (B) Fixed and stained SH-SY5Y cells. All squares are 750 μm in length.

To test this strategy, we generated a pattern containing an AT known to bind cells, PEG acid-AT,[4b] using the cytophobic fluoro-AT[9] as the background (Figure 1D). To test the importance of the density of the cytophilic AT, solutions of varying molar ratios of PEG acid-AT diluted with a hydrophilic AT that does not interact with cells, polyol-AT,[7] were spotted on bare gold areas formed by photopatterning a SAM. Neuroblastoma (SH-SY5Y) and fibroblast (Swiss 3T3) cell lines were plated on the patterned surfaces, allowed to proliferate, fixed, and stained (Figure 2). For both cell lines, adhesion decreases as the mole fraction of the acid within the spots approaches 0.2. These data illustrate the feasibility of the spotting method, and they indicate that SAMs composed of mixtures of ATs can be screened using this approach.

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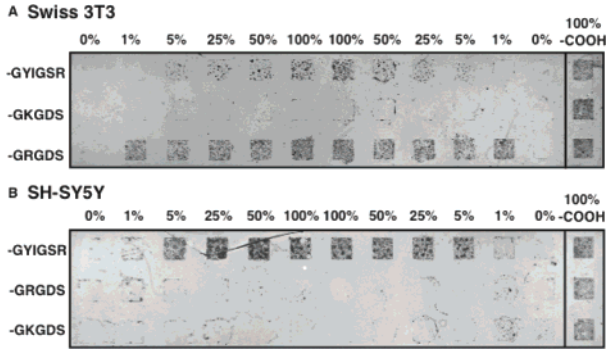

Figure 3. Two cell lines plated on arrays formed by spotting with various percentages of peptide-ATs and polyol-AT on bare gold squares surrounded by a fluoro-AT SAM. (A) Fixed (2% paraformaldehyde) and stained (Coomassie) Swiss 3T3 fibroblast cells. (B) Fixed and stained SH-SY5Y neuroblastoma cells. "100% -COOH" indicates PEG acid-AT. The label "-GYIGSR" denotes the peptide-AT derivatized with the sequence GSDPGYIGSR. All squares are 750 μm in length.

In physiological settings, cells interpret cues from the extracellular matrix. Different cell types interact with different matrix proteins. The sequence RGD, which is found in a number of proteins, including fibronectin and vitronectin, binds a family of integrins on the surface of some cell lines, including Swiss-3T3 fibroblasts. This binding is specific; a simple Arg to Lys substitution obliterates adhesion.[10] Alternatively, the peptide YIGSR, a sequence found in the matrix protein laminin, interacts with neuroblastoma cell lines but not with fibroblasts.[11] Therefore, surfaces displaying YIGSR are expected to bind the neuroblastoma cell line and the fibroblast cell line is expected to adhere preferentially to RGD surfaces over those presenting KGD.

To test whether surfaces displaying different peptides interact selectively with different cell types, a surface displaying fluoro-AT SAM was photopatterned. Mixtures of polyol-AT and peptide-AT[12] were spotted on the bare gold. An array element known to bind cells, 100% PEG acid-AT, and one that resists cell binding, 100% polyol-AT, were also spotted. Swiss 3T3 and SH-SY5Y cells were plated on the resulting chips, allowed to proliferate, fixed, and stained. The Swiss 3T3 fibroblasts (Figure 3A) preferentially bound the RGD surface; minimal binding was detected to the KGD element. Modest fibroblast adhesion to the YIGSR-modified surface was observed; as the percentage of polyol-AT was increased, however, cell binding fell off more rapidly than did binding to RGD-presenting surfaces. The neuroblastoma cell line, SH-SY5Y (Figure 3B), adheres to the YIGSR-substituted surface but not to either the RGD or KGD elements. Thus, cell-line specific binding profiles can be established using these arrays.

Human embryonic stem (ES) cells are developmental precursors to all cell types and thus termed "pluripotent".[13] The ability to culture human ES cells on synthetic surfaces would eliminate the complications of using mouse embryonic fibroblasts as a feeder layer during ES expansion.[14] To determine whether our method can be used to identify surfaces upon which to culture human ES cells, solutions of different molar ratios of PEG acid-AT and polyol-AT were employed to generate arrays upon which human ES cells were plated. Again, a gradient of cell attachment was observed (Figure 4). The human ES cells, however, require a more acidic surface than the two previously investigated cell lines. Interestingly, alkaline phosphatase staining of human ES cells indicates that these cells remain undifferentiated for at least 2 days after plating (Figure 4B). It is intriguing that such a simple surface can propagate human embryonic stem cells. This result bodes well for finding optimized surfaces for long-term ES cell proliferation and controlled differentiation.

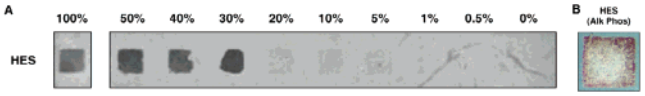

Figure 4. Human embryonic stem cells plated on arrays formed by spotting various percentages of PEG acid-AT (indicated) and polyol-AT on bare gold squares surrounded by a fluoro-AT SAM. (A) Fixed (2% paraformaldehyde) and stained (Coomassie) Human ES cells (the 100% spot was spatially separated but in the same array). (B) Human ES cells on 100% PEG Acid-AT SAM stained for alkaline phosphatase activity. All squares are 750 μm in length.

The data indicate that our method for fabricating ligand-presenting arrays can be used for cell-based screens. We have shown that arrays generated by this strategy can present combinations of ligands; different cell lines can "read" the same surfaces differently. Given the flexibility of our design, we anticipate that it can be used to present virtually any single AT or combination of synthetic ATs. We expect that this method can be used to fabricate arrays that address fundamental questions critical for understanding and controlling the adhesion, proliferation, and differentiation of cells.

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Supporting Information Available: Synthetic methods and experimental details for the development of the methodology (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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