APPROACHES TO ORGANIZE CELLS ON SURFACES

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ABSTRACT - **The binding of cell surface receptors to local bioactive ligands is the principal mechanism by which cells survey their microenvironment and accordingly modulate their behaviors, such as proliferation, differentiation, migration, and suicide. Using microfabrication approaches to engineer cellular microenvironments, we are examining how the spatial positioning of cells and their intercellular contacts can control cellular functions. Here, we describe methods to pattern the adhesivity of surfaces to 1) control the position of cells on surfaces, 2) measure forces generated by cells against deformable substrata, and 3) control the extent of cell-to-cell contact. We hope to use the microengineered substrates to improve the interface between artificial surfaces and living cells.**

I. INTRODUCTION

Cell adhesion to the surrounding extracellular matrix (ECM) plays a central role in the regulation of many cellular processes, such as proliferation, differentiation, migration, and suicide (apoptosis). For example, in the absence of adhesion, most human cells will rapidly undergo apoptosis, with the exception of those in the immune system [1]. It is also well known that adhesion itself, in addition to appropriate soluble growth factors, is a requirement for cell proliferation [2]. Yet, despite the increasing recognition of cell adhesion as a ubiquitous regulator of all forms of signal transduction, the exact mechanisms by which cell-matrix interactions exert regulatory effects remain poorly defined: It appears that cell function is determined not only by the identity of the extracellular matrix ligands and the integrin receptors that bind them, but also by the spatial presentation of those ligands. For example, cells switch from a suicide program to cell proliferation when they are attached to progressively larger islands coated with the same extracellular matrix [3]. Based on evidence from the developing embryo and the complex structure of adult tissues, one would also suspect that how cells are organized in relation to each other and how mechanical forces are imparted on cells could also direct their function.

Using conventional and non-conventional microfabrication approaches to engineer well-defined cellular microenvironments, we are examining how cells recognize and respond to these different aspects of adhesion. We will discuss our approaches to control the architecture and geometry of the adhesive interactions, as well as the resulting progress in identifying and elucidating the mechanisms by which cells sense the physical, chemical, and structural information conveyed by the ECM. We use methods to pattern the adhesivity of the surface to control the attachment of individual cells and the organization of groups of cells. By engineering well-defined cellular microenvironments, we hope to improve the interface engineered between artificial surfaces and living cells.

II. METHODS

To address the need to provide strict c ontrol of cell-surface interactions, we typically use microcontact printing (μCP) to pattern self-assembled monolayers (SAMs) of alkanethiolates on gold surfaces to fabricate "islands" of ECM proteins surrounded by nonadhesive regions. Single cells biospecifically attach and spread to the size and shape of the engineered islands [1, 4]. In this approach, a stamp of polydimethylsiloxane (PDMS) is cast from a silicon-photoresist master to produce a bas-relief, negative replica of the master (Fig. 1A). A hydrophobic alkanethiol, such as hexadecanethiol, is inked onto the stamp, and the stamp is placed against a thin, transparent film of gold evaporated onto glass. Because the sulfur

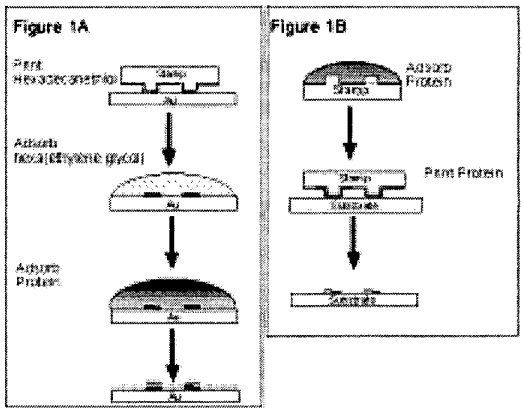

Figure 1. A) Schematic of the patterning of SAMs on gold surface. B) Schematic of directly printing proteins onto a surface.

atoms in alkanethiols exhibit a strong affinity for gold atoms, the thiol molecules transfer from the stamp to the gold, with the hydrophobic CH_3- endgroup of the alkanethiol oriented away from the surface of the gold. Subsequent immersion of the stamped surface into a

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solution of hexa(ethylene glycol)-terminated alkanethiol $HS(CH_2)_{11}(OCH_2CH_2)_6OH$ derivatizes the unstamped regions with the hexa(ethylene glycol) moiety, which resists adsorption of protein. When the stamped surface is placed in a solution of extracellular matrix (ECM) protein (fibronectin, laminin, or collagen), protein adsorbs onto the hydrophobic, CH_3-terminated regions only. Cells that are seeded attach and spread only on the ECM-coated regions of the surface. In this way, we can control the shapes and degrees of spreading of cells.

Recently, we have explored the use of other materials in conjunction with μCP to eliminate two obstacles that have prevented widespread use of the technique – the need for evaporation of gold onto surfaces and synthetic capabilities to produce alkanethiols. In addition, for some applications, the use of gold and SAMs are not appropriate. For example, cells cultured on elastomeric materials can generate traction forces that visibly deform these materials [5]. To pattern these surfaces using gold would render the substrates too rigid. To circumvent the dependence on gold and alkanethiols, we have used μCP to directly print protein from a stamp onto a substrate (Fig. 1B) [6]. One can then adsorb non-adhesive detergents such as an ethylene oxide-propylene oxide-ethylene oxide copolymer (trade name Pluronics) onto the unprinted regions of the surface, rendering them inert [7]. This process is simple, but has the disadvantage that the protein must be dried onto the stamp before the protein is printed, which can damage the protein. We have used this approach to pattern polymeric surfaces such as PDMS itself. The flexibility of PDMS allows us then to observe local deformations in the substrate as a result of the isometric tensions generated by cells against the surface.

We have also used patterning tools to try to control the degree of cell-cell contact between pairs of cells. Using SAMs on gold, if we juxtapose pairs of islands and place a narrow constriction between the islands (such as in the projected shape of an hourglass), cells will attach to the islands and form contacts between each other. However, the cells quickly strengthen those contacts and increase the area of contact between them beyond the constricted shape of the island. We believe that the flatness of the patterned surfaces allows cells to form bridges over the nonadhesive regions, so we developed another approach to control cell-cell contact: A PDMS stamp containing raised bowtie-shaped features is placed against a glass coverslip such that the bowties sealed to the glass. A hot solution of agarose is then wicked underneath the stamp and polymerized by cooling. When the stamp is peeled off, the surface contains a thick (20 μm tall) layer of agarose in the regions surrounding the bowties. Because the non-adhesive agarose is raised from the glass surface, cells should not be able to form bridges over these regions, and thus, cell-cell contact is restricted to the constriction in the bowtie.

III. RESULTS AND DISCUSSION

Using direct printing of proteins onto PDMS substrates, the placement of cells can be well controlled (Fig. 2). The patterning was comparable in quality to that of SAMs on gold. The gross pattern of attachment closely matches that of fibronectin adsorbed onto the printed hexadecanethiol. Using several different cell types, we have learned that the fidelity of patterning immediately after plating is extremely robust even in the presence of serum, ECM and growth factors in the media. However, there remain several challenges and limitations in these systems. For example, the number of cells that land on each island is completely stochastic, and therefore, it is difficult to obtain a completely uniform density of cells across the surface or to purposefully non-uniformly coat different regions with different cell densities. In addition, we have found that cells eventually escape the pattern by gaining the ability to spread across the nonadhesive regions in a period of several days to weeks, depending on the cell type. The loss of pattern fidelity may be a result of an active degradative process caused by the cells, but it could also be the natural gradual oxidation and destruction of the ethylene glycol moiety. Another feature of this form of patterning is that cells can bridge non-adhesive regions if the distance between adhesive regions is less than 5 to 20 μm, depending on the cell type. One of the applications enabled by directly printing proteins onto surfaces, as opposed to SAMs on gold, is to pattern cells on deformable substrates. We are able to observe the deformations (appearing as wrinkles,

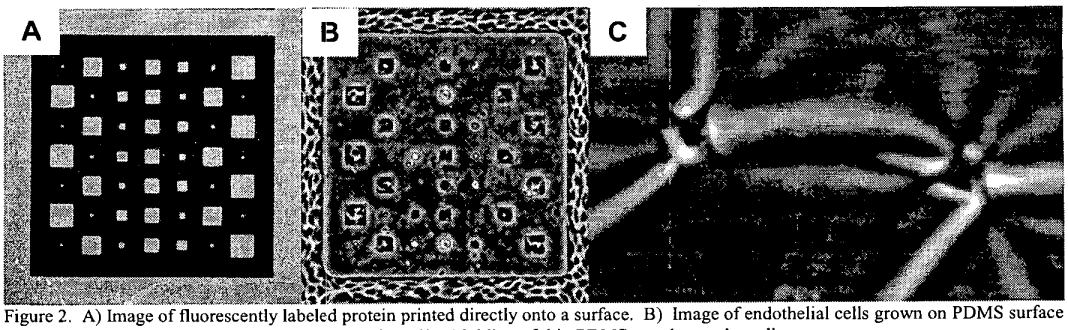

Figure 2. A) Image of fluorescently labeled protein printed directly onto a surface. B) Image of endothelial cells grown on PDMS surface printed with fibronectin and blocked with Pluronics. C) Wrinkling of thin PDMS membranes by cells.

Fig. 2C) of thin PDMS membranes caused by cells, and hope to combine the patterning technology directly with the thin-membrane substrates to address how the organization of cells controls their contractile behavior.

To control the degree of intercellular contact between neighboring cells, we plate pairs of cells in bowties made with either the SAMs-on-gold, or agarose-on-glass technique. We found that the constrictions in the bowties

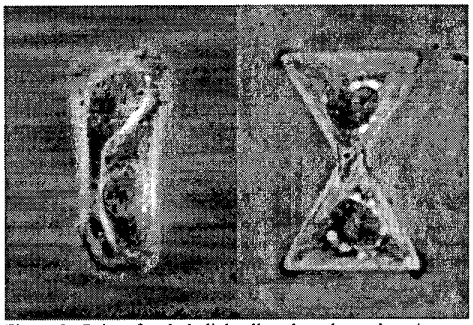

Figure 3. Pairs of endothelial cells cultured onto bowtie-shaped patterns made with SAMs on gold (left) or agarose on glass (right).

were ineffective at controlling the extent of intercellular contact between cells on SAMs (Figure 3). In contrast, pairs of cells formed well-defined contacts on the agarose substrates. We believe that the extent of these contacts influence how cells perceive their environment. One drawback of the current agarose patterning method is that the total area of substrate that can be patterned is limited to less than one square centimeter, presumably because the hot agarose does not wick easily under large areas of PDMS. Using standard forced microfluidic delivery, this limitation should be circumvented.

IV. CONCLUSIONS

Our long term goal is to develop a more complete paradigm of how spatial cues from cell adhesion and mechanical forces regulate single-cell behavior and multicellular organization. Although we have developed robust, sophisticated tools to pattern cells on surfaces, many of these approaches have not been widely adopted in the biomedical research community. Here, we demonstrate the use of inexpensive, accessible materials to obtain comparable patterning fidelity for two specialty applications – patterning elastomeric substrates and controlling intercellular contact. The adoption of such tools in the biological research community will be an essential step in the integration of microfabrication technology with biological applications.

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