14 Self-Assembled Monolayers in Mammalian Cell Cultures

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CONTENTS

I. INTRODUCTION

Self-assembled monolayers (SAMs) are materials comprising ordered monolayers of organic molecules supported on solid substrates.[1,2] The most extensively investigated types of SAMs form when molecules having the general formula $HS(CH_2)_nY$ self-assemble on films of gold, silver, or palladium.[1,3,4] In general, for SAMs formed from XRY, –X is a functional group on the molecule that has a high chemical affinity to a solid substrate, –Y represents a group whose functions can be tailored to generate a surface that has a particular property, and –R is an organic linker in between. Interactions between the –R groups, as well as those between the –Y groups, also contribute to the stability of the system. SAMs offer better control of surface chemistry than polymers, metals, and metal oxides. By making it possible to control the molecular level structure of surfaces, SAMs have allowed applications ranging from the fabrication of surfaces that are tailored to present biomolecular ligands, through to the development of supports for attached tissue cultures, to the testing of substrates for tissue engineering. SAMs are also widely used in a range of non-biological applications (micro and nanolithography,[5] preparation of substrates for studies of wetting,[6,7] studies of adhesion and tribology,[8,9] studies of nucleation sites for crystallization,[10] modification of surfaces of electrodes,[11] and studies of mechanisms of electron transport in organic molecules).[12,13] This chapter focuses on applications of SAMs in cell and tissue cultures.

II. PREPARATIONS OF SAMs AND TYPES OF SAMs

SAMs are formed by immersing gold-coated substrates in solutions of organic disulfides or alkanethiols.[14] Table 14.1 lists a number of different types of SAMs.

A. SAMs of Alkanethiols on Gold and Silver

Alkanethiolates with the general formula $HS(CH_2)_nY$ self-assemble into ordered monolayers on gold and silver. This type of SAM is the most widely used in studies related to biology,[15,16] Although silver forms more ordered SAMs than gold, silver has two characteristics that make it less attractive than gold for biological applications: it is toxic to cells, and it oxidizes to silver oxide

TABLE 14.1
Substrates and Ligands that Form SAMs

Substrate	Organic Precursor	Reference
Au	RSSR′ R = aliphatic or aromatic	103
—	RSH	103
—	RSR	103
Ag	RSH	103
Cu	RSH	103
Pd	RSH	4
Pt	RNC/RSH	103
SiO_2/glass	$RSiX_3$, X = Cl, OH, OCH_3, OCH_2CH_3	104
Si/SiH	$(RCOO)_2$	105
—	RCH = CHR′	106
—	RLi, RMgX	107
GaAs	RSH	108
InP	RSH	109
In_2O_3/SnO_2	RPO_3H_2	110
Al_2O_3/other metal oxides	$RCOOH/RCONHOH/ROPO_3^{2-}/RPO_3^{2-}$	103

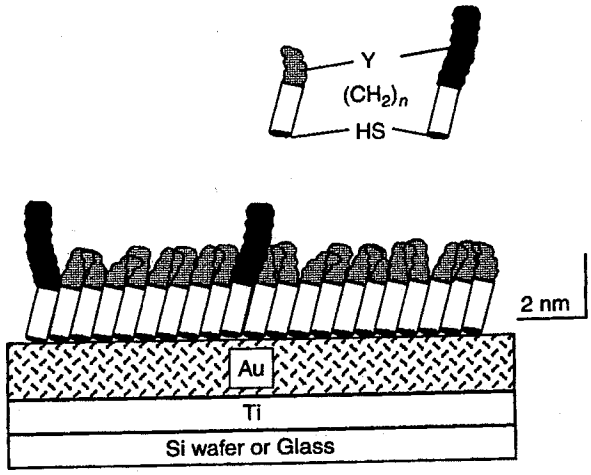

FIGURE 14.1 SAMs are formed by $HS(CH_2)_mY_1$ and $HS(CH_2)_nY_2$ on gold. A generic scenario of a mixed SAM formed by two different types of alkanethiols is shown.

upon contact with air. SAMs on gold are therefore the preferred materials for studies in biology. This chapter will focus on the SAMs formed by thiols on gold.

Mixed SAMs can be formed from a mixture of $HS(CH_2)_mY_1$ and $HS(CH_2)_nY_2$[17] (where $-Y_1$ and $-Y_2$ represent different types of chemical groups, and m and n can be the same or different; Figure 14.1). Mixed SAMs have important applications in cell and tissue engineering (see Sections VI–VII in this chapter).

B. SAMs of Alkylsiloxanes

SAMs of alkylsiloxanes on Si/SiO_2 are useful for functionalizing surfaces of glass and silicon wafers.[15] These SAMs have found many applications in controlling cell attachment,[18] patterning neurons,[19,20] and patterning DNA on chips.[21,22] This class of SAMs has been extensively reviewed elsewhere and will not be addressed in this chapter.[23]

C. Other Types of SAMs

Table 14.1 lists some of the other types of SAMs. These SAMs are not as widely used as the SAMs formed by thiols on gold, but we list them for completeness; some of them have been extensively characterized while others have not.[5]

III. CHARACTERIZATION OF SAMs

SAMs on gold are molecularly well-defined interfacial materials. They are thin films that self-assemble onto a solid substrate with semicrystalline or polycrystalline packing, and whose chemical and physical properties are largely controlled by the characteristics of the terminal groups.[24] Many techniques used for analyzing surfaces have been employed to characterize the structure and composition of SAMs and systems involving SAMs.

Contact angle goniometry gives information about the homogeneity of the SAM by examining the hydrophobicity (i.e., the wetting properties) of the surface; this property is largely controlled by the terminal groups presented on the SAMs.[1,14] Infrared (IR) spectroscopy is used to characterize the ordering of SAMs.[25] Ellipsometry measures the thickness of an organic film formed on a metallic surface, and it is particularly useful for analyzing the amount of the adsorbent

on the surface.[26,27] Mass spectrometry (MS) identifies the types of organic functional groups that can be ejected in ionized form from the surface.[28,29] X-ray photoelectron spectroscopy (XPS) provides the elemental composition of the surface. It is useful for confirming the elemental composition of various types of SAMs and characterizing the adsorbed proteins or polymers on SAMs.[30–32] Scanning probe microscopy provides information about the composition of SAMs on the atomic scale.[33,34]

Surface plasmon resonance (SPR) spectroscopy allows *in situ* measurements of the thickness of adsorbed organic films on the surfaces of metals based on the shift in the resonance angle of surface plasmons.[35–37] Quartz crystal microbalance (QCM) is an acoustic method that gives real-time information about the amount and conformation of the proteins adsorbed,[38] based on the change in resonance frequency of a crystal as the mass and viscoelastic properties of the adsorbed material on the quartz are varied.[39] SPR and QCM have become popular methods for measuring the adsorption of proteins on surfaces for the following reasons: they provide thermodynamic and kinetic information about adsorption, they do not require the proteins to be labeled, and they require only modest amounts of proteins. Typically, SPR provides higher signal-to-noise ratios than QCM, although QCM provides additional information about the change in conformation of the proteins as they adsorb, and about the viscoelastic properties of the adsorbed protein film.

These methods generate qualitative and quantitative information — especially composition and coverage — about the properties of SAMs. Some of them are also used to study the interactions of biomolecules and cells with SAMs.

IV. INTRODUCTION OF FUNCTIONAL GROUPS TO THE SURFACES OF SAMs

SAMs have the capacity to accommodate a wide range of chemical groups on a surface. Thus, diverse biochemical functionalities and macroscopic properties can be incorporated. Because SAMs enable molecular level control of interfaces, they allow the study of structure–property relationships. This capability to fine-tune the surface-properties of SAMs is the major advantage of SAMs over organic polymers, metals, or metal oxides for fundamental studies.

There are two main methods of introducing chemical groups onto surfaces using SAMs. One method is to synthesize the alkanethiol or organic disulfide that already terminates in the appropriate functional group. The second method is to introduce functional groups onto the surface

TABLE 14.2
Biologically Important Ligands that Have Been Introduced onto the Termini of SAMs

Molecule on SAM	Molecule in Solution/Function	Reference
Benzensulfonamide	Carbonic andydrase	70
Nitrilotriacetic acid	Ni (II) and His-tag	71
Biotin	Avidin/strepavidin/antibiotin	52
RGD	Integrin	42
Ang-1	Integrin (α5)	111
Mannose	FimH adhesin	43
Globotriose	IgG/IgM	44
Acridine	DNA	112
D-Ala-D-Ala	Vancomycin	113
PEG and others	Resist nonspecific adsorption of proteins	26
Carbohydrates	Lectins	77
Kinase substrates	Kinase	75
Phosphonate	Cutinase	72

after the formation of a SAM. Using these reactions, small molecules, peptides, sugars, proteins, and DNA can be incorporated onto surfaces (Table 14.2).

A. The Synthesis of SAMs

Many types of thiols have been synthesized. For example, we synthesized thiols that resist the non-specific adsorption of proteins, such as polyethylene glycol (PEG)-terminated thiols.[40,41] We also made thiols that terminate in a peptide sequence (e.g., RGD) and promote adhesion of cells.[42] We, among others, synthesized thiols that terminate in carbohydrate groups to carry out fundamental studies of the interactions between proteins and surfaces.[43,44]

B. *In Situ* Transformation

An alternative strategy for introducing functional groups onto a surface is to carry out chemical reactions on the terminal groups of the SAM after its formation on the surface. The approach using activated esters and related groups is one of the most commonly used strategies to introduce functional groups onto the surfaces of SAMs. Conversion to functional moieties usually involve intermediates such as succinimidyl or pentafluorephenyl esters,[45,46] interchain anhydrides,[47] maleimide,[48] aldehydes,[49] or acid chlorides/fluorides.[50]

Mrksich and coworkers used electrochemistry to immobilize or release functional groups on SAMs. In one such system, a monolayer of hydroquinone was electrooxidized to quinone, and the quinone subsequently reacted with a solution of cyclopentadiene to give the Diels–Alder adduct on the surface.[51] In a second example, a monolayer of a quinone propionic ester was selectively reduced by applying a reductive potential to the gold substrate, and this reduction caused the release of biotin from the SAM.[52]

Other types of chemical reactions have been employed to introduce functional groups onto surfaces, for example, photochemical reactions that carry out changes on SAMs.[53] A more extensive review on chemical reactions on SAMs covers the details of all types of *in situ* transformations on SAMs.[54]

Each of the two strategies used for introducing functional groups onto the termini of SAMs has its advantages and disadvantages. The synthesis of desirable thiols is often laborious and cannot be used to introduce functional groups that are incompatible with sulfides or disulfides. However, the formation of the SAM is convenient (simple immersion of the gold coated substrate in solutions of desired thiols) once the thiols are made. *In situ* transformation of SAMs is often more straightforward in synthesis and allows several layers of transformations, but it is difficult to achieve 100% conversion and to control the exact densities of ligands in mixed SAMs.[54] *In situ* transformation, therefore, typically leads to heterogeneous surfaces. Many types of biologically relevant groups have been introduced onto the surfaces of SAMs; Table 14.2 lists some of them.

V. Chemical Transformations That Modify the Interactions Between the SAM and the Substrate

This section covers chemical reactions that change the sulfur–gold bond and alter the overall structures of SAMs. The ability to selectively change the properties of a surface using this class of chemical reactions has important applications in biology.

A. Using Electrochemistry to Desorb SAMs

When an electrical potential is applied across the monolayer, the thiols making up the SAM desorb and the integrity of the SAM is destroyed.[55] Electrochemical desorption of SAMs is used to release biological ligands from surfaces,[56,57] and this process has been extensively studied, characterized, and applied to various systems.[55,58,59] Electrochemists have focused on the desorption of SAMs on

the dependence of the strength of the applied potential, the length of the thiol chains making up the SAM, and the conditions in solution (such as pH and temperature).[60,61] Desorption of PEG-terminated SAMs enables adhesion of proteins and attachment of cells in areas that were originally inert, and allows patterned cells to be released from areas of confinement.[59,62] This process is useful for studying the spreading of cells.

B. Oxidation of SAMs and Other Systems

Light and air can irreversibly oxidize thiols and cause them to desorb from surfaces of gold.[63] SAMs were used as photoresists for the patterning of micrometer sized features and can be used to study the adhesion and patterning of mammalian cells.[64,65]

Langer and coworkers[66] made a system of "switchable" SAMs that can reversibly modify the wettability of a surface by controlling electrochemical changes on the surface.

VI. INTERACTIONS OF PROTEINS WITH SAMs

By enabling molecular level control of interfaces, SAMs allow systematic studies of the interactions between proteins and surfaces. Because the interactions between cells and surfaces are mediated by proteins, these studies also provide the basis for studies of interactions between cells and surfaces. SPR, QCM, and ellipsometry are the main tools used to quantify the amounts of proteins adsorbed onto a given surface (see Section III).

A. Using SAMs as Model Surfaces to Study Adsorption of Proteins

Proteins adsorb irreversibly to many kinds of surfaces.[26] Adsorption of proteins on surfaces is a complex issue, dominated by hydrophobic, electrostatic, and van der Waals interactions. The adsorption is highly dependent on the structures and properties of both the proteins and the surfaces. We will only present a brief overview of this issue and point out related studies important for tissue engineering.[16]

Since SAMs allow the incorporation of different types and densities of chemical functional groups onto surfaces, macroscopic properties of the surface, such as wettability, can be systematically varied. Studies of the wettability of surfaces using SAMs have improved the understanding of the physical parameters that determine the adsorption of proteins. For instance, in general, the more hydrophobic a surface is, the more proteins adsorb (with some exceptions).[67]

B. "Inert" Surfaces

We refer to surfaces that resist the non-specific adsorption of protein as "inert" surfaces. PEG-terminated SAMs were initially identified as being inert.[26] We have screened many types of surfaces to identify additional types of inert surfaces using the anhydride chemistry approach (see Section IV.B).[68] There are certain common features associated with surfaces that are inert (such as overall electrical neutrality, polarity, H-bond donor/acceptor), but there are still no general principles for designing inert surfaces. PEG and related surfaces are the most inert surfaces that have been studied thus far.[68]

C. Non-Specific Adsorption of Proteins on Hydrophobic SAMs

SAMs allow systematic studies of interactions of proteins with surfaces presenting hydrophobic groups of well-defined shapes. We used mixed SAMs presenting hydrophobic headgroups in a background of PEG-terminated thiols as a model to study the adsorption of proteins using SPR. The extent to which adsorbed proteins undergo conformational rearrangements appears to depend

on the density of the hydrophobic groups at the surface, and on the concentration of proteins in solution.[69]

D. Biospecific Adsorption of Proteins on SAMs

Using inert surfaces, it is possible to generate surfaces that present specific ligands that immobilize only desired proteins.[36,70] These surfaces allow studies of the thermodynamics and kinetics of the interactions between a type of ligand and its receptor on the surface.[71,72]

E. Electroactive Proteins on SAMs

It is also possible to probe the conformation of redox active proteins on surfaces through the use of electrochemistry and SAMs. The formal redox potential of cytochrome c (cyt c), when it is adsorbed onto a SAM gives information about the conformation and orientation of the protein. The conformation and orientation of the protein adsorbed, and thus redox potential, changes depending on the type of monolayer presented on the surface.[73] For instance, differences in formal redox potentials of cyt c shows that the conformation of cyt c adsorbed on SAMs terminating in methyl, amine, and aromatic groups is different than on SAMs terminating in trimethylammonium and carboxylic acid groups. The rates of electron transfer in these SAMs are also different.

F. Controlling the Orientation of Proteins on Surfaces

How is the conformation of an adsorbed protein determined by the properties of the surface? Some studies on the adsorption of proteins on SAMs have demonstrated how conformations of proteins can be determined by the charge and hydrophobicity of the surface. It is still difficult to determine, *a priori*, the conformation of any given protein on a surface with known physical and chemical properties, although in certain cases, controlling the orientation of proteins on surfaces with either electrostatic forces or biospecific binding is certainly possible.[72,74]

G. Other Biomolecules: DNA, Lipids, and Others

In addition to proteins, it is possible to immobilize other classes of biologically important molecules on SAMs, such as peptides,[75] DNA,[56] lipids,[76] and carbohydrates.[77] Some of these molecules have found uses in tissue engineering.[16]

SAM-based systems have dramatically improved the understanding of how proteins interact with surfaces, especially the influence of charge and hydrophobicity of the surface on the adsorption of proteins. The effect of pH and ionic strength on the adsorption of certain proteins yielded information about the mechanism of adsorption.[39] Principles derived from these studies have enabled the development of new types of inert surfaces, as well as the design of materials having properties that interact favorably with proteins.[16]

VII. Controlling Adsorption of Proteins and Patterning of Cells; Application of SAMs in Cell Biology

Most mammalian cells need to attach to a surface and spread, in order to go through the cell cycle and maintain basal cellular functions.[78] The attachment of mammalian cells can be controlled using SAMs. There are several levels in controlling the attachment of cells: controlling the composition and density of ligands for cell-surface receptors, minimizing non-specific attachment, and spatially confining the patterns of cells.[42,79,80] These technologies provide the opportunity to study in detail the biology of attached cells.

A. Controlling the Composition and Density of Ligands on the Surface

It is possible to achieve biospecific attachment of mammalian cells using a SAM presenting the peptide sequence arginine–glycine–aspartate (RGD) in a background of PEG groups.[42] By controlling the relative amount of RGD-terminated thiols in the mixed SAM, we can control the adhesion of cells on the substrate. This system allows systematic studies of the fundamental mechanisms of adhesion on surfaces, as well as the spreading and detachment of cells.

B. Soft Lithography, μCP, and Patterning Proteins

"Soft lithography" refers to a set of technologies capable of generating micro- and nanofeatures rapidly using flexible polymers such as poly(dimethylsiloxane) (PDMS).[5] Micro-contact printing (μCP) is an important technique in soft-lithography, where a stamp carrying an ink is used to "print" thiols onto gold to form spatially confined SAMs (Figure 14.2).[81] This method has become a standard technology to generate patterns of proteins and cells, and is generally applicable to many types of proteins. Once the stamp is made, it is straightforward to form patterns of SAMs on a surface. This process does not require the use of a clean room and can be implemented under ambient conditions of most biology laboratories. Figure 14.2 shows how this process can be used to generate arrays of proteins.

To pattern islands of proteins, a thiol that terminates in a methyl group (such as $HS(CH_2)_{17}CH_3$) is first printed onto the gold using a PDMS stamp. The surface is then incubated with a second thiol

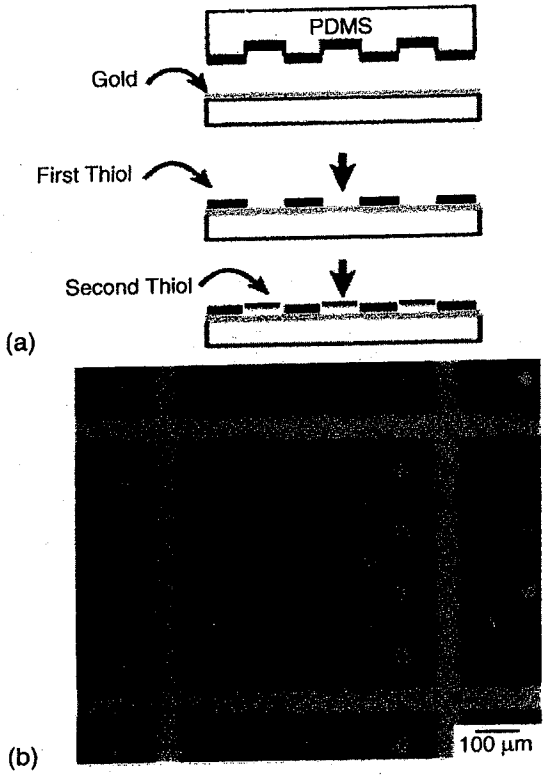

FIGURE 14.2 Generation of micropatterns of proteins by μCP of SAMs. (a) A schematic illustration of the procedure of μCP. (b) Incubation of a chip that has SAMs patterned by μCP with a solution of fibronectin, an extracellular matrix protein, results in arrays of proteins. The proteins were visualized by antibodies against fibronectin.

that terminates in an inert group (such as PEG, $-(CH_2CH_2O)_{n=3-6}OH$) in order to fill the uncovered surfaces of gold with PEG. Patterns of proteins are made by the adsorption of proteins from a solution containing them.

C. Patterning Cells

Most mammalian cells require adsorbed extracellular matrix (ECM) proteins or adhesion molecules to attach to surfaces.[78] Defined patterns of these proteins or molecules can restrict the spreading of cells.[79,82] The geometry (i.e., the sizes and shapes) of attached cells can be conveniently controlled by the geometry of the stamps used in μCP. Figure 14.3 illustrates the result of a single bovine endothelial (BCE) cell patterned using μCP of SAMs.

By varying the sizes and total attachment areas of a single cell using patterned arrays of ECM proteins, we determined that it is the total area of projection of a cell, instead of the total area of attachment, that is important in determining whether a cell undergoes growth or programmed death (i.e., apoptosis).[83]

D. Dynamic Patterning of Cells and the Patterning of Multiple Proteins and Cells

When cells are patterned onto SAMs, it is possible to release them controllably from their patterns of confinement; we call this process "dynamic patterning."[59,84] Cells can be released by electrochemical desorption of SAMs,[59] and we believe that this straightforward method will allow cell-based assays based on cell motility for the screening of drugs. Mrksich and coworkers[84] electrooxidized a mixed SAM of hydroquinone and PEG (that was overall inert to the spreading and attachment of cells) to immobilize RGD-tethered cylcopentadiene; the attachment of this peptide enabled the release of cells controllably from their patterns of confinement.

There have been other methods to pattern cells into defined geometries, for example, using agar,[85] siloxane-based chemistry on glass or silicone chips,[20,86] polymers,[87] and elastic membranes.[88] Micropatterning of cells with SAMs on gold is the only process to date that is

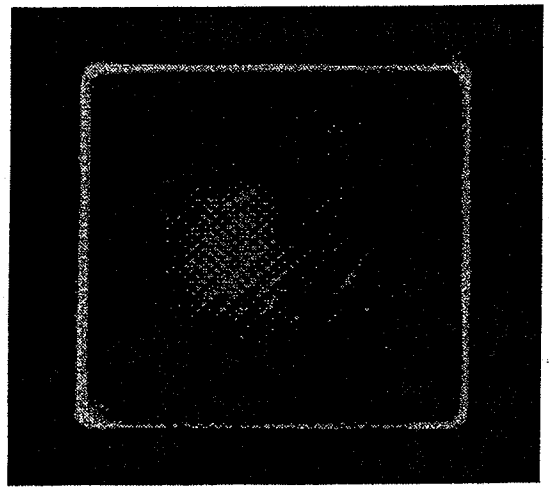

FIGURE 14.3 A single bovine capillary endothelial cell patterned on a square microisland. The cell is stained with a fluorescent probe that binds actin. Also shown is the nucleus.

simple to implement, allows control of cell adhesion, achieves high levels of fidelity, is able to maintain long-term stability, and is convenient for the execution of dynamic patterning.

E. Directional Control of Cell Migration

Defining the shape of a cell can direct cell migration.[89] When cells that are confined to square patterns are stimulated by growth factors, they extend their lamellipodia, filopodia, and microspikes preferentially to the corners of the squares. Their behaviors are similar on other shapes having corners, such as triangles, pentagons, and hexagons.[90]

Technologies based on SAMs can be used to control the attachment of cells that have defined sizes, shapes, and extent of spreading. These technologies yield information about the cell that is difficult to obtain through biochemical methods.

VIII. THE BRIDGE TO TISSUE ENGINEERING

A tissue is a complex structure made up of different types of cells with specific arrangements that carry out certain functions. The generation of tissues from a few undifferentiated cells is a complex, well-regulated process that is only partially understood.[91] Tissue engineers have not yet been able to "grow" vascularized organs *in vitro*. There have only been a few examples of successful demonstrations of simple man-made tissues generated *in vitro*.[92,93] Many complex problems limit our ability to generate vascularized organs *in vitro*. One major issue is the ability to control the interaction between cells and substrates. Using SAMs for the control of this interaction has opened up avenues to the *in vitro* production of several tissue-like biomaterials, although the area of culturing different types of cells with the correct structure and arrangements using SAMs, or any other technology, is still in its infancy. In some cases, patterned cells mimic certain structures found in tissues or organs. There are some initial demonstrations of tissue-like functions of cultured cells in SAM-based and non-SAM-based systems. This section outlines cultured cells that mimic the structures and functions of certain tissues.

A. Angiogenesis

Angiogenesis is the process by which new blood vessels form; it is an important physiological process in areas ranging from tissue generation to the growth of tumors.[94] By controlling the area of cell spreading, we can control switching between growth, differentiation, and apoptosis during angiogenesis.[95] When cells are allowed to spread fully (on islands $>1500\ \mu m^2$), they grow, and when spreading is restricted to small areas (on islands $<500\ \mu m^2$), they undergo apoptosis. When spreading of an endothelial cell is confined to intermediate areas (narrow lines of 10 μm), the cells neither divide nor undergo apoptosis. Rather, they differentiate into hollow tubes that resemble blood vessels, but when cells are confined to lines of 30 μm, they do not differentiate into tubular structures (Figure 14.4).

B. Patterning of Neurons

Patterning networks of neurons provide insight into the cellular basis of neuroscience. A future idea is to interface defined patterns of neurons with silicon-based computers. Neurons from both invertebrates (such as aplysia) and vertebrates (such as goldfish and rat) can be patterned into defined geometries. Some of these neurons produce functional synapses that mimic synapses in the brain. These primitive neuronal networks are useful in understanding how electrical signals propagate in the nervous system.[19,96]

One important aspect of the patterning of neurons and the understanding of neuronal development is to specify the polarity of neurons on a given substrate. Micro-fluidic gradients of chemoattractants or repellants can control the polarity of cultured rat hippocampal neurons.[97]

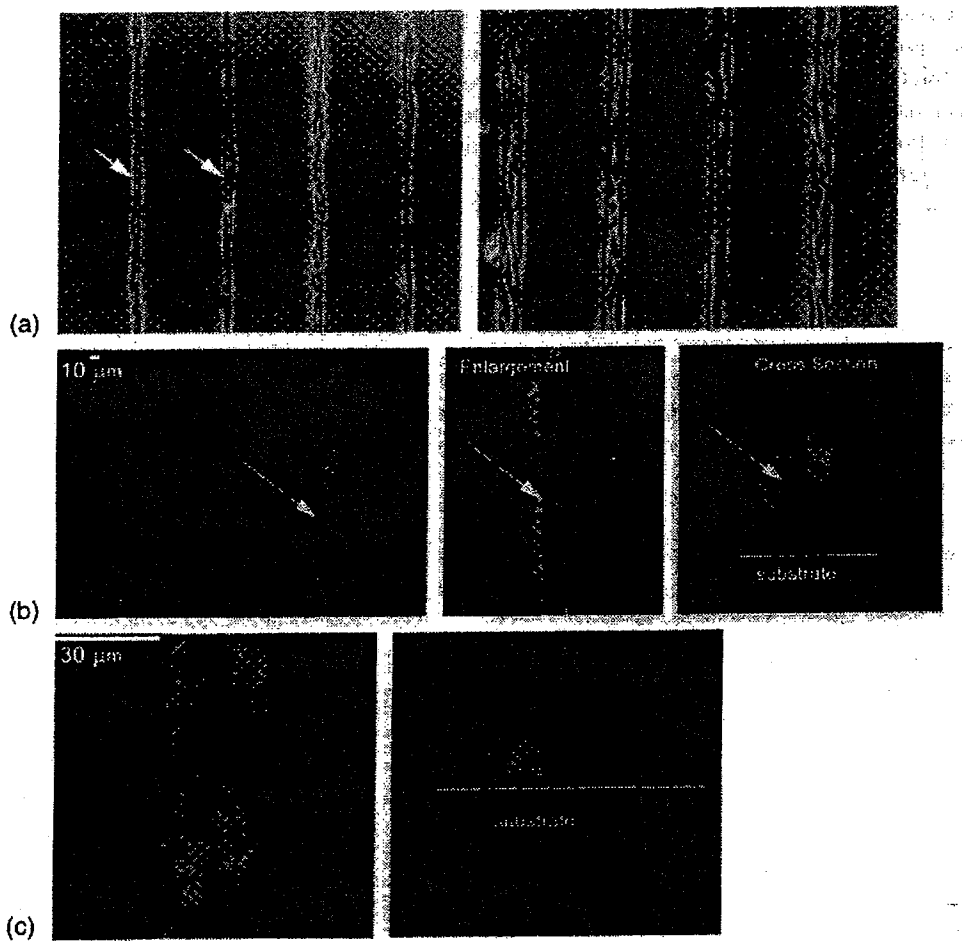

FIGURE 14.4 Formation of capillary tubes by endothelial cells on striped patterns. Cells cultured on 10 μm lines differentiate into capillaries, while those on 30 μm lines do not. (a) Phase-contrast images of endothelial cells cultured on 10 or 30 μm lines. White arrows indicate chords of cells that formed a tube with a central lumen, while black arrows indicate those that did not. (b) and (c) are fluorescent micrographs illustrating the tubes on 10 μm lines (b) vs. those on 30 μm lines (c).

C. Coculturing of Different Types of Cells

The ability to achieve controlled patterns of two or more types of cells is a key issue in tissue engineering because the development of tissues usually involves the mutual interactions of several different types of cells.[91] The ability to pattern two or more types of cells is one route to achieve artificial tissue *in vitro*. Using SAMs and related methods, it is possible to pattern several different types of cells on a solid substrate.

Using electrochemical methods on SAMs,[84] Mrksich and coworkers[98] demonstrated culturing of different populations of cells using SAMs. There have been other methods that utilize the printing from elastic stamps or membranes to pattern several different types of cells. For instance, Chen and coworkers[99] used multi-level stamps to pattern multiple types of proteins to control the attachment of different types of cells, and Toner and coworkers[100] used elastic membranes and stamps to make cocultures of hepatocytes and non-parenchymal cells. Their strategy can define the extent of the contacts and the ratio of population between hepatocytes and non-parenchymal cells,

and provide information on how the interaction between the two types of cells determines the phenotype of hepatocytes.

Applications of SAMs in tissue engineering are limited by the fact that SAMs can only provide two-dimensional surfaces, while most tissues need to fold into three-dimensional structures to function properly. Methods using SAMs and related systems allowed the fabrication of pseudo three-dimensional structures.[101]

IX. OTHER APPLICATIONS OF SAMs IN BIOCHEMISTRY AND BIOTECHNOLOGY

SAMs are widely applicable in many fields of biochemistry and biotechnology.[102] Methods that render the surface inert, and methods that enable biospecific binding on surfaces, are both useful in various types of screening assays involving both proteins and cells.[15]

X. CONCLUSIONS AND SUMMARY

SAMs are a class of materials that can be molecularly controlled. They are robust under a variety of physiological conditions for up to a few weeks, but long-term robustness of SAMs in tissue implants have not been carefully evaluated. The key application of SAMs in tissue engineering is likely to stem from its capacity to tune the extent and geometry of attachment of various types of cells.

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