Molecular Self-assembly

Molecular self-assembly is ubiquitous in nature and has now emerged as a new approach in chemical synthesis, nanotechnology, polymer science, materials, and engineering. Molecular self-assembly systems lie at the interface between molecular biology, chemistry, polymer science, materials science, and engineering. Many self-assembling systems have been developed. These systems range from bi- and triblock copolymers to complex DNA structures and simple and complex proteins and peptides. Molecular self-assembly systems represent a significant advance in the molecular engineering of simple molecular building blocks useful for a wide range of applications. This field is extremely broad and is growing at an accelerating pace. This article therefore restricts molecular self-assembly to biological building block systems only.

1. The Basis of Self-assembly and Molecular Self-assembly

Self-assembly is everywhere in nature at both macroscopic and microscopic scales—for example, from the assembly of schools of fish in the ocean, flocks of birds in the sky, and herds of wild animals to oil droplets in water. Self-assembly describes the spontaneous association of numerous individual entities into a coherent organization and well-defined structure to maximize the benefit of the individual without external instruction.

Molecular self-assembly, by definition, is the spontaneous organization of molecules under thermodynamic equilibrium conditions into structurally well-defined and rather stable arrangements through a number of noncovalent interactions (Lehn 1993, Whitesides *et al.* 1991, Ball 1994). These molecules undergo self-association forming hierarchical structures. The key engineering principle for molecular self-assembly is artfully to design the molecular building blocks that are able spontaneously to undergo stepwise interactions and assemblies through the formation of numerous noncovalent weak chemical bonds. These typically include hydrogen bonds, ionic bonds, and van der Waals bonds (Pauling 1960) to assemble these molecules into well-defined and stable hierarchical macroscopic structures. Although each of the bonds is rather weak, the collective interactions can result in very stable structures and materials. The key elements in molecular self-assembly are chemical complementarity and structural compatibility. Like hands and gloves, both the size and the correct orientation i.e., chirality, are important in order to have a complementary and compatible fitting.

2. Molecular Self-assembly in Nature

Biomimicry and designing nature-inspired materials through molecular self-assembly is an emerging field at the beginning of the twenty-first century. Nature is a grand master at designing chemically complementary and structurally compatible constituents for molecular self-assembly through eons of molecular selection and evolution. Chemical evolution from the first groups of primitive molecules through countless iterations of molecular self-assembly and disassembly has ultimately produced more and more complex molecular systems.

In the last few years, considerable advances have been made in the use of peptides, phospholipids, and DNA as building blocks to produce potential biological materials for a wide range of applications (Zhang *et al.* 1993, Aggeli *et al.* 1997, Schnur 1993, Winfree *et al.* 1998). The constituents of biological origins, such as phospholipid molecules, amino acids, and nucleotides have not been generally considered to be useful materials for traditional materials science and engineering. The advent of biotechnology and genetic engineering coupled with the recent advancement in chemistry of nucleic acids and peptide syntheses has resulted in a conceptual change. Molecular self-assembly is emerging as a new route to produce novel materials and to complement other materials, i.e., ceramics, metals and alloys, synthetic polymers, and other composite materials. Several recent discoveries and rapid developments in biotechnology, however, have rekindled the field of biological materials engineering (Urry 1997, Huc and Lehn 1997, Petka *et al.* 1998).

There are numerous examples of molecular self-assembly in nature. One of the well-known examples is silk assembly. The monomeric silk fibroin protein is approximately one micrometer long but a single silk worm can spin fibroins into silk materials over two kilometers in length, two billion times longer! (See Feltwell 1990, Winkler *et al.* 1999, *Silk Produced by Engineered Bacteria*.) Such marvelous engineering skill is hardly matched even by current technology. These building blocks are often on the nanometer scale. However, the resulting materials could be measured on meter and kilometer scales. Likewise, the size of individual phospholipid molecules is approximately 1.5 nm in length, but they can self-assemble into millimeter-size lipid tubules with defined helical twist, many million times larger, and a number of applications have been developed (Schnur 1993). Each nucleotide is approximately 0.34 nm in size. Two single-stranded DNA double helices in human chromosome 22 (34.4 million base pairs) can extend to as long as approximately 1.2 cm, about 35 million times longer (Dunham *et al.* 1999).

The power of molecular self-assembly can never be underestimated. Molecular self-assembly can also build sophisticated structures and materials. For

example, collagen and keratin can self-assemble into ligaments and hair, respectively (see *Natural Protein Fibers* and *Keratin*). In cells, many individual chaperone proteins assemble into a well-defined ring structure to sort out, fold, and refold proteins (Sigler *et al*. 1998). The same is true for other protein systems such as seashell biomineralization (Morse 1999, *Bone and Natural Composites: Properties*, *Bone Mineralization*, *Shell: Properties*, *Pearl*). Likewise, mammals build their teeth through self-assembly of a protein scaffold that is made up of many individual proteins first and then recruit calcium ions to the sites for biomineralization (see *Marine Teeth* (*and Mammal Teeth*), *Ivories*, *Pearl*, *Fibrous Ceramics*).

3. *Self-assembling Peptide Systems*

A new class of oligopeptide-based biological materials was serendipitously discovered from the self-assembly of ionic self-complementary oligopeptides (Zhang *et al*. 1993). A number of peptide molecular self-assembly systems has been designed and developed. This systematic analysis has provided insight into the chemical and structural principles of peptide self-assembly. These peptides are short, simple to design, extremely versatile, and easy to synthesize. Three types of self-assembling peptides have been systematically studied thus far. Some believe that additional different types will be discovered and developed in the coming years. This new class of biological materials has considerable potential for a number of applications, including scaffolding for tissue repair and tissue engineering, drug delivery of molecular medicine, and biological surface engineering. Similar systems have also been described where these peptide systems undergo self-assembly to form a gel with regular β-sheet tapes of well-defined structure (Aggeli *et al*. 1997). The self-assembly of peptide nanotubes which allow ions to pass through and to insert themselves into lipid bilayer membranes was also described (Ghadiri *et al*. 1994, Bieri *et al*. 1999). Furthermore, a number of fascinating biomimetic peptide and protein structures have been engineered, such as helical coil–coils and di-, tri-, and tetrahelical bundles (O'Shea *et al*. 1989, Hecht *et al*. 1994, Baker and DeGrado 1999). However, their applications for materials science and engineering remain under-explored. It is likely that these stable coiled coils will be developed as nanomaterials in the future.

3.1 *Type I Self-assembling Peptides*

Type I peptides, also called "molecular Lego," form β-sheet structures in aqueous solution because they contain two distinct surfaces: one hydrophilic and the other hydrophobic. Like Lego bricks that have pegs and holes and can only be assembled into particular structures, these peptides can be assembled at the molecular level. The unique structural feature of these peptides is that they form complementary ionic bonds with regular repeats on the hydrophilic surface (Fig. 1). The complementary ionic sides have been classified into several moduli, i.e., modulus I, II, III, IV, etc., and mixed moduli. This classification is based on the hydrophilic surface of the molecules that have alternating positively and negatively charged amino acid residues, alternating by 1, 2, 3, 4, and so on. For example, molecules of modulus I have $-+-+-+-+$, modulus II $--++--++$, and modulus IV $----++++$. These well-defined sequences allow them to undergo ordered self-assembly, resembling a situation found in well-studied polymer assemblies.

Upon the addition of monovalent alkaline cations or the introduction of the peptide solutions into physiological media, these oligopeptides spontaneously assemble to form macroscopic structures which can be fabricated into various geometric shapes (Fig. 2) (Zhang *et al*. 1995). Scanning electron microscopy reveals that the matrices are made of interwoven filaments that are about 10–20 nm in diameter and pores about 50–100 nm in diameter (Zhang 1993, Leon *et al*. 1998).

The molecular structure and proposed complementary ionic pairings of the Type I peptides between positively charged lysines and negatively charged glutamates in an overlap arrangement are modeled in Fig. 1. This structure represents an example of this class of self-assembling β-sheet peptides that spontaneously undergo association under physiological conditions. If the charged residues are substituted, i.e., the positively charged lysines (Lys) are replaced by positively charged arginines (Arg) and the negatively charged glutamates (Glu) are replaced by negatively charged aspartates (Asp), there are essentially no drastic effects on the self-assembly process. However, if the positively charged residues, Lys and Arg, are replaced by negatively charged residues, Asp and Glu, the peptide can no longer undergo self-assembly to form macroscopic materials although it can still form β-sheet structures in the presence of salt. If the alanines (Ala) are changed to more hydrophobic residues, such as Leu, Ile, Phe, or Tyr, the molecules have a greater tendency to self-assemble and form peptide matrices with enhanced strength (Leon *et al*. 1998).

The fundamental design principles of such self-assembling peptide systems can be readily extended to polymers and polymer composites, where copolymers can be designed and produced. Mankind has learned a great deal from nature, has gone many steps further, and will continue to create new materials.

3.2 *Type II Self-assembling Peptides*

Several Type II peptides have been developed as "molecular switches" in which the peptides can

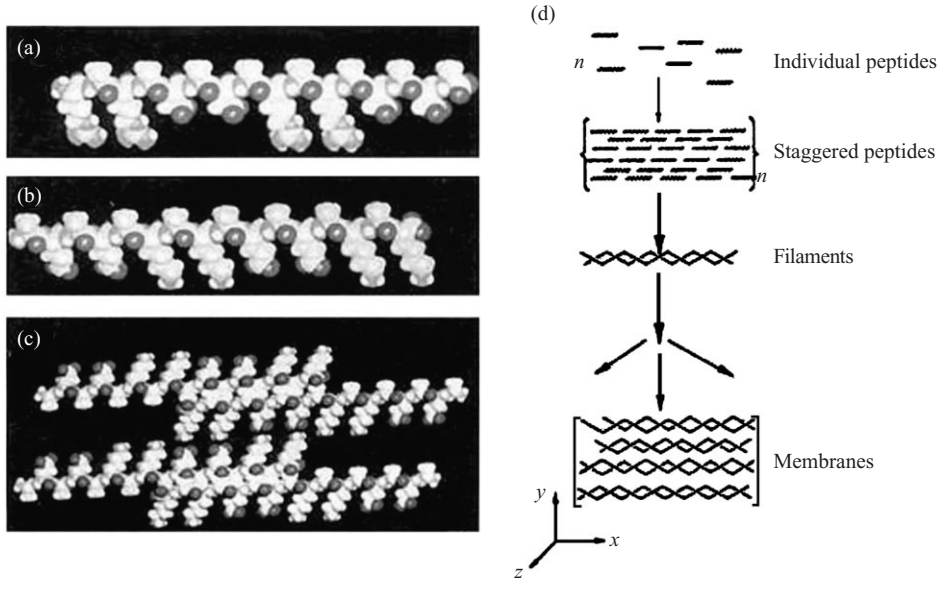

Figure 1
Molecular models of the extended β-strand structures of individual molecules are shown for RAD16 (a) and EAK16 (b). The distance between the charged side chains along the backbone is approximately 6.8 Å; the methyl groups of alanines are found on one side of the sheet and the charged residues are on the other side. Conventional β-sheet hydrogen bond formation between the oxygen and hydrogen on the nitrogen of the peptide backbones are perpendicular to the page; (c) a proposed staggered assembly of molecular models for EAK16. The complementary ionic bonds and hydrophobic alanines are shown. Although an antiparallel β-sheet is illustrated, a parallel β-sheet model is also possible; (d) a proposed model of sequential events which could lead to assembly of macroscopic matrices showing one of the possible pathways of matrix formation. The three dimensions of the materials are indicated by *x*, *y*, and *z*. Geometric shapes other than membrane can also be produced as suggested by the diverging thin arrows (see Fig. 2).

drastically change their molecular structure (Fig. 3). One of the peptides with 16 amino acids, DAR16-IV, has a β-sheet structure 5 nm in length at ambient temperature but can undergo an abrupt structural transition at high temperatures to form a stable α-helical structure 2.5 nm long (Zhang and Rich 1997). Similar structural transformations can be induced by changes in pH. This suggests that secondary structures of some sequences, especially segments flanked by clusters of negative charges on the N-terminus and positive charges on the C-terminus, may undergo drastic conformational transformations under the appropriate conditions. These findings can not only provide insights into protein–protein interactions during protein folding and the pathogenesis of some protein conformational diseases, including scrapie and Huntington's, Parkinson's, and Alzheimer's diseases (Dobson 1999) (Fig. 4), but can also be developed as molecular switches for a new generation of nano-actuators.

The peptides of DAR16-IV (DADADADARARARARA) and EAK12 (AEAEAEAEAKAK) have a cluster of negatively charged glutamate residues close to the N-terminus and a cluster of positively charged Arg residues near the C-terminus. It is well known that all α-helices have a helical dipole moment with a partially negative C-terminus toward a partially positive N-terminus (Hol *et al.* 1981). Because of the unique sequence of DAR16-IV and EAK12, their side chain charges balance the helical dipole moment, therefore favoring helical structure formation. However, they also have alternating hydrophilic and hydrophobic residues as well as ionic self-complementarity, which have been previously found to form stable β-sheets. Thus the behavior of these Type II molecules is likely to be more complex and dynamic than other stable β-sheet peptides. Additional molecules with such dipoles have been designed and studied, and the results confirmed the initial findings.

Others have also reported similar findings that proteins and peptides can undergo self-assembly and disassembly or change their conformations depending on the environmental influence, such as its location, pH change, and temperature, or crystal lattice packing (Minor and Kim 1996, Tan and Richmond 1998, Takahashi *et al.* 1999).

3.3 Type III Self-assembling Peptides

Type III peptides, like "molecular paint" and

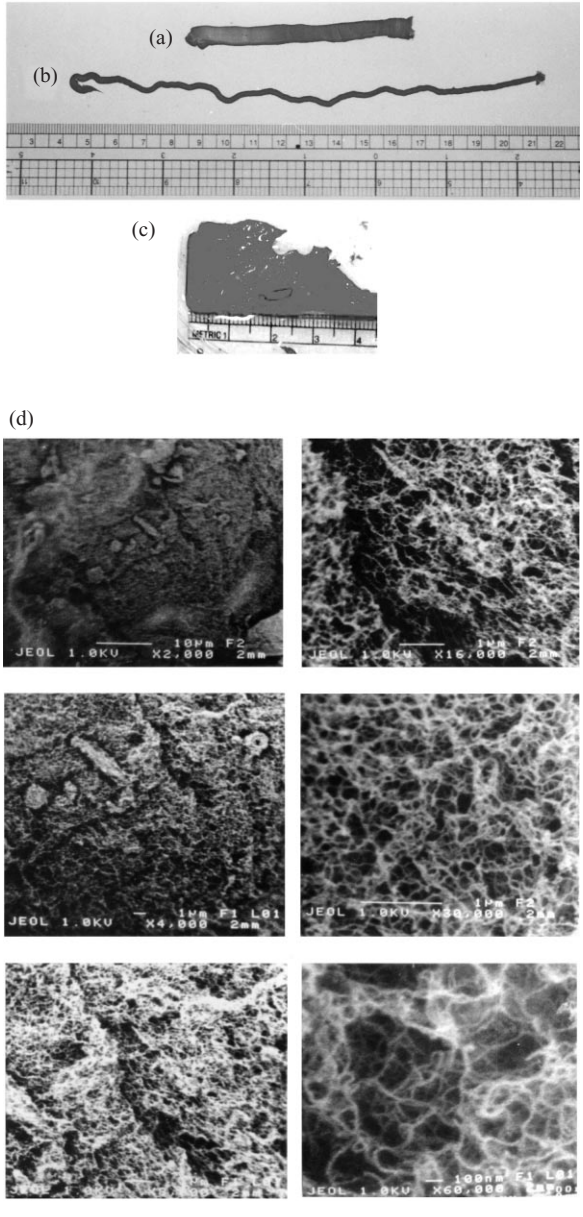

Figure 2
Hydrogel form of biological materials from self-assembly of the Type I peptides (a)–(c); (d) the SEM structure of peptide filaments. The material is self-assembled from individual interwoven fibers. The diameter of the fiber is about 10–20 nm and the enclosures are about 50–100 nm. Under high resolution by atomic force microscopy the filaments are revealed to be a twisted helix with regular helical repeats.

"molecular Velcro," undergo self-assembly on to surface rather than with themselves. They form monolayers on surfaces for a specific cell pattern formation

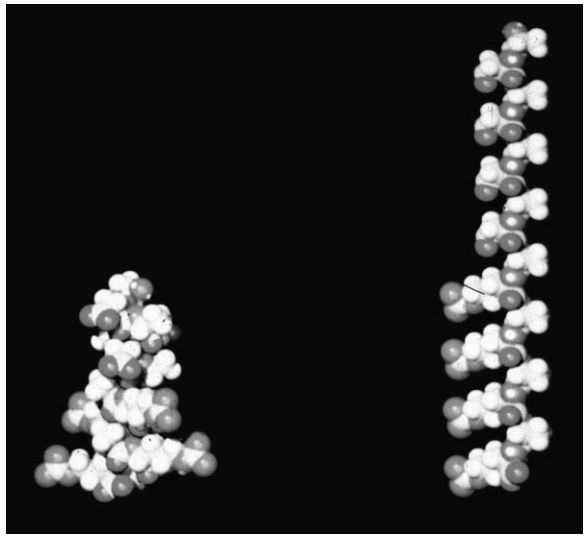

Figure 3
Molecular models of DAR16-IV in a β-strand form and an α-helical form. The length of the fully extended β-strand is about 5 nm and the length of the α-helix is about 2.5 nm. Therefore, β-form can be viewed as ON and the α-form as OFF.

or to interact with other molecules. These oligopeptides have three distinct features (Fig. 5). The first feature is the terminal segment of ligands that incorporate a variety of functional groups for recognition by other molecules or cells. The second feature is the central linker where a variable spacer is not only used to allow freedom of interaction at a specified distance away from the surface but also controls the flexibility or rigidity. The third feature is the surface anchor where a chemical group on the peptide can react with the surface to form a covalent bond. This simple system using Type III self-assembly peptides and other substances to engineer surfaces is an emerging technology that will be a useful tool in biomedical engineering and biology. This biological surface engineering technique will provide new methods to study cell–cell communication and cell behavior (Fig. 6) (Zhang *et al*. 1999).

Others previously pioneered similar kinds of molecular self-assembly systems through incorporating a segment of organic linker for surface anchoring (Whitesides *et al*. 1991).

3.4 Additional Self-assembling Peptide Systems

Several other types of peptide self-assembly systems are currently being developed as emerging materials. In one of these systems part of the peptide binds to and condenses nucleic acids, while the other parts facilitate membrane trafficking to translocate them across the

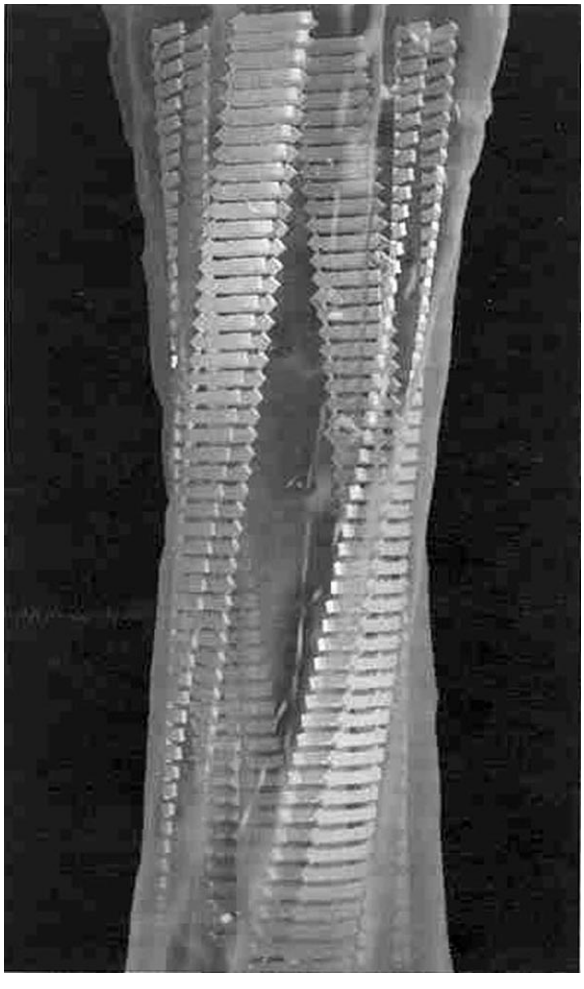

Figure 4
Molecular model of molecular self-assembly from an SH3 domain in a protein. The fibril consists four "protofilaments" that twist one another to form a hollow helical tube with a diameter approximately 6 nm. The model shown here represents one way in which regions of the polypeptide chains involved in β-sheet structure can be assembled within the fibrils (after Dobson 1999).

cellular lipid membranes. This system will likely have applications for delivery of molecular drugs, including DNA and RNA for gene therapy as well as protein therapy and delivery of other therapeutics. Another system is aiming to produce new biological scaffold material to facilitate biomineralization. In this system the peptides/proteins have two segments, one hydrophobic and the other negatively charged. The hydrophobic segments of the chain in aqueous solution can self-assemble into regular intersections of the scaffold, much like the joints in the construction of buildings, therefore forming negatively charged compartments.

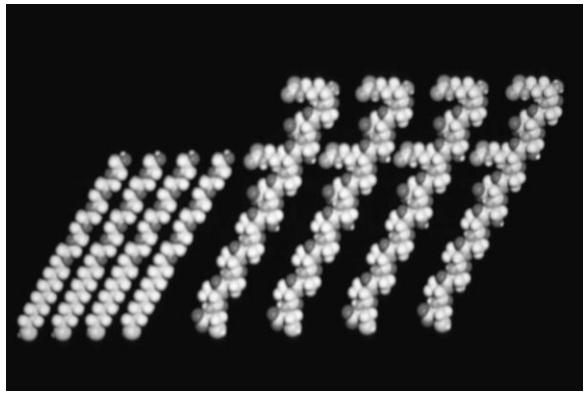

Figure 5
Self-assembling peptides for biological surface engineering. Molecular models of the oligopeptide RADSC-14 with the sequence RADSRADSAAAAAC (right) and of ethylene glycol thiolate (EG6SH) (left). The N-terminal segment $(RADS)_2$ is the ligand for cell attachment, and the five-alanine segment, AAAAA, is a linker to the anchoring cysteine. The cysteine anchor is covalently bound to the gold atoms on the surface. Molecular models are shown on the surface where both molecules form self-assembled monolayers with different height. The extended lengths of RADSC-14 and EG6SH are approximately 5 and 4 nm, respectively.

These negative compartments can then attract the positively charged ions, thus building the mineral phases.

4. Other Molecular Self-assembling Systems

Molecular self-assembly systems using nucleic acids on a chip have been developed. This new technology is based entirely on the principles of nucleic acid molecular self-assembly. Numerous new devices and technologies have been advanced. The most well-known example is the biochip technology—"Lab on a Chip," "GeneChip," or "Microarray Technology" (Affymetrix, Orchid Biocomputers) (Fig. 7). In such a biochip short oligonucleotides, mostly DNA with as large as 10^7 complexity, are anchored on the solid surface. These fragments of genes self-assemble with their complementary pairs through specific molecular recognition, selecting the relevant genes. This microarray system is widely used in gene expression analysis, the human genome project, diagnostics, discovery of new functions of genes, and high-throughput drug discovery and screenings (Schena *et al.* 1998). This breakthrough molecular self-assembly system has revolutionized the concepts of molecular biology, medical technology, the pharmaceutical industry and accelerated drug discovery and has fostered an array of new technologies.

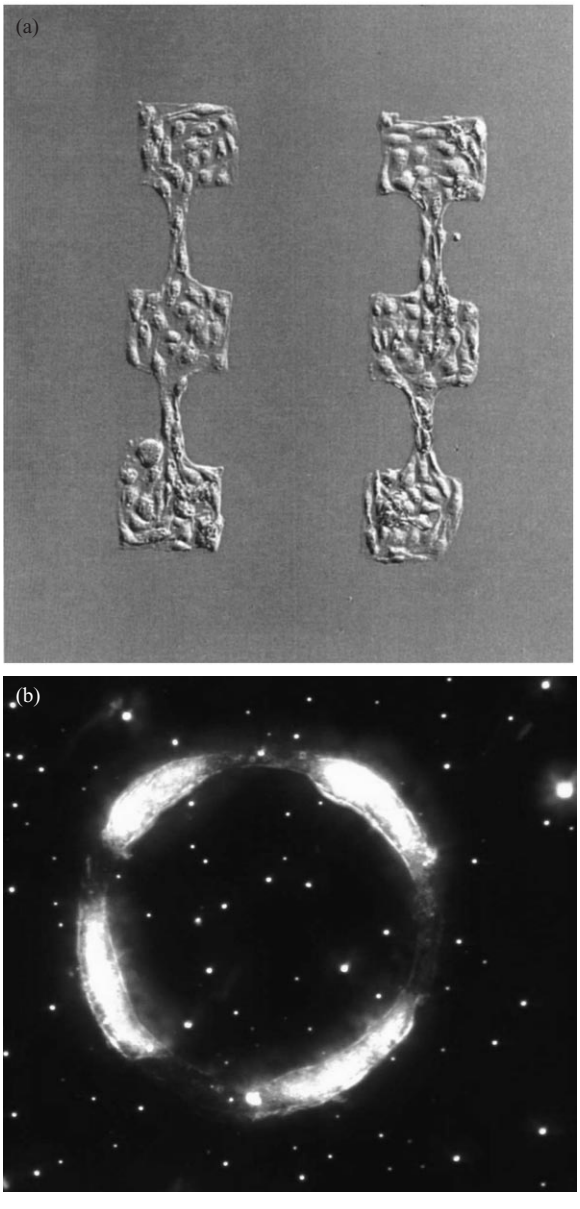

Figure 6
Cell arrays and patterns. The images of cells were taken with a Normarski microscope: (a) endothelial cells form the patterns of squares connected with linear cell tracks in line and patch form; (b) four individually separated cells form a circle.

5. Emerging Biological Materials and Technologies

Development of new materials and technologies often broadens the questions we can address and therefore deepens our understanding of seemingly intractable phenomena. Molecular self-assembly systems will create a new class of materials at the molecular level. Application of these simple and versatile molecular self-assembly systems will provide us with new opportunities to study some complex and previously intractable biological phenomena. Molecular engineering through molecular design and self-assembly of biological building blocks is an enabling technology that will likely play an increasingly important role in the future and will no doubt change our lives.

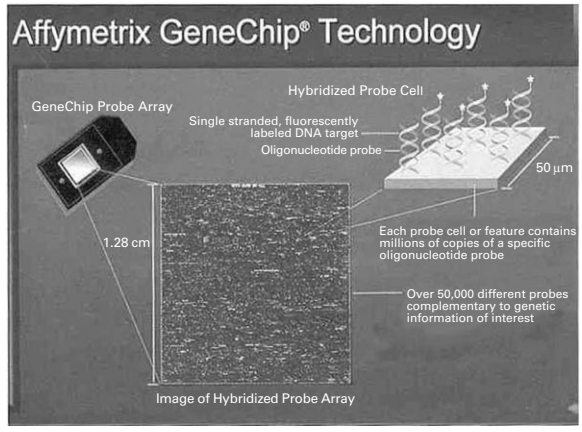

Figure 7
Schematic illustration of molecular self-assembly of DNA on a gene chip. The Genechip microarray technology produces biochips that are able molecularly to self-assemble fluorescent-labeled DNA or RNA fragments through complementary interactions with various nucleotide probes (after Affymetrix).

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S. Zhang

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