Expanding Frontiers in Biomaterials

Samuel I. Stupp, Jack J.J.M. Donners, Liang-shi Li, and Alvaro Mata

Abstract

This article celebrating Arthur von Hippel's career considers the expanding frontiers in the field of biomaterials, a subject that intrigued him given his interests in the molecular engineering of materials. The interface of materials science and biology started to develop decades ago when synthetic materials were first used to repair parts of the human body. An exciting transformation is now occurring in the field, as advances in biology are used to engineer bioactive materials at the molecular level. The transformation is going further to other frontiers that include the use of sophisticated materials to obtain biological information and learn biology, the creation of materials that imitate biological microstructures and functions, and the manipulation of organisms to create artificial materials.

Keywords: *Arthur von Hippel, bioactive materials, biomaterials, biomimetics.*

Introduction

Science historians will identify the turn of this century as a time when most scientists became intrigued by the ingenuity and complexity of biology. In our time, some scientists have the ambition to understand biology from the molecular to the systems level, while others hope to mimic, control, or modify biological systems to develop things that are useful to society. In this same period, the field of materials and its close ally, nanoscience, occupy a center-stage position that is no longer just the traditional domain of materials science, but one that has expanded to chemistry, physics, and all areas of engineering. This makes the field of *biomaterials* one of the most exciting scientific challenges today.

Arthur von Hippel, whose life is celebrated in this issue of *MRS Bulletin,* was a pioneer who decades ago had the vision to advocate all of these key scientific transformations. The Laboratory for Insulation Research, which he founded at the Massachusetts Institute of Technology in 1937, planted the seeds of molecular engineering of materials, a strategy that makes synthetic chemistry today an important dimension of materials science. Biology is, of course, the ultimate expression of molecular engineering, and von Hippel extended his vision by raising questions on how nature proceeds with molecular design to create living systems. In the later stages of his career, his interests in living matter were wisely expressed through research on the structure and properties of *water,* which he saw as critical to our understanding of biological structures. As a testimony of von Hippel's vision, his last publication was titled *From Atoms toward Living Systems*, published in 1979.[1] Honoring von Hippel's foresight, we gaze in this article at the many faces of the expanding and highly inspiring field of biomaterials.

The Genesis of Biomaterials

The scope of biomaterials has traditionally consisted of using materials in medicine and dentistry with the purpose of restoring the structure and/or function of tissues and organs. In fact, this use of materials can be traced back to prehistoric times, with evidence of sutures dating back more than 30,000 years.[2] More than 2000 years ago, gold was used by the Greeks (described in early Greek literature by Galen of Pergamon) as wires for ligatures, and by the Romans, Chinese, and Aztecs in dentistry.[2,3] More recently, in 1816 Philip Physick from the University of Pennsylvania used lead wire sutures, while in 1849 J. Marion Sims used, more successfully, silver wires developed by a jeweler.[2] In 1829, H.S. Levert performed a significant study describing the *in vivo* biocompatibility of gold, silver, lead, and platinum.[2] In 1860, relatively good success was achieved with the invention of contact lenses made from glass, giving form to a concept that was originally proposed by Leonardo da Vinci in the 16th century.[2]

During the 20th century, accessibility to more sophisticated materials like stainless steel, cobalt chromium alloys, and the development of synthetic polymers resulted in revolutionary advances.[2,4–6] Among the highlights one can mention is the development of the first dialysis machine using cellulose membranes in 1943 by Kolff, the first intraocular lenses made from poly(methyl methacrylate) (PMMA) in 1949 after Ridley's observation that the eyes of aviators containing pieces of plastic from shattered cockpits of fighter planes had healed with little reaction,[2,7] and the first successful vascular grafts made from parachute fabric in 1952.[2] Other major landmarks were the first successful hip joint replacement using an acetabular cup made from high-molecular-weight polyethylene;[2,5] the introduction of bone cement made from PMMA;[2] the first commercially available hydrogel-based contact lenses (made from hydroxyethylmethacrylate);[2] and artificial heart valves made from materials such as silicone rubber, nylon fabric, PMMA, and polycarbonate in the 1960s.[2,8]

By the early 1980s, more than 40 apparently biocompatible materials had been identified and used in implantable devices such as pacemakers and artificial blood vessels, including silicone elastomers, polyurethanes, poly(tetrafluoroethylene), hydrogels, poly(ethylene glycol), poly(lactic-*co*-glycolic acid), hydroxyapatite, titanium, and bioglass.[2]

The scope of the field is no longer about taking materials from technology at large and using them in medicine to make humans more functional; instead, the field has now moved forward to the use of tailored materials that elicit a specific biological response. This direction is creating a platform in which materials will be used to mediate the regeneration of tissues and organs for regenerative medicine.[9] The concept could be described as an effort to use materials to repair human biology. This expanding scope does not end there, since all of our new biological know-how, complemented with the ability to control the micro-, nano-, and molecular-scale structure of matter, would allow us to build configurations that mimic those found in biology, as well as to use sophisticated materials to learn biology, to obtain biological information, and to control bio-

logical processes. Moreover, it is also now possible to consider the use of biology to make abiotic materials. One example would be the use of bacteria to synthesize artificial proteins designed to have specific physical properties using unnatural amino acids; another would be the use of cells to synthesize semiconducting nanocrystals. This adds up to many exciting possibilities that certainly resonate with the von Hippel spirit. This article is not meant to be an exhaustive review of efforts in all of these expanding areas; it is only a brief account of what is happening today in this very exciting field, recognized early on by von Hippel. Our view of the expanding field of biomaterials is summarized in Figure 1, showing examples of the important interface between materials and biology.

Materials to Repair Human Biology

The biomaterials described in the previous section are still in widespread clinical use. They are inert materials used to create permanent implants to restore structure and/or function of tissues lost to trauma or disease. However, in recent years, two new trends have emerged that could transform the role of materials to repair

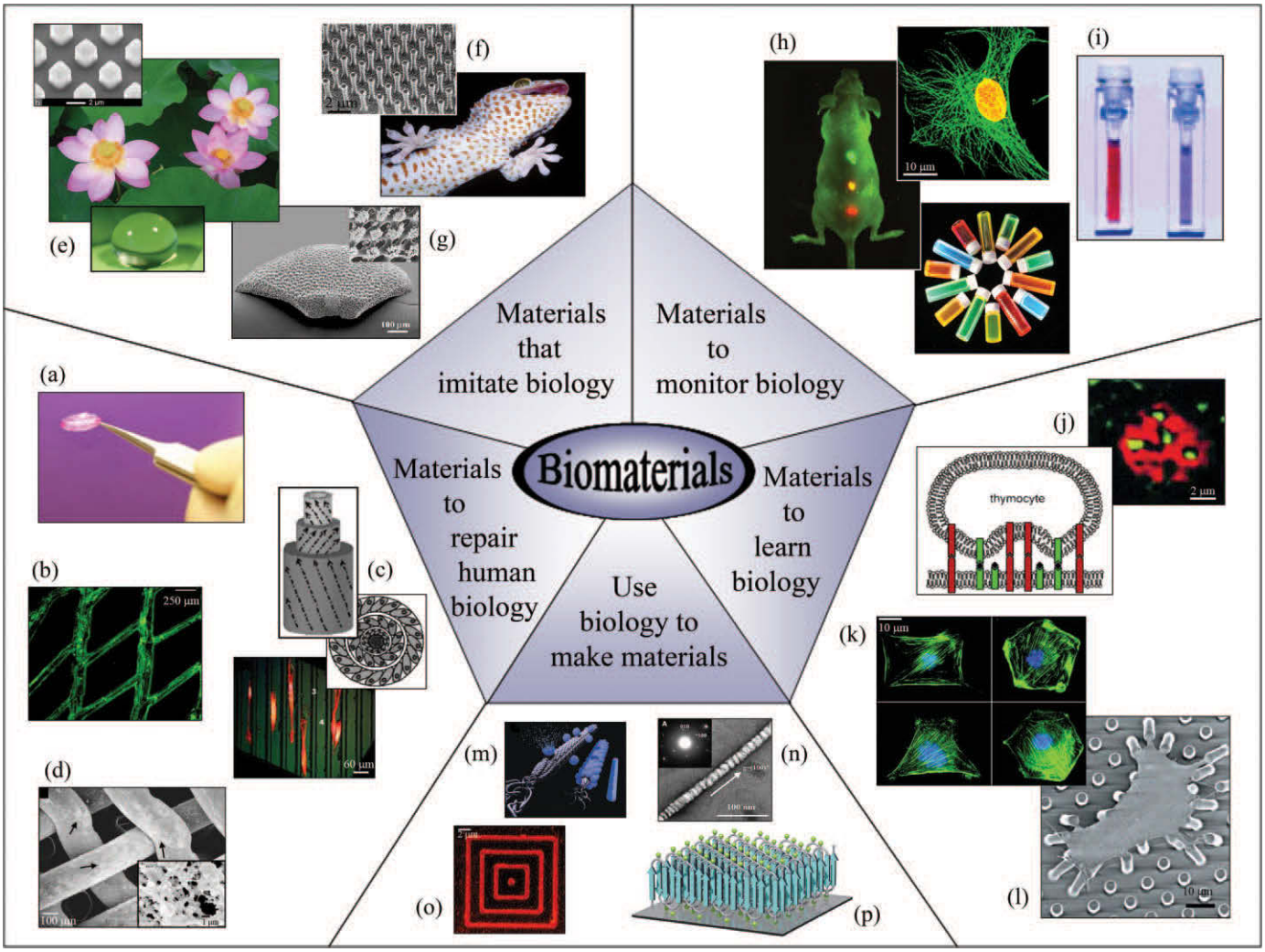

Figure 1. The field of biomaterials is undergoing an enormous expansion in new directions to create bioactive materials that can interact with cells by design in order to repair and regenerate tissues and organs. Some examples include (a) materials molecularly designed to be bioactive that self-assemble from liquids to gels once injected in tissues;[10] (b),(c) microfabricated 3D structures for cell and tissue guidance;[11,12] and (d) titanium implants modified with bioactive hybrid materials containing hydroxyapatite and poly(amino acids).[13] There are also expanding efforts to create materials that imitate biological ones, such as synthetic recreation of (e) superhydrophobic surfaces found on the leaves of water lilies,[14–16] (f) adhesive surfaces of gecko feet,[17,18] and (g) top-down manufacturing of optical microlenses that imitate those in the brittlestar.[19] In other efforts, nanomaterials are used to obtain biological information using, for example, (h) quantum dots[20–22] and (i) metallic nanoparticles[23] to detect the presence of specific proteins and genes. In some laboratories, sophisticated micropatterned materials with well-defined surfaces are being used to learn biology; in one example, (j) suspended lipid bilayer membranes patterned with "corrals" are used to study cells of the immune system such as T-cells and their precursors (thymocytes);[24] other studies explore (k) the effect of chemically patterned substrates on cell shapes[25] and (l) the effects of microtexturing of surfaces[26] to investigate cell mechanics. Finally, advances in molecular and cell biology are showing us pathways to use biology to make materials such as (m) the engineering of viruses to nucleate specific inorganic materials and (n) nanowires templated by viruses,[27] (o) peptides from viruses that bind different semiconductors,[28] and (p) the use of genetic engineering of bacteria to synthesize artificial proteins with the properties of materials such as silk.[29]

human biology. The first trend has been to consider designing bioactive, as opposed to inert, biomaterials that elicit a specific biological response in order to create better interfaces with natural tissues. This connects to von Hippel's idea of molecular engineering of materials to achieve specific functions.

The best example is the modification of metal surfaces with materials such as hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] in orthopedic implants in order to promote bone bonding to the implant surface, or bone ingrowth into porous metal surfaces, thus producing stronger implant–tissue interfaces. In our own laboratory, we have developed techniques to grow osteoconductive coatings on titanium known as organoapatites. These new materials are hybrids of calcium phosphate mineral phases and poly(amino acids) that have been shown to significantly enhance bone cell migration and proliferation within the pores of the metal.[13,30,31] Using finite element models, it is predicted that bony infiltration would generate a more stable interface that avoids stress concentration.[134]

Another recent trend has been to use materials merely as biodegradable scaffolds that can harbor the right cells in order to regenerate tissue. This approach falls within the umbrella of tissue engineering[32] and advocates the regeneration of tissue either *in vitro* in bioreactors with subsequent transplantation or *in vivo* by placing cell-seeded biodegradable scaffolds in the right location of the body. Scaffolds were originally based on natural polymers such as collagen, but it is now more common to use biodegradable synthetic polymers because of immunological concerns and the ability to tailor the mechanical properties, pore sizes, and degradation rate of the scaffolds. This particular approach to repairing tissues and organs has only been used to a very limited extent in humans; most of the systems are currently being studied in animal models. One challenge in this approach to human repair is to develop strategies to promote the rapid growth of blood vessels (angiogenesis) within the scaffold in order to feed the large numbers of cells required to grow a macroscopic segment of tissue. A second challenge is to learn how best to incorporate necessary growth factors (proteins) to achieve tissue regeneration in the volume defined by the scaffold. For example, a method for dual growth-factor delivery was reported by Richardson et al., as illustrated in Figure 2a.[33]

Other directions that may become important for tissue engineering will be based on current capabilities to control surface chemistry and topography in three-dimensional (3D) structures. The implementation of precise surface chemistries and topographies within 3D structures could significantly enhance the *in vivo* performance of scaffolds. Techniques have been developed recently to create 3D structures with precise microscale architectures that can be specifically designed to direct the growth and organization of cells and tissues.[11,12,34–36] For example, Shin et al. have combined microfabrication with computational analysis to engineer a network of microscopic channels that resembles vascular geometry.[12] Similar techniques were used by Folch et al. to fabricate 3D structures with precise microarchitecture for potential use as scaffolds in tissue engineering.[37] An even higher degree of cell and tissue manipulation may be achieved by combining both a precise microscale architecture with surface topographies that not only guide cell and tissue growth, but also can selectively stimulate their behavior. Mata et al. have used an innovative fabrication process to develop 3D polymeric structures with well-defined micro-architectures in which surfaces have precise textures that enhance mesenchymal stem cell growth and proliferation (Figure 2b).[34,35,38] Mesenchymal stem cells are those that develop into connective tissues, blood vessels, and lymphatic tissue, and upon differentiation can give rise to bone, muscle, cartilage, and fat cells.

There are other interesting recent ideas for the use of materials to repair biology that are not related to the use of scaffolds for tissue engineering. A minimally invasive implantation strategy is always highly desirable from a clinical point of view, and an interesting approach has been suggested using shape-memory polymers (Figure 2d). These materials consist of two components, each with a different phase transition temperature. They are able to memorize a permanent shape but temporarily adopt a substantially different initial shape. The phase with the higher transition temperature (near body temperature) is responsible for the permanent shape, whereas the other phase determines the initial shape. Using this strategy, polymeric sutures that tie themselves have been generated.[39] Other applications envisioned are materials that will acquire the shape of a stent.

Another interesting emerging concept is the use of cell sheets for tissue regeneration[40] that are produced *in vitro* using a thermoresponsive polymer covalently grafted to a culture dish. Reducing the temperature to room temperature leads to swelling and hydration of the polymer matrix, which in turn result in detachment of the cell sheet from the matrix. This approach is particularly promising for growing tissues in which cell monolayers are important, for example, in regeneration of the cornea. This method has in fact been shown to be effective in human trials.[41] It is believed that the approach may be useful also in the regeneration of heart muscle. Layering four sheets of cardiomyocytes resulted in a synchronously beating tissue that was maintained for one year when implanted in the backs of immunodeficient rats.[42] Here, synthetic materials play an indirect role in the context of regeneration, serving as a substrate that releases cell layers as a result of thermoresponsive properties.

The next stage in materials to repair human biology has to involve structures that are sophisticated enough to recruit and activate cells in their surroundings to jump-start regenerative processes. Ideally, they would be introduced noninvasively, possibly as liquids that self-assemble into elastic or viscoelastic solids in the environment of living tissues. These biomaterials would be molecularly designed as artificial extracellular matrices for regenerative medicine. The cell-seeded forms of these materials, in analogy to tissue engineering scaffolds, will most likely be the matrices that will carry stem cells to specific targets for tissue and organ regeneration. A sophisticated bioactivity crafted through molecular and supramolecular structure would guide stem cells into the right proliferation and differentiation pathways to achieve regeneration of body parts.

Our laboratory has begun work to design such biomaterials,[43,44] and the first efforts have used molecules termed peptide amphiphiles (PAs) that upon contact with physiological fluids self-assemble into cylindrical nanofibers with well-defined diameters, creating 3D networks and thus inducing a liquid-to-gel transformation.[9,44–46] The highly hydrated gels (Figures 1a and 2c) provide signals to cells that direct the differentiation of neural progenitor cells into neurons and at the same time discourage the production of astrocytes.[45,47] Astrocytes are the brain's glial cells (cells that protect and support neurons). They have many useful functions, but after spinal cord injury, they form scar tissue that impedes the regeneration of sensory and motor axons. This type of bioactivity is therefore likely to be critical in the regeneration of the spinal cord so that paralysis of humans after trauma can be prevented or reversed. Other systems have been designed to promote the formation of blood vessels *in vivo*[48] and the regeneration of bone.[44]

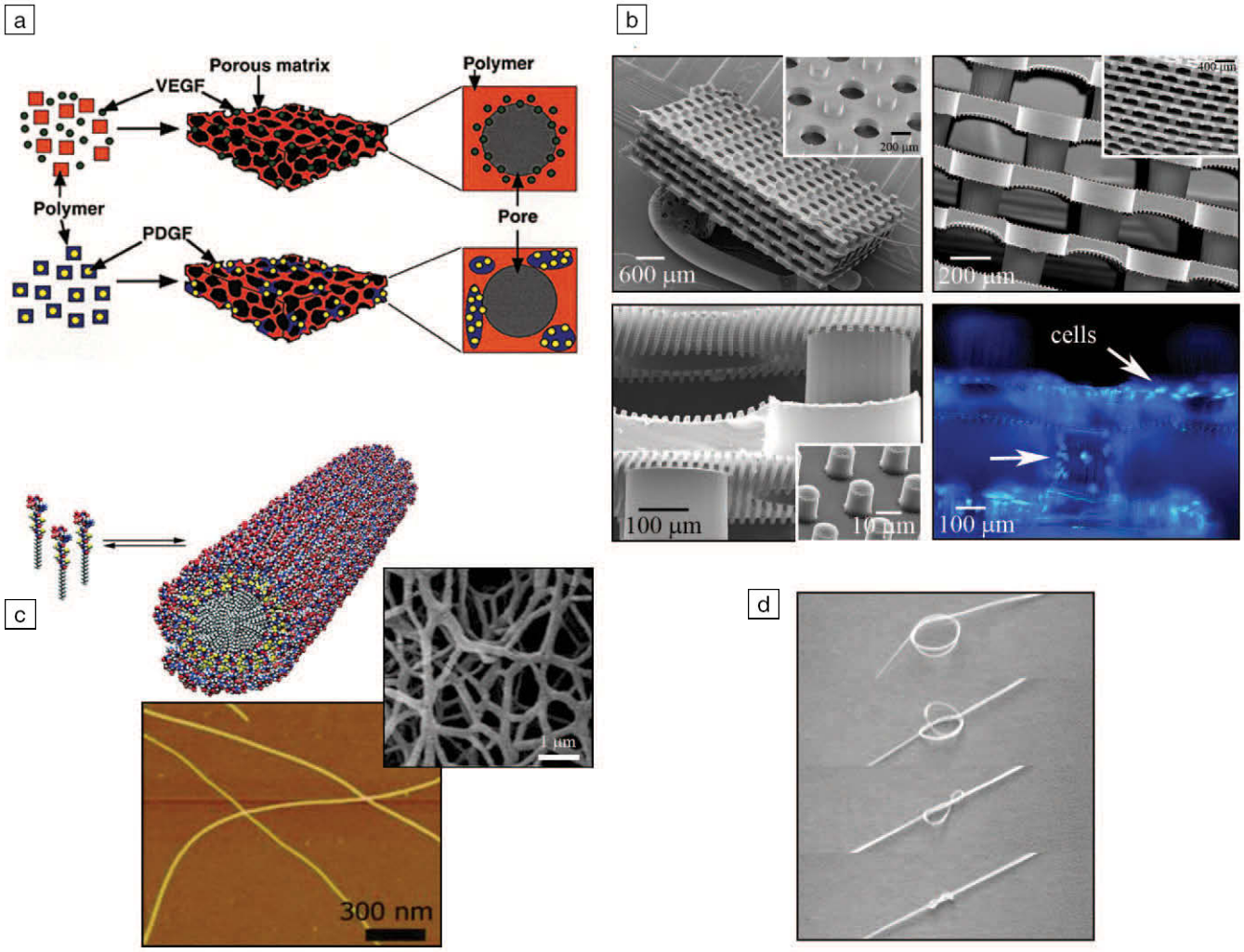

Figure 2. Contemporary approaches under development to create bioactive or smart biomaterials by design to repair or regenerate tissues and organs. (a) Heterogeneous scaffold for temporal release of angiogenic growth factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF-2); one growth factor is pre-encapsulated in polymer microspheres, whereas the other is entrapped in the pores of a hydrogel.[33] (b) Three-dimensional structures with interconnected levels (top left, inset) of up to twelve layers (top right, inset) comprising both precise micro-architecture and surface microtextures (bottom left, inset). These structures can be used as scaffolds to stimulate and direct 3D growth of both cells and tissues.[34,35,38] (c) Bioactive peptide amphiphile nanofibers formed by self-assembly from a dilute aqueous solution upon injection in tissues.[45] These materials create networks in the extracellular space that can signal cells to promote regeneration. (d) Self-tying biodegradable surgical sutures based on temperature-sensitive shape-memory polymers.[39,135]

The design of these bioactive biomaterials utilizes supramolecular chemistry, the chemistry of noncovalent interactions among molecules pioneered by Lehn, Pedersen, and Cram.[49] Furthermore, cells cultured within these nanofiber networks displaying the RGD epitope (the tripeptide sequence of arginine, glycine, and aspartic acid) remain viable for weeks and can proliferate.[10] Our laboratory has also prepared PAs with peptide sequences discovered using the technique known as phage display, which can bind growth factors with varying strengths.[50] For reasons explained before, this feature would be extremely useful in creating matrices with the capacity to regenerate tissues and organs. Finally, the PA-based materials have enormous flexibility for molecular engineering, and we have recently modified them so that once injected they can be tracked through the use of magnetic resonance imaging (MRI).[51] It may also be possible to use PA-based biomaterials to deliver genes to cells.[52]

Materials that Imitate Biology

As greater capabilities emerge for the design and characterization of microstructures and nanostructures, one new direction in the field of biomaterials has been the development of biomimetic materials. The objective in this area is to emulate the complex properties of biological structures using completely synthetic systems. Examples of this developing area include the use of surface topographies to recreate functions of biological structures such as the self-cleaning superhydrophobic properties of leaves[14] or the adhesive surfaces found on the feet of geckos and flies.[17,53,54]

Natural enzymes are highly efficient, low-temperature catalysts with high selectivity. The geometric control, rather than reactivity control, of chemical selectivity

has inspired the development of artificial systems[55] in which reactive sites are brought into proximity using specific templates. In one recent example, the two parts of the active sites in silicatein, an enzyme responsible for the synthesis of silica in the orange puffball sponge *Tethya aurantia*, were each grafted onto a different gold nanoparticle (Figure 3).[56] This was found to catalyze the formation of silica at locations where the nanoparticles are in proximity to each other. Another recent example has been the growth of micrometer-long crystals of hydroxyapatite with their *c*-axes aligned, mimicking the crystals of dental enamel. This was achieved using as a template aggregates of the protein known as amelogenin, known to be involved in enamel biosynthesis.[57]

DNA macromolecules, the storage media for genetic information in living organisms, offer nearly perfect fidelity in terms of Watson–Crick pairing of their bases, and they are also a double helix polymer with very large persistence length (a parameter commonly used in polymer science to measure the extended nature of chains) (~50 nm). These properties of the genetic polymer make it an interesting template to create artificial structures built with DNA segments or by coupling DNA derivatized inorganic particles to a template. DNA segments are therefore versatile building blocks to program the formation of secondary and tertiary structures;[58,59] there are examples in the literature of tiles,[60] linked rings,[61] polyhedra,[62] nanomechanical devices,[63,64] and thin gold wires using the template idea.[65,66]

Biomimetic structures can also be formed with self-assembling peptides in completely abiotic environments such as organic solvents. Using this idea, our laboratory recently reported on the formation of nanofibers in organic solvents[67] with peptide–lipid molecules that contain β-sheets (parallel or anti-parallel arrays of extended peptide segments) in the interior of the fiber and form outer surfaces that can be used as templates to organize lipophilic inorganic particles. By modifying the surfaces of the nanofibers and of gold nanoparticles with hydrogen-bond mimics of Watson–Crick pairs, we were able to organize micrometers-long 1D arrays of close-packed gold nanoparticles. Self-assembling synthetic structures of this type that mimic certain structures of biology could be useful in optical or electronic devices. The 1D array of gold nanoparticles is shown in Figure 4a.

A very interesting example of a biomimetic structure was reported recently by Aizenberg and co-workers,[68] who utilized synthetic systems to recreate the microlenses of the brittlestar (Figure 4b). In the brittlestar, highly organized arrays of birefringent calcite crystals with their optical *c*-axes aligned prevent double image formation and thus lead to improvement of the optical properties of the lens. Aizenberg used the amorphous-to-crystalline transition of amorphous calcium carbonate to generate mimics of the microlenses by depositing amorphous calcium carbonate on a micropatterned substrate, followed by crystallization. The advantage of using the amorphous-to-crystalline transition is that the crystalline phase preserves the shape of the amorphous precipitate. The resulting lenses proved to have strong focusing ability.[68] In addition, light-absorbing liquids can penetrate into the pores between the crystals, which allows fine-tuning of the optical properties of the lens. This work represents a clear example of a functional material based on biomineralization principles.

Materials to Monitor Biology

The rapid development of nanomaterials in recent years has generated a num-

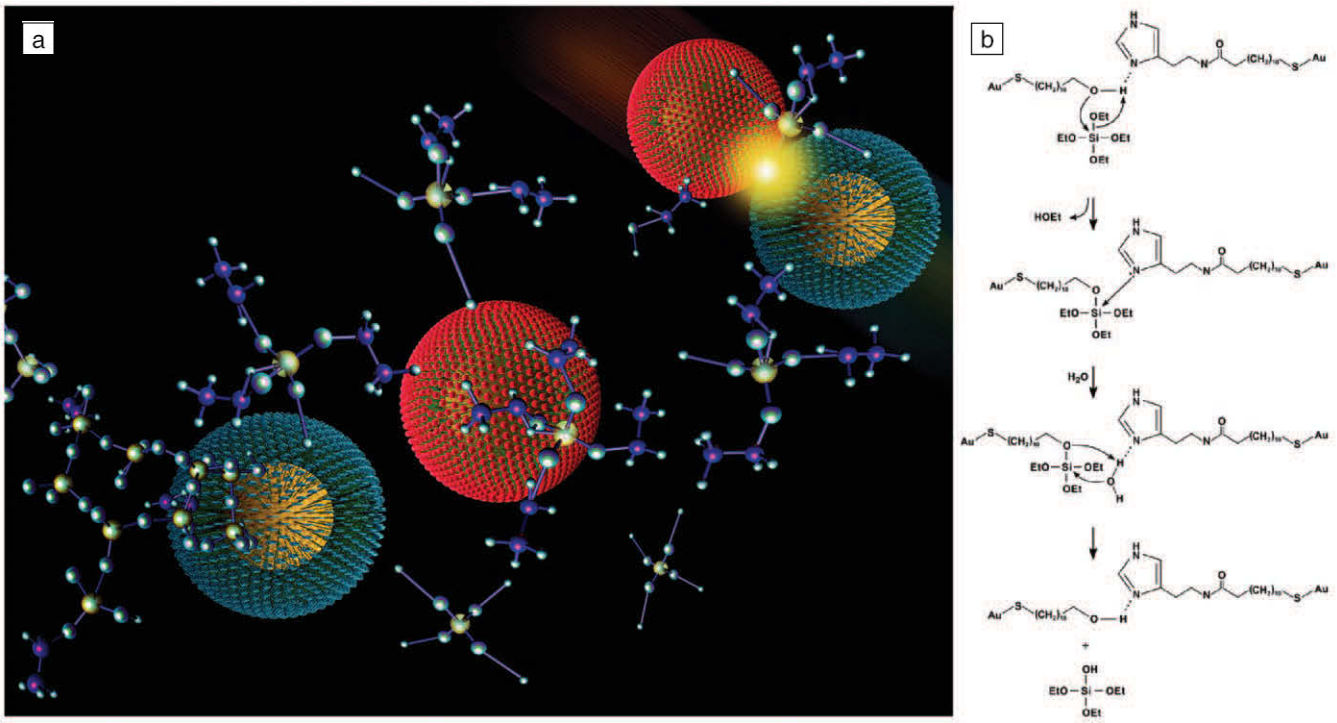

Figure 3. Enzyme mimics for the synthesis of materials. (a) Alkyl thiol molecules functionalized by a hydroxy group and an imidazole moiety, respectively, are grafted to gold nanoparticles to mimic histidine and serine residues that are responsible for the synthesis of silica catalyzed by silicatein in the orange puffball sponge, Tethya aurantia. *(b) Silica forms only when nanoparticles bearing the two different thiol molecules are in proximity to each other.*[56]

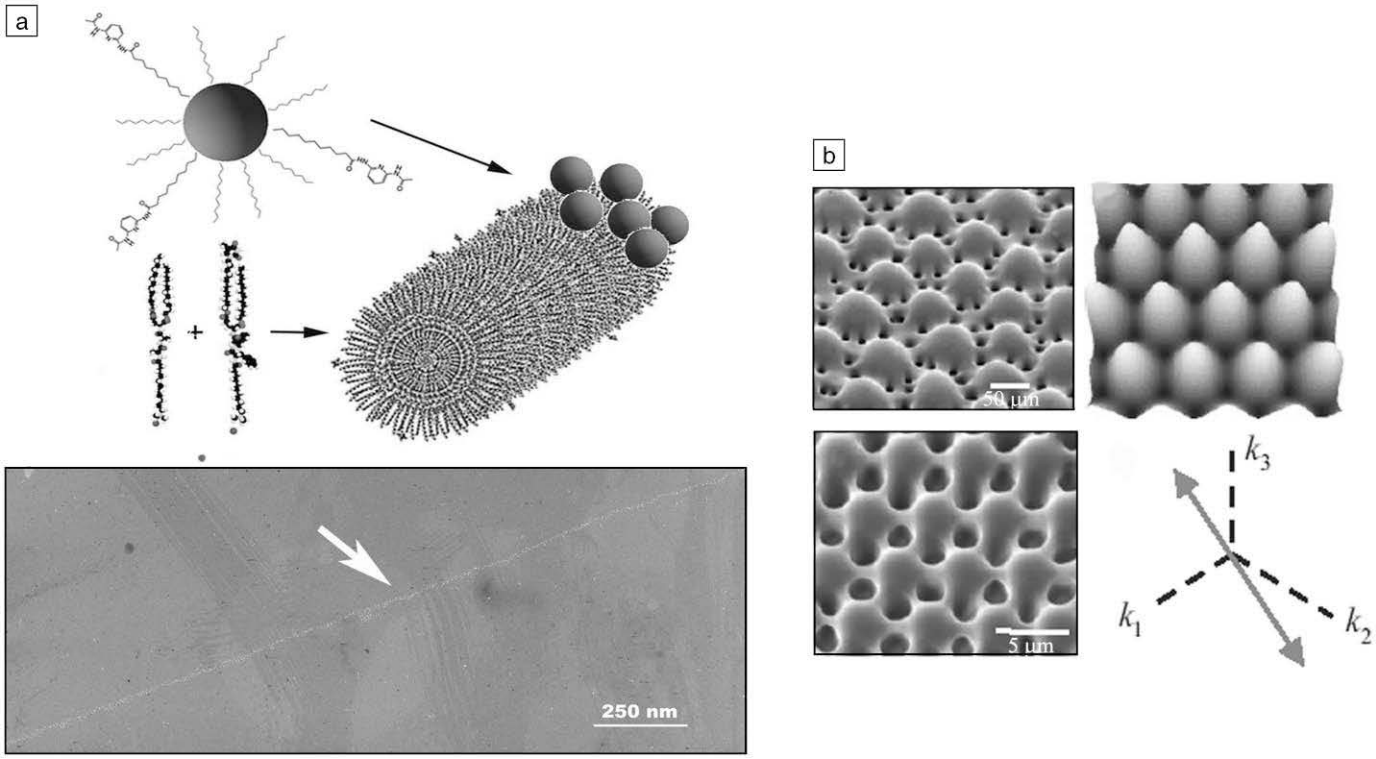

Figure 4. (a) Linear array of gold nanoparticles (white arrow) templated by self-assembled nanofibers in apolar solvents.[67] The nanofibers are formed by peptide–lipid molecules that form β-sheets and display on the surface the nucleotide thymine recognized by molecularly modified gold nanoparticles. (b) Natural (top left and top right) and biomimetic (bottom left) porous microlens arrays resembling the highly organized arrays of birefringent calcite crystals in brittlestars.[68] The biomimetic lenses prevent double image formation and lead to improved optical properties similar to those of the natural brittlestar. Schematic illustrates the beam polarization used in three-beam interference lithography to generate the biomimetic lens.

ber of opportunities to use synthetic materials in biosensing applications. This area is important because of its potential impact on medical diagnostics by increasing both the sensitivity and selectivity of probes that monitor biology. The ultimate goal is single-molecule detection *in vivo* and also the possibility of detecting *in vitro* many analytes in a single probing event. Given the nature of the nanomaterials discovered over the past two decades, the signals one may use to develop new diagnostic techniques could be optical, electrical, or magnetic.[23,69]

Optical signals such as fluorescence and absorption have played important roles in studies of biological processes, for example, in the development of assays for biological research. Fluorescent dyes that are commonly used for these purposes suffer from a few disadvantages, such as narrow excitation spectra but broad emission spectra and low photobleaching thresholds. For this reason, semiconducting inorganic nanoparticles (quantum dots) were proposed[70,71] as alternatives and have been widely used since in research for cellular imaging and diagnostics.[72,73] Compared with organic dyes, these quantum dots have material- and size-tunable emission spectra, a larger two-photon excitation cross section, and allow for simultaneous multicolor detection as well as deep tissue imaging with two-photon fluorescence or IR-emitting nanoparticles.[74]

Metallic nanoparticles are also used for biological sensing, because of their large light-scattering cross section.[75,76] In addition, their aggregation-dependent color of scattered light,[77] as well as the enhanced Raman scattering on their surfaces,[78–80] has been used in the detection of nucleic acids and other organic molecules.

Bio-barcode assays have been developed in which gold nanoparticles are functionalized with molecules binding specifically to the target molecules and DNA oligomers. The target molecules to be detected can be either DNA or proteins, while the DNA oligomers serve as surrogates for the target molecules in final readout with a variety of methods. Because the ratio between the number of barcode DNA molecules and the target-binding molecules on the nanoparticles can be fairly large (up to thousands), amplification is achieved for each binding event. Further, when magnetic microbeads that also carry target-binding molecules are combined with the barcode probes to enrich the probes, a detection limit of just 10 molecules in solution[81] has been demonstrated (Figure 5), which significantly excels PCR (polymerase chain reaction) or ELISA (enzyme-linked immunosorbent assay) techniques that are normally used for detection purposes.

Subwavelength waveguides made of single-crystalline 1D inorganic nanowires or nanoribbons[82] provide another way of optically sensing molecules, in which the evanescent wave around the waveguide can be used to excite molecules deposited on the waveguide.[83]

Also, electrical current through carbon nanotubes[84–86] and nanowires[87] can be sensitively perturbed by substances adsorbed on the surfaces, and have been used as sensitive sensors for protein-binding events.

Another important area of nanomaterials to monitor biology is the use of particles for MRI. Superparamagnetic nanoparticles

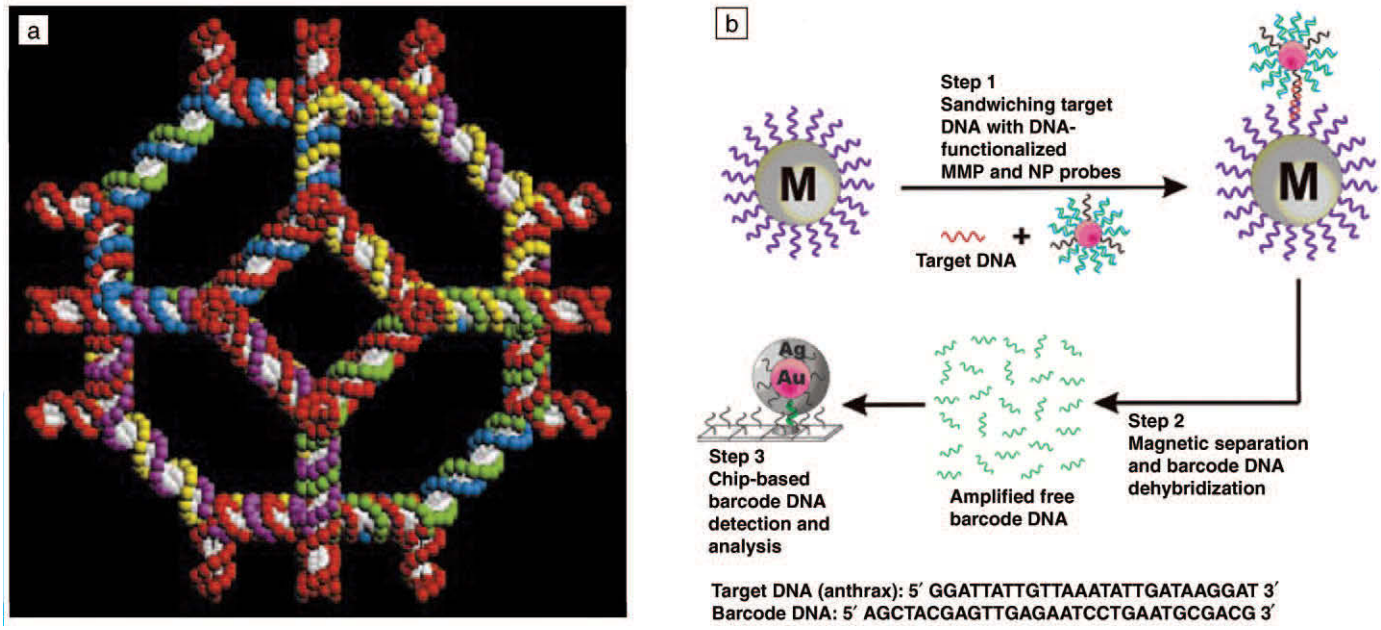

Figure 5. Use of DNA to create synthetic structures and sensing devices. (a) Three-dimensional structure synthesized from three-armed DNA.[59] (b) Chip-based barcode DNA detection. Microparticles (MPs) and gold nanoparticles (NPs) were functionalized respectively with oligonucleotides capable of binding complementarily to the 3′ and 5′ ends of a target DNA.[81] The NPs also carry the "barcode" binding nucleotides for further analysis. The sandwiched target DNA was subsequently separated magnetically and the target DNA amplified through the barcode-binding DNA, which enables a reported detection limit of ~10 target DNA molecules.

have been widely used[88–90] as contrast agents in MRI. Recently, our laboratory has reported on PA nanofibers that are conjugated to gadolinium(III) complexes to enhance magnetic contrast, which could be used to monitor noninvasively the fate of bioactive matrices for regeneration.[51]

Materials to Learn Biology

Another important interface of materials and biology is the use of our current capabilities in the control of synthetic structures and patterns to learn more about biology using unconventional experiments designed by physical scientists and engineers.

Current surface-patterning techniques provide reproducible tools to engineer the surface chemistry of biomaterials with molecular precision.[91] Printing methods such as soft lithographic techniques have been used to generate self-assembled monolayers (SAMs) that enable specific arrangements of well-defined geometrical regions with distinct chemical properties. Soft lithographic techniques include microcontact printing (μCP), replica molding (REM), microtransfer molding (μTM), micromolding in capillaries (MIMIC), and solvent-assisted micromolding (SAMIM).[92] These methods have been used to study cellular mechanics including relations between cell shape and cell survival,[25] cell spreading and cell proliferation,[93] focal adhesion formation and cell spreading,[94] and interactions between different cell types and cell function.[95]

An increased level of manipulation and complexity is becoming available through patterning of surface chemistries that can be dynamically controlled and regulated. This approach uses different switching mechanisms to organize ligands and influence cells. Electrical transduction consists of applying an electrical potential to change the properties of the underlying substrate; electrochemical transduction uses potentials to cause redox reactions at the surface; photochemical transduction uses light to change the activity of immobilized ligands; thermal transduction is used to control surface properties based on conformational changes of ligands due to temperature variations; and mechanical transduction makes use of forces exerted by adherent cells on a surface to physically control the interaction between cells and substrates.[43,96] These techniques recreate some of the dynamic interactions between cells and surfaces. They offer great promise for a deeper understanding of cell behavior that could then be applied to biosensing platforms and to the design of new scaffolds for cell and tissue regeneration.[43,96]

In addition to these surface-patterning techniques to study cell behaviors such as haptotaxis (the directed movement of cell motility), concentration gradients of biomolecules have also been used to study cellular behavior.[97–99] The inherent laminar character of microfluidic systems permits the controlled diffusive mixing of soluble factors. These systems have allowed the patterning of surfaces[98,99] as well as investigations of cell behaviors of a chemotactic nature.[100,101]

Another form of patterning that shows great potential for the study of cellular behaviors is the use of well-ordered, supported membrane lipid bilayers. This technique makes use of the spontaneous assembly of lipid bilayer vesicles into a continuous lipid membrane[24] to resemble the structure and lateral fluidity of living cell membranes.[102] These lipid membranes permit the diffusion of lipids and membrane-linked proteins and have enabled investigations of structure[102–105] and manipulation[106] of the cell membrane; they have also offered fresh mechanistic insights on how the immune system works.[24,107]

Cell behaviors have also been studied and manipulated through the use of surface topographies at both the micro- and nanoscale.[108] The first study to recognize an effect of surface topography on cell behavior was by Harrison, who in 1912 described cell motion along the fibers of

spider webs.[109] Since then, and especially within the last 20 years, when microfabrication started to be used to develop surfaces for tissue culture,[110] more studies have increasingly looked at the effect of surface topography on cell behaviors (Figure 6).[111,112] These investigations have important implications in the understanding of cell biology and tissue engineering.[115] Microfabricated topographies have been used to investigate the fundamentals of cellular mechanics such as focal adhesions, which involve the formation of localized plaques of proteins that connect the cell through its cytoskeleton to the external environment, resulting in survival signals,[26,114,115] cell migration,[111] and cell proliferation.[112] In addition, topographical features have been used to selectively stimulate cells to perform desired functions such as a more *in vivo*–like myocyte morphology for heart tissue replacements[116] and increased mesenchymal stem cell control and manipulation for connective tissue reconstruction.[111,112] The incorporation of precise topographies on biomaterial surfaces provides an attractive approach to selectively enhance specific cell behaviors without changes in composition.[34]

Use of Biology to Make Materials

Recent advances in molecular and cell biology have allowed the possibility of manipulating or modifying cells and viruses in order to make materials. These materials can take the form of artificial proteins, inorganic particles, or highly organized composite materials. For example, recombinant DNA techniques have been used extensively in protein engineering,[117,118] and some of the targets have been polypeptides with sequences that target specific physical properties[119] and phases such as liquid crystals.[120]

A common approach is to introduce gene segments that encode for the targeted material in a plasmid, which is then introduced in bacteria for large-scale expression of the desired polypeptide. Using this method, several spider silks that are not naturally available in large quantities have been produced in non-native hosts.[29] Very recently, it has also been possible to incorporate in the sequences artificial amino acids using the bacterial methodology,[121–125] which would expand the range of properties possible in these artificial protein materials. Living cells have also been used to synthesize inorganic nanoparticles. It has been known for some time that microorganisms reduce or eliminate the toxicity of heavy-metal ions by reduction or precipitation.[126] This has been taken advantage of to synthesize nanoparticles of gold[127,128] and silver[129] as well as some sulfides and oxides.[130–132] Using phage display methodology,[133] it has been possible to discover peptides capable of binding to different inorganic materials, including semiconductors.[28] It is too early to judge if biology-based syntheses of materials will become an important source of what Arthur von Hippel would have called molecularly engineered materials.

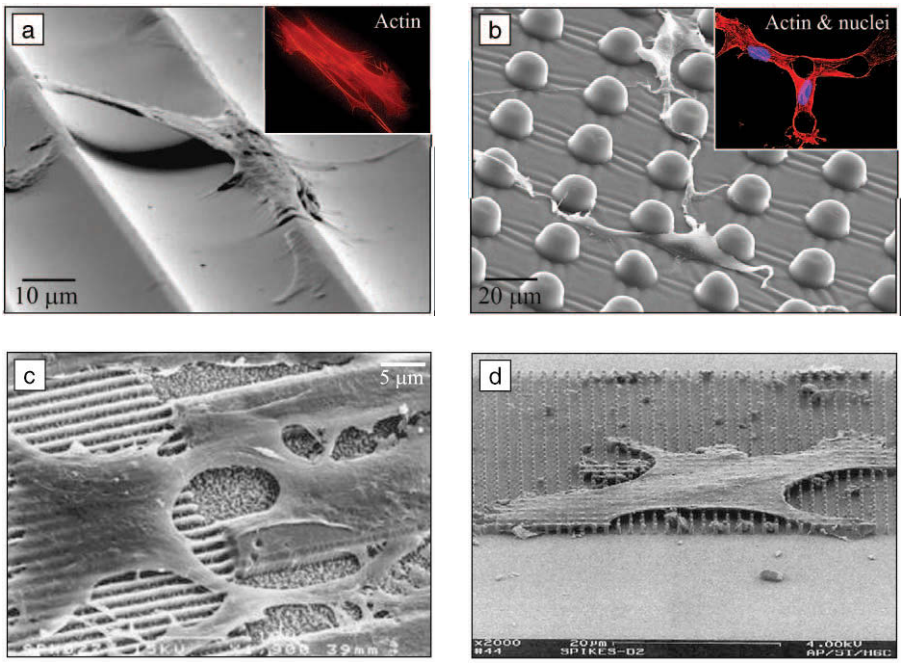

Figure 6. Examples of surface-microfabricated topographies used to investigate fundamental cellular phenomena such as cell adhesion, migration, proliferation, and differentiation. These studies provide important information about the behavior of cells in artificial environments that could be useful in the design of biomedical devices. (a),(b) Connective tissue progenitor cells aligning and migrating along 11-μm-high rounded channels[34,111] and proliferating within 10 μm posts microfabricated on poly(dimethylsiloxane) (PDMS) surfaces.[34,112] Insets illustrate the distinctive assembly of the cells' actin cytoskeleton on each surface. (c) Fibroblasts aligning to various-sized titanium microgrooves.[113] (d) Astrocyte preferentially attaching and spreading on the tips of 1-μm-high silicon columns.[114]

Conclusions

The field of biomaterials had humble beginnings, when polymers developed for inexpensive consumer goods, corrosion-resistant metals, and parachute fabrics were first used to repair joints, bones, and blood vessels in humans. The concept of the molecular engineering of materials that Arthur von Hippel was interested in has been slowly penetrating the field in order to achieve a specific biological response at tissue–material interfaces. In recent years, this concept has driven the biomaterials community to design bioactive materials, which in the extreme take the form of temporary scaffolds whose main role is to orchestrate cells into rebuilding tissues and organs. With further advances in the physical and life sciences, there is enormous potential for these materials to revolutionize regenerative medicine in areas ranging from the repair of spinal cord injuries to the cure of neurodegenerative diseases and diabetes. Led by a vibrant research community, the field is rapidly expanding into other areas that benefit each other in highly synergistic ways, discovering strategies to create biomimetic materials, using nanomaterials to diagnose disease and obtain genomic information, fabricating patterned materials to learn biology, and using biology itself to create sophisticated materials.

References

1. A. von Hippel, *Mater. Res. Bull.* **14** (1979) p. 273.
2. B.D. Ratner, *Biomaterials Science: An Introduction to Materials in Medicine* (Elsevier Academic Press, San Diego, 2004).
3. D.G. Anderson, J.A. Burdick, and R. Langer, *Science* **305** (2004) p. 1923.
4. L.L. Hench and E.C. Ethridge, *Biomaterials: An Interfacial Approach* (Academic Press, San Diego, 1982).

5. S. Dumitriu, ed., *Polymeric Biomaterials,* 2nd ed. (Marcel Dekker, New York, 2002).
6. J.D. Bronzio, *The Biomedical Engineering Handbook* (CRC Press, Boca Raton, Fla., 1995).
7. H.P. Williams, *Br. J. Ophthalmol.* **85** (9) (2001) p. 1022.
8. R.A. DeWall, N. Qasim, and L. Carr, *Ann. Thorac. Surg.* **69** (2000) p. 1612.
9. S.I. Stupp, *MRS Bull.* **30** (7) (2005) p. 546.
10. E. Beniash, J.D. Hartgerink, H. Storrie, J.C. Stendahl, and S.I. Stupp, *Acta Biomater.* **1** (2005) p. 385.
11. S. Sarkar, M. Dadhania, P. Rourke, T.A. Desai, and J.Y. Wong, *Acta Biomater.* **1** (2005) p. 93.
12. M. Shin, K. Matsuda, O. Ishii, H. Terai, M. Kaazempur-Mofrad, J. Borenstein, M. Detmar, and J.P. Vacanti, *Biomed. Microdevices* **6** (4) (2004) p. 269.
13. H. Storrie and S.I. Stupp, *Biomaterials* **26** (2005) p. 5492.
14. R. Furstner, W. Barthlott, and P. Walzel, *Langmuir* **21** (2005) p. 956.
15. S.C.S Lai, "Mimicking Nature: Physical Basis and Artificial Synthesis of the Lotus-Effect," Universiteit Leiden (August 2003), home.wanadoo.nl/scslai/lotus.pdf (accessed September 2005.)
16. Photograph of a water lily, www.chiba-u.ac.jp/JP/gakuseibu/kyoumuka/original/lotus-2.jpg (accessed September 2005).
17. A.K. Geim, S.V. Dubonos, I.V. Grigorieva, K.S. Novoselov, A.A. Zhukov, and S.Y. Shapoval, *Nature Mater. Lett.* (2003) p. 461.
18. "How Geckos Stick to Walls," www.lclark.edu/,autumn/private/u38j47a0t/ (accessed September 2005).
19. J. Aizenberg, *Adv. Mater.* **16** (2004) p. 1295.
20. The Whitaker Foundation, "Imaging Prostate Cancer with Quantum Dots," www.whitaker.org/news/nie2.html (accessed September 2005).
21. Office of Naval Research, "Image Gallery: General Science and Technology," www.onr.navy.mil/media/gallery_category.asp?Cat=general (accessed September 2005).
22. X. Wu, H. Liu, J. Liu, K.N. Haley, J.A. Treadway, J.P. Larson, N. Ge, F. Peale, and M.P. Bruchez, *Nature Biotech.* **21** (2003) p. 41.
23. N.L. Rosi and C.A. Mirkin, *Chem. Rev.* **105** (2005) p. 1547.
24. J.T. Groves, *Angew. Chem. Int. Ed.* **44** (2005) p. 3524.
25. A. Brock, E. Chang, C.C. Ho, P. LeDuc, X. Jiang, G.M. Whitesides, D.E. Ingber, *Langmuir* **19** (2003) p. 1611.
26. J.L. Tan, J. Tien, D. Pirone, D.S. Gray, and C.S. Chen, *Proc. Natl. Acad. Sci. USA* **100** (2003) p. 1484.
27. C. Mao, D.J. Solis, B.D. Reiss, S.T. Kottmann, R.Y. Sweeney, A. Hayhurst, G. Georgiou, B. Iverson, and A.M. Belcher, *Science* **303** (2004) p. 213.
28. S.R. Whaley, D.S. English, E.L. Hu, P.F. Barbara, and A.M. Belcher, *Nature* **405** (2000) p. 665.
29. J. Cappello, J. Crissman, M. Dorman, M. Mikolajczak, G. Textor, M. Marquet, and F. Ferrari, *Biotechnol. Progr.* **6** (1990) p. 198.
30. E.D. Spoerke and S.I. Stupp, *Biomaterials* **26** (2005) p. 5120.
31. E.D. Spoerke and S.I. Stupp, *J. Biomed. Mater. Res.* **67A** (2003) p. 960.
32. R.P. Lanza, R. Langer, and J. Vacanti, *Principles of Tissue Engineering* (Academic Press, San Diego, 2000).
33. T.P. Richardson, M.C. Peters, A.B. Ennett, and D.J. Mooney, *Nature Biotech.* **19** (2001) p. 1029.
34. A. Mata, doctoral dissertation, Cleveland State University (2005).
35. A. Mata, A.J. Fleischman, and S. Roy, "Microfabricated 3D Scaffolds for Tissue Engineering Applications," in *Nanoscale Materials Science in Biology and Medicine,* edited by C.T. Laurencin and E.A. Botchwey (*Mater Res. Soc. Symp. Proc.* **845**, Warrendale, PA, 2005) p. AA4.3.1.
36. W. Tan and T.A. Desai, *Biomaterials* **25** (2004) p. 1355.
37. A. Folch, S. Mezzour, M. During, O. Hurtado, M. Toner, and R. Muller, *Biomed. Microdevices* **2** (3) (2000) p. 207.
38. A. Mata, A.J. Fleischman, and S. Roy, *Proc. Am. Soc. Precision Eng. Spring Topical Meeting on Precision Micro/Nano-Scale Polymer-Based Component and Device Fabrication, Vol. 35* (2005) p. 15.
39. A. Lendlein and R. Langer, *Science* **296** (2002) p. 1673.
40. J. Yang, M. Yamato, and T. Okano, *MRS Bull.* **30** (3) (2005) p. 189.
41. K. Nishida, M. Yamato, Y. Hayashida, K. Watanabe, N. Maeda, H. Watanabe, K. Yamamoto, S. Nagai, A. Kikuchi, Y. Tano, and T. Okano, *Transplantation* **77** (2004) p. 379.
42. T. Shimizu, M. Yamato, Y. Isoi, T. Akutsu, T. Setomaru, K. Abe, A. Kikuchi, M. Umezu, and T. Okano, *Circ. Res.* **90** (2002) p. e40.
43. J. Lahann and R. Langer, *MRS Bull.* **30** (3) (2005) p. 185.
44. J.D. Hartgerink, E. Beniash, and S.I. Stupp, *Science* **294** (2001) p. 1684.
45. G.A. Silva, C. Czeisler, K.L. Niece, E. Beniash, D. Harrington, J.A. Kessler, and S.I. Stupp, *Science* **303** (2004) p. 1352.
46. J.D. Hartgerink, E. Beniash, and S.I. Stupp, *Proc. Natl. Acad. Sci. USA* **99** (2002) p. 5133.
47. K.L. Niece, J.D. Hartgerink, J. Donners, and S.I. Stupp, *J. Am. Chem. Soc.* **125** (2003) p. 7146.
48. R.F. Service, *Science* **308** (2005) p. 44.
49. J.-M. Lehn, *Les Prix Nobel* (1988) p. 129.
50. H.A. Behanna, J. Donners, A.C. Gordon, and S.I. Stupp, *J. Am. Chem. Soc.* **127** (2005) p. 1193.
51. S.R. Bull, M.O. Guler, R.E. Bras, T.J. Meade, and S.I. Stupp, *Nano Lett.* **5** (2005) p. 1.
52. M.O. Guler, J.K. Pokorski, D.H. Appella, and S.I. Stupp, *Bioconjug. Chem.,* **16** (2005) p. 501.
53. R. Spolenak, G. Stanislav, and E. Arzt, *Acta Biomater.* **1** (2005) p. 5.
54. K. Autumn and A.M. Peattie, *Integr. Comp. Biol.* **42** (2002) p. 1081.
55. R. Breslow, *Chem. Rec.* (2000) p. 3.
56. D. Kisailus, M. Najarian, J.C. Weaver, and D.E. Morse, *Adv. Mater.* **17** (2005) p. 1234.
57. C. Du, G. Falini, S. Fermani, C. Abbott, and J. Moradian-Oldak, *Science* **307** (2005) p. 1450.
58. N.C. Seeman, *Trends Biochem. Sci.* **30** (2005) p. 119.
59. C.M. Niemeyer, *Curr. Opin. Chem. Biol.* **4** (2000) p. 609.
60. E. Winfree, F. Liu, L.A. Wenzler, and N.C. Seeman, *Nature* **394** (1998) p. 539.
61. C. Mao, W. Sun, and N.C. Seeman, *Nature* **386** (1997) p. 137.
62. J. Chen and N.C. Seeman, *Nature* **350** (1991) p. 631.
63. C. Mao, W. Sun, Z. Shen, and N.C. Seeman, *Nature* **397** (1999) p. 144.
64. H. Yan, X. Zhang, Z. Shen, and N.C. Seeman, *Nature* **415** (2002) p. 62.
65. M.G. Warner and J.E. Hutchison, *Nature Mater.* **2** (2003) p. 272.
66. H. Nakao, H. Shiigi, Y. Yamamoto, S. Tokonami, T. Nagaoka, S. Sugiyama, and T. Ohtani, *Nano Lett.* **3** (2003) p. 1391.
67. L.-S. Li and S.I. Stupp, *Angew. Chem. Int. Ed.* **44** (2005) p. 1833.
68. S. Yang, G. Chen, M. Megens, C.K. Ullal, Y.J. Han, R. Rapaport, E.L Thomas, and J. Aizenberg, *Adv. Mater.,* **17** (4) (2005) p. 435.
69. P. Alivisatos, *Nature Biotechnol.* **22** (2004) p. 47.
70. M. Bruchez Jr., M. Moronne, P. Gin, S. Weiss, and A.P. Alivisatos, *Science* **281** (1998) p. 2013.
71. W.C.W. Chan and S. Nie, *Science* **281** (1998) p. 2016.
72. X. Michalet, F.F. Pinaud, L.A. Bentolila, J.M. Tsay, S. Doose, J.J. Li, G. Sundaresan, A.M. Wu, S.S. Gambhir, and S. Weiss, *Science* **307** (2005) p. 538.
73. I.L. Medintz, H.T. Uyeda, E.R. Goldman, and H. Mattoussi, *Nature Mater.* **4** (2005) p. 435.
74. S. Kim, Y.T. Lim, E.G. Soltesz, A.M. De Grand, J. Lee, A. Nakayama, J.A. Parker, T. Mihaljevic, R.G. Laurence, D.M. Dor, L.H. Cohn, M.G. Bawendi, and J.V. Frangioni, *Nature Biotechnol.* **22** (2004) p. 93.
75. T.A. Taton, C.A. Mirkin, and R.L. Letsinger, *Science* **289** (2000) p. 1757.
76. C.S. Thaxton, N.L. Rosi, and C.A. Mirkin, *MRS Bull.* **30** (5) (2005) p. 376.
77. R. Elghanian, J.J. Storhoff, R.C. Mucic, R.L. Letsinger, and C.A. Mirkin, Science 277 (1997) p. 1078.
78. Y.W.C. Cao, R.C. Jin, and C.A. Mirkin, *Science* **297** (2002) p. 1536.
79. J.B. Jackson, S.L. Westcott, L.R. Hirsch, J.L. West, and N.J. Halas, *Appl. Phys. Lett.* **82** (2003) p. 257.
80. L.R. Hirsch, J.B. Jackson, A. Lee, N.J. Halas, and J. West, *Anal. Chem.* **75** (2003) p. 2377.
81. J.-M. Nam, S. Stoeva, and C.A. Mirkin, *J. Am. Chem. Soc.* **126** (2004) p. 5932.
82. P.D. Yang, *MRS Bull.* **30** (2) (2005) p. 85.
83. D.J. Sirbuly, M. Law, P. Pauzauskie, H. Yan, A.V. Maslov, K. Knutsen, C.-Z. Ning, R.J. Saykally, and P. Yang, *Proc. Natl. Acad. Sci.* USA **102** (2005) p. 7800.
84. J. Kong, N. Franklin, C. Zhou, S. Peng, J.J. Cho, and H. Dai, *Science* **287** (2000) p. 622.
85. P.G. Collins, K. Bradley, M. Ishigami, and A. Zettl, *Science* **287** (2000) p. 1801.
86. R.J. Chen, S. Bangsaruntip, K.A. Drouvalakis, N. Wong Shi Kam, M. Shim, Y. Li, W. Kim, P.J. Utz, and H. Dai, *Proc. Natl. Acad. Sci.* USA **100** (2003) p. 4984.
87. Y. Cui, Q.Q. Wei, H.K. Park, and C.M. Lieber, *Science* **293** (2001) p. 1289.
88. O. Clément, N. Siauve, C-A. Cuénod, and G. Frija, *Top. Magn. Reson. Imaging* **9** (1998) p. 167.
89. Y.-X. Wang, S.M. Hussain, and G.P. Krestin, *Eur. Radiol.* **11** (2001) p. 2319.

90. R. Lawaczeck, M. Menzel, and H. Pietsch, *Appl. Organomet. Chem.* **18** (2004) p. 506.
91. A. Chilkoti and J.A. Hubbell, *MRS Bull.* **30** (3) (2005) p. 175.
92. Y. Xia and G.M. Whitesides, *Angew. Chem. Int. Ed.* **37** (1998) p. 550.
93. C.M. Nelson and C.S. Chen, *FEBS Lett.* **514** (2002) p. 238.
94. C.S. Chen, J.L. Alonso, E. Ostuni, G.M. Whitesides, and D.E. Ingber, *Biochem. Biophys. Res. Commun.* **307** (2003) p. 355.
95. S.N. Bhatia, U.J. Balis, M.L. Yarmush, and M. Toner, *FASEB J.* **13** (1999) p. 1883.
96. M. Mrksich, *MRS Bull.* **30** (3) (2005) p. 180.
97. S. Raghavan and C.S. Chen, *Adv. Mater.* **16** (15) (2004) p. 1303.
98. C.S. Chen, X. Jiang, and G.M. Whitesides, *MRS Bull.* **30** (3) (2005) p. 194.
99. X. Jiang and G.M. Whitesides, *Eng. Life Sci.* **3** (12) (2003) p. 475.
100. G.M. Walker, J. Sai, A. Richmond, M. Stremler, C.Y. Chung, and J.P. Wikswo, *Lab Chip* **5** (2005) p. 611.
101. S. Takayama, E. Ostuni, LeDuc, K. Naruse, D.E. Ingber, and G.M. Whitesides, *Nature* **411** (2001) p. 1016.
102. J.T. Groves, N. Ulman, and S.G. Boxer, *Science* **275** (1997) p. 651.
103. C. Yoshina-Ishii, G.P. Miller, M.L. Kraft, E.T. Kool, and S.G. Boxer, *J. Am. Chem. Soc.* **127** (2005) p. 1356.
104. H. Schonherr, J.M. Johnson, P. Lenz, C.W. Frank, and S.G. Boxer, *Langmuir* **20** (2004) p. 11600.
105. M.M. Baksh, M. Jaros, and J.T. Groves, *Nature* **427** (2004) p. 139.
106. L. Kam and S.G. Boxer, *Langmuir* **19** (2003) p. 1624.
107. A. Grakoui, S.K. Bromley, C. Sumen, M.M. Davis, A.S. Shaw, P.M. Allen, and M.L. Dustin, *Science* **285** (1999) p. 221.
108. A. Curtis and C. Wilkinson, *Biomaterials* **18** (1998) p. 1573.
109. R.G. Harrison, *J. Exp. Zool.* **17** (4) (1912) p. 521.
110. D.M. Brunette, *Exp. Cell Res.* **164** (1) (1986) p. 11.
111. A. Mata, C. Boehm, A.J. Fleischman, G.M. Muschler, and S. Roy, *Biomed. Microdevices* **4** (4) (2002) p. 267.
112. A. Mata, C. Boehm, A.J. Fleischman, G.M. Muschler, and S. Roy, *J. Biomed. Mater. Res.* **62** (2002) p. 499.
113. E.T. den Braber, H.V. Jansen, M.J. de Boer, H.J.E. Croes, M. Elwenspoek, L.A. Ginsel, and J.A. Jansen, *J. Biomed. Mater. Res.* **40** (1998) p. 425.
114. H.G. Craighead, S.W. Turner, R.C. Davis, C. James, A.M. Perez, P.M. St. John, M.S. Isaacson, L. Kam, W. Shain, J.N. Turner, and G. Banker, *Biomed. Microdevices* **1** (1998) p. 49.
115. C.D.W. Wilkinson, M. Riehle, M. Wood, J. Gallagher, and A.S.G. Curtis, *Mater. Sci. Eng.* **C19** (2002) p. 263.
116. D. Deutsch, B. Motlagh, B. Russell, and T.A. Desai, *J. Biomed. Mater. Res.* **53** (2000) p. 267.
117. J. Sambrook, E.F. Fritsch, and T. Maniatis, *Molecular Cloning* (Cold Spring Harbor Laboratory Press, New York, 1986).
118 J.C.M. van Hest and D.A. Tirrell, *Chem. Commun.* (2001) p. 1897.
119. W.A. Pelka, J.L. Harden, K.P. McGrath, D. Wirtz, and D.A. Tirrell, *Science* **281** (1998) p. 389.
120. S.J.M. Yu, V.P. Conticello, G.H. Zhang, C. Kayser, M.J. Fournier, T.L. Mason, and D.A. Tirrell, *Nature* **389** (1997) p. 167.
121. A.J. Link, M.L. Mock, and D.A. Tirrell, *Curr. Opin. Biotechnol.* **14** (2003) p. 603.
122. T. Hohsaka and M. Sisido, *Curr. Opin. Chem. Biol.* **6** (2002) p. 809.
123. D.A. Dougherty, *Curr. Opin. Chem. Biol.* **4** (2000) p. 645.
124. L. Wang, A. Brock, B. Herberich, and P.G. Schultz, *Science* **292** (2001) p.498.
125. L. Wang and P.G. Schultz, *Angew. Chem. Int. Ed.* **44** (2005) p.34.
126. S. Silver, *Gene* **179** (1996) p. 9.
127. J.L. Gardea-Torresdey, J.G. Parsons, E. Gomez, J. Peralta-Videa, H.E. Troiani, P. Santiago, and M.J. Yacaman, *Nano Lett.* **2** (2002) p. 397.
128. P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, S.R. Sainkar, M.I. Khan, R. Ramani, R. Parischa, P.V. Ajayakumar, M. Alam, M. Sastry, and R. Kumar, *Angew. Chem. Intl. Ed.* **40** (2001) p. 3585.
129. P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, S.R. Sainkar, M.I. Khan, R. Parischa, P.V. Ajayakumar, M. Alam, R. Kumar, and M. Sastry, *Nano Lett.* **1** (2001) p. 515.
130. C.T. Dameron, R.N. Reese, R.K. Mehra, A.R. Kortan, P.J. Carroll, M.L. Steigerwald, L.E. Brus, and D.R. Winge, *Nature* **338** (1989) p. 596.
131. A. Ahmad, P. Mukherjee, D. Mandal, S. Senapati, M.I. Khan, R. Kumar, and M. Sastry, *J. Am. Chem. Soc.* **124** (2002) p.12108.
132. T. Douglas, D.P.E. Dickson, S. Betteridge, J. Charnock, C.D. Garner, and S. Mann, *Science* **269** (1995) p. 54.
133. R.H. Hoess, *Chem. Rev* **101** (2001) p. 3205.
134. E.D. Spoerke, N.D. Murray, H. Li, L.C. Brinson, D.C. Dunand, and S.I. Stupp, *Acta Biomater.* **1** (2005) p. 523.
135. Atomic force microscope and scanning electron microscope images provided by Hongzhou Jiang and Dr. Daniel A. Harrington, respectively. □