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A Modern-Day Venus
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The New Atlantis and the Frontiers of Medicine

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IN THE EARLY 17TH CENTURY, SIR FRANCIS BACON SET OUT ON A QUEST to unravel the secrets of the natural world. His approach was singularly encyclopedic, involving careful scrutiny of every imaginable phenomenon from the currents of streams and the recesses of caves to the decay of bodies, the condensation of rain, and the refraction of light. What was his ultimate purpose? Nothing less, he expounded in *The New Atlantis*, than "the knowledge of causes, and secret motions of things; and the enlarging of the bounds of human empire, to the effecting of all things possible."[1]

Interestingly, Bacon's pursuit has parallels in our own era. The Human Genome Project, with its encyclopedic approach to gene identification, is reminiscent of the scope of Bacon's early explorations. While Bacon's lofty goals seem remarkably grandiose, it is not entirely clear that our ambitions today are more modest. For example, James Watson, as head of the Human Genome Project, ventured that the characterization of the human genome would eventually provide us with "the ultimate answers to the chemical underpinnings of human existence."[2]

One of the most conspicuous differences between Bacon's time and ours is the advent of modern technology. The research tools now available to scientists and clinicians offer unprecedented magnifying power for the study of biological processes. As a result, medicine has progressed from gross anatomical descriptions of disease to precise characterization of physiologic and pathologic phenomena at microscopic and molecular levels. As the editors of the *New England Journal of Medicine* recently concluded, "It is hard not to be moved by the astounding course of medical history over the past thousand years. No one alive in the year 1000 could possibly have imagined what was in store."[3]

This month, *MSJAMA* examines several emerging technologies that are assuming increasing importance in the practice of clinical medicine. George Scarlatis comments on recent advances in the use of retinal and cortical stimulation to restore vision. Max Diehn, Ash Alizadeh, and Patrick Brown examine how complementary DNA microarray technology is redefining molecular medicine. In the closing article, Steve S. W. Han and Itzhak Fischer review advances in stem cell research and describe preliminary results on the repair of traumatic spinal cord injury in an animal model. See the *MSJAMA* Web site for Field Willingham's discussion of the technological innovations transforming medical education at the University of Maryland.

Medical students and physicians have access to a rapidly growing armamentarium of medical technologies. With these expanded capabilities comes a responsibility to introduce new innovations with discretion. Medical advances will never achieve their full potential unless they are well understood, thoughtfully applied, and critically evaluated. Only by staying abreast of new advances can medical students and physicians rise to the challenges and opportunities afforded by emerging health care advances.

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Optical Prosthesis: Visions of the Future

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SINCE ANTIQUITY, PEOPLE HAVE DREAMED OF RESTORING vision to the blind. Only recently, however, with the development of optical prostheses, has this prospect become a foreseeable reality. Early studies have demonstrated that direct electrical stimulation to neurons of the visual system will cause a subject to perceive points of light (phosphenes).[1] This observation spurred investigation into the use of electrical stimulation to overcome visual loss. Current approaches to optical prosthesis involve stimulation of nerves at either the retina or the visual cortex.[2] While both approaches are theoretically feasible, retinal prostheses have the advantage of being far less invasive and are the focus of this essay.

Visual impairment can result from lesions anywhere along the visual pathway. In the normal eye, light passes through the cornea, anterior chamber, lens, and vitreous and then stimulates the photoreceptors. The photoreceptors, which comprise the outer layer of the retina, transduce light energy into an electrical signal and propagate this signal through the layers of the retina to the retinal ganglion cells. From there, the electrical signal travels along the optic nerve, through the visual pathways, and eventually reaches the visual cortex, where sight perceptions are formed.

Retinal approaches to prosthetic vision are most readily applicable to those causes of blindness that involve injury to the outer retinal layer, where the photoreceptors are located. In age-related macular degeneration and retinitis pigmentosa, the photoreceptors of the outer retinal layer are destroyed and the inner retinal layer is preserved. These diseases thus disrupt the normal visual pathway at the point where light energy is transduced into neuroelectrical signals. Retinal prosthetics exploit the selective survival of the inner retinal layer neurons by bypassing the defective photoreceptors and directly stimulating the still-viable inner neuroretina.

To emulate the functions of the photoreceptors, optical prostheses must collect and deliver visual information efficiently. One approach to collecting visual information involves capturing images with a camera located outside the eye. These images are then translated by an image processor and sent via transmitter to the implanted device.[3] Another approach uses light-activated microphotodiodes[4] that are implanted within the eye and aligned geometrically to the information delivery apparatus. The camera method has the advantage of allowing multiple levels of image processing; the microphotodiode method obviates the need for external equipment and, due to its location, records fewer extraneous stimuli.

After the visual information has been collected, it is delivered to the surviving cells of the neuroretina by way of a microelectrode array. This array may be placed either just behind the retina (subretinal) or immediately anterior to it (epiretinal). Subretinal placement of semiconductor microphotodiodes is the more invasive method, but it is also technically simpler, allows for prolonged function in the absence of an external power supply, and does not significantly alter inner retinal function or architecture.[5,6] Epiretinal placement has the advantage of involving only very minimal surgical damage to the underlying retina.[7,8] Unlike the subretinal devices, however, the epiretinal devices have not yet been shown to be capable of generating in vivo a current in response to light stimulation over an extended period of time.

The available data indicate that visual prostheses have considerable potential for restoring rudimentary vision.[3] Clinical studies have shown that when electrical signals are applied to a small area of the retina with a microelectrode, otherwise blind patients will perceive a small spot of light (phosphene). When multiple electrodes are activated by light in a 2-dimensional array, the patient perceives a series of small spots of light. Subretinal devices currently under study contain over 1000 pixels per square millimeter.[4] The vision mediated by optical prostheses is analogous to the image formed on a scoreboard or on a dot-matrix printer, and it could allow blind patients to regain vision of basic geometric forms sufficient for restoration of ambulatory mobility and reading typed text.

While many issues relating to the biocompatibility, efficacy, and safety of visual prostheses need to be resolved, preliminary results are promising. Future research efforts will attempt to address current concerns and define stimulus patterns that will enable subjects to perceive complex images.[3] The development of optical prosthetics is an important advance that may eventually make possible the restoration of vision for patients with outer retinal disorders.

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Examining the Living Genome in Health and Disease With DNA Microarrays

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WITHIN A YEAR WE WILL KNOW VIRTUALLY THE ENTIRE sequence of the human genome—the genetic instructions that specify the molecular components, the design, and the operating software for the human body. This knowledge will transform medicine, giving us the means to see and to understand human anatomy, specialization, physiology, and pathophysiology in molecular detail. The genomes of more than 30 frequently studied organisms, including many human pathogens, have already been fully sequenced, and almost half of the sequence of the human genome is currently available in fragmentary form in public databases (NCBI GeneMap at http://www.ncbi.nlm.nih.gov/genemap). Not only will this new knowledge open a molecular window on a largely unexplored world of human biology, but it will also provide a way to see and to understand the molecular scripts that guide normal physiology and development and their alterations in disease. Here we focus on the use of DNA microarrays, or DNA chips as "microscopes," to observe the physiology of the living genome.

The Basics of DNA Microarrays

DNA microarrays are microscopic, physically ordered arrays of thousands of DNAs of known sequences, attached to solid surfaces. All of the genes in a genome can be arrayed in an area no larger than a standard microscope slide. Today, the largest DNA microarrays contain elements representing almost 40 000 genes, roughly half of the predicted number of genes in the human genome. A few years from now, when we know the complete catalog of human genes, DNA microarrays will allow us to watch every gene in our genomes.

To survey the expression of genes, RNA transcripts are isolated from cells, labeled with a fluorescent dye, and hybridized to a DNA microarray (**FIGURE**). During this hybridization process, the DNA sequences of the immobilized elements "capture" their complementary cognates in the fluorescent probe mixture. The fluorescent signal at the "spot" in the array representing each individual gene provides a quantitative readout of the level of expression of that gene in the sample.[1] This straightforward procedure provides a systematic, quantitative way to monitor expression of tens of thousands of genes simultaneously.

Each cell in the human body expresses a specific set of genes according to a precisely controlled program that gives each cell its distinctive design and functional capabilities. Cells further employ signal transduction systems to collect information about their condition, including the presence of infection, stress, drugs, injury, growth factors or hormones, and convert these inputs to changes in gene expression. The gene expression patterns thus reflect a cell's internal state and microenvironment, creating a molecular "picture" of the cell's state. Since DNA microarrays detect gene expression patterns, they can be used to capture these molecular pictures and thus to deduce the condition of cells.

Because the expression pattern of a gene is closely tied to its biological role, systematic microarray studies of global gene expression can provide remarkably detailed clues to the functions of specific genes. This is an important advance, since we currently know the functions of fewer than 5% of the genes in the human genome.

Figure. Use of DNA Microarray to Detect Differences in Gene Expression in Normal vs Malignant Breast Epithelial Cells.
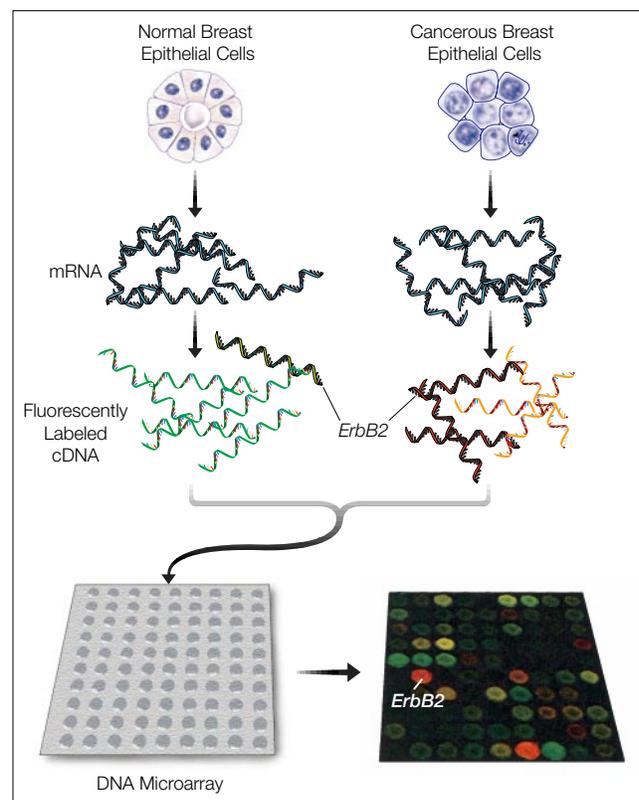
Messenger RNA from normal breast tissue is labeled with green fluorescent dye and messenger RNA from the malignant breast tissue is labeled with red fluorescent dye using a reverse transcription reaction. The resulting fluorescent complementary DNAs from the 2 samples are then combined and hybridized to the DNA microarray. The relative abundance of different genes in the 2 samples is reflected by the color of the corresponding spots in the microarray and can be quantitated using a scanning laser microscope. In the example shown, the microarray spot denoting *ErbB2* fluoresces red, indicating that the oncogene *ErbB2* is expressed at abnormally high levels in the malignant breast tumor cells.

A broad range of clinical applications has been suggested for DNA microarrays, and many have already been demonstrated in recent studies. These include applications such as messenger RNA expression profiling for improved disease classification,[2,3] genotyping of polymorphisms affecting disease susceptibility,[4] identification of genetic lesions within malignancies,[5,6] design and discovery of therapeutics, and sequencing of DNA.[7]

Gene Expression Profiling

Most disease processes are accompanied not only by characteristic macroscopic or histological changes but also by systematic changes in gene expression patterns. For some pathological processes, such as cancer, inappropriate gene expression is fundamental to pathogenesis. For others, the gene expression programs, both in cells directly affected by a disease and in healthy cells responding to the local and systemic effects of a disease, can give us a detailed molecular picture of the pathogenic process.

Subtle but critically important molecular differences that have heretofore gone unrecognized enable us to distinguish superficially similar disease processes that differ importantly in their natural histories and therapeutic responses. We expect that the detailed molecular pictures provided by genomic expression analysis will revolutionize molecular medicine just as high-resolution radiographic imaging methods have revolutionized diagnosis and treatment at the gross anatomic level.

Several studies have used gene expression signatures captured using DNA microarrays for the molecular classification of cancer. One study recently demonstrated the ability of these profiles to distinguish distinct pathological entities, such as acute myeloid leukemia and acute lymphoblastic leukemia, on the basis of their distinctive gene expression programs.[3] More promisingly, DNA microarrays have revealed distinct new diseases. For example, a recent study showed that diffuse large B-cell lymphoma, the most common non-Hodgkin lymphoma, is actually comprised of at least 2 distinct diseases with distinct expression profiles and strikingly different clinical courses.[2] Because discrete disease variants will often require different therapies, the ability to classify diseases on the basis of gene expression profiles will undoubtedly improve management of many disorders.

Drug Development

A common strategy in the development of new therapeutics is to screen candidate compounds for activity against disease-specific cellular targets. However, this approach has been limited by the scarcity of known molecular targets. Microarray-based gene expression analyses will facilitate the rapid identification of disease-specific genes and reveal the cellular pathways involved in pathophysiology. The discovery of disease-specific genes and pathways has immediate implications for drug development. In the simplest scenario, genes overexpressed in diseased cells (such as the *Her-2/neu* in breast cancers) could serve as potential drug targets. In addition, established drugs that act through unknown molecular mechanisms can be studied using DNA microarrays. The gene expression responses of cells exposed to these agents should help elucidate their mechanisms of action and facilitate the development of new drugs with similar specificities.[8]

Other applications of DNA microarrays include pharmacogenomic methods for improved drug development and measurements of DNA variation associated with pathogenesis or involved in disease predisposition. See the full-length Web version of this article online at www.msjama.org for an in-depth discussion of these and related microarray applications.

Beyond Nucleic Acids

The ability to use a DNA sequence directly as a reagent for detecting and assaying copies of that sequence in a biological sample has been exploited in the first wave of genomic assays and diagnostics. Genome sequences also provide a less immediate but equally valuable route to assays for the protein products of every gene. We are thus on the threshold of a formidable new challenge and opportunity: discovering the biological activities of proteins on a genomic scale. This rapidly expanding enterprise has been termed "proteomics." Its tools include diverse mass spectroscopic methods,[9] antibody microarrays, which simultaneously assay the presence or absence of multiple disease-marker proteins within bodily fluids, and genetic and "chemical genetic" technologies.[10,11] Such tools, combined with the continued use of DNA microarrays, will have an immense impact on clinical diagnostics and therapeutics in coming years.

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Neural Stem Cells and Gene Therapy: Prospects for Repairing the Injured Spinal Cord

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BECAUSE OF THE ADULT CENTRAL NERVOUS SYSTEM'S (CNS'S) limited ability to repair itself following traumatic injury, spinal cord injuries can be devastating, and the prospects for recovery are generally grim. However, the observation that a few regions in the CNS continue to produce neurons throughout life offers exciting prospects for repairing an injured spinal cord. Considerable progress has been made in developing efficient methods for culturing the neural stem cells of rodents, genetically modifying them to produce therapeutic genes, and transplanting them into animal models of brain diseases. These same gene therapy and grafting methods are now being pursued for restoring function following traumatic spinal cord injury.

Neural Stem Cells

Stem cells are multipotential cells that have the capacity to proliferate in an undifferentiated state, to self-renew, and to give rise to all the cell types of a particular tissue.[1] In the developing embryo, neuroepithelial cells of the neural tube generate a variety of lineage-restricted precursor cells that migrate and differentiate into neurons, astrocytes, and oligodendrocytes (**FIGURE 1**).[2] CNS stem cells have now been discovered in the human CNS and appear to behave similarly to their rodent counterparts.[3] These stem cells could potentially be used to promote neurogenesis following injury and disease.

Transplantation studies have demonstrated that neural stem cells and precursors have the capacity to alter their fate in response to the environment into which they are reintroduced and to integrate appropriately with the host tissue.[4] Neural stem cells can be isolated from different areas and propagated for long periods in culture without losing their multipotentiality. Thus, when transplanted back into the CNS, these stem cells have the capacity to migrate, to integrate with the host tissue, and to respond to local cues for differentiation.

Transplantation of Stem Cells

Neural stem cell grafts have been studied in a variety of animal models. One application involves grafting neural stem cells into a specific area of degeneration to replace a missing or deficient product. For example, in an animal model of Parkinson disease, precursor cells grafted into the striatum can replace degenerated dopamine-producing neurons in the nigrostriatal pathway and promote limited functional recovery.[5] Grafts of neural stem cells may also be effective in cases of widespread neural degeneration. For example, in a genetic model of demyelination, both the pathology and symptoms can be reversed by transplantation of neural stem cells into the cerebral ventricles at birth.[6] The grafted stem cells migrate extensively throughout the brain,

integrate into the host cytoarchitecture, and correct the myelination process during subsequent developmental stages.

Grafted neural stem cells could potentially replace cells lost to injury, reconstitute the neuronal circuitry, and provide a relay station between the injured pathways above and below the lesion. Furthermore, intraspinal stem cell transplants can be genetically modified to provide therapeutic factors that prevent cell death and promote regeneration.

Cells to be transplanted into the injured spinal cord need to be readily obtained, easily expanded and stored, and amenable to genetic modification. They should also be able to survive for extended periods within the injury site, to integrate with host tissue, to rescue injured neurons from cell death and

Figure 1. Therapy With Transplanted Genetically Modified Stem Cells
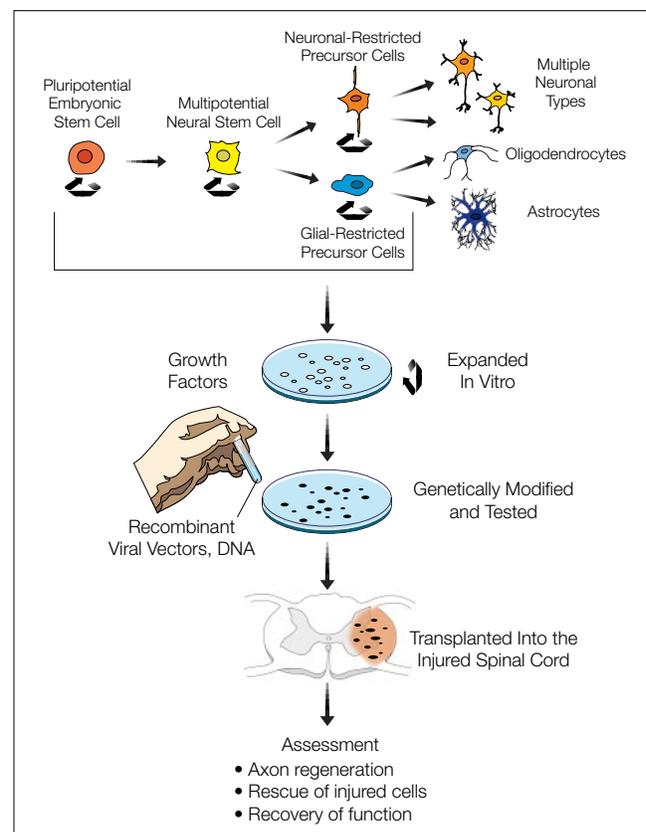
Multipotential neural stem cells have the ability to self-renew (curved arrows) and to generate all the mature cell types of the central nervous system—neurons, oligodendrocytes, and astrocytes. Neuronal-restricted precursor cells and glial-restricted precursor cells are more limited in their potential and ability to self-renew. These cells alone or in combination with ex vivo gene therapy are being evaluated for their potential to promote axon regeneration, rescue injured cells, and enhance functional recovery after spinal cord injury.

Figure 2. Transplantation of Neuronal-Restricted Precursor Cells Into a Rat Model of Spinal Cord Injury
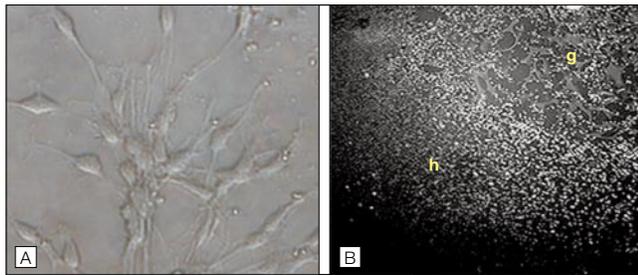
A partial cervical hemisection was performed in adult Sprague-Dawley rats, and labeled neuronal-restricted precursor (NRP) cells were grafted into the lesion cavity (see reference 8 for methods). (A) Phase-contrast microscopy of cultured NRP cells showing typical morphology (original magnification ×400; see reference 7 for details). (B) Longitudinal tissue section of the spinal cord visualized under fluorescence microscopy showing survival of grafted bisBenzimide-labeled donor cells (g) at one month. Some cells (h) migrate out of the cavity into the host tissue (original magnification ×50).

atrophy, to promote axonal regeneration, and, ultimately, to restore function. Neural stem cells and neural precursors theoretically fit many of the above requirements; the challenge is to demonstrate their efficacy and safety for clinical applications.

Spinal Cord Repair

Among the most promising sources of cells for spinal cord repair are neuronal-restricted precursors (NRPs) derived from the developing spinal cord. These cells can be expanded in vitro and have the potential to differentiate into numerous neuronal types (**FIGURE 1**), including motoneurons.[7] In the ex vivo modality of gene therapy, therapeutic genes are introduced into cultured cells that are subsequently transplanted into the CNS. Researchers in our laboratory, in collaboration with Mahendra Rao, MBBS, PhD, at the University of Utah School of Medicine, are studying the developmental potential of NRP cells and plan to use the ex vivo approach to examine the therapeutic potential of these cells grafted into a rat model of spinal cord injury. Preliminary observations demonstrate survival of grafted NRP cells in the lesion site for at least 1 month (**FIGURE 2**).

Genetically modified stem cells have not yet been grafted into the injured spinal cord; however, transplantation of brain-derived neurotropic factor–producing fibroblasts has been carried out in our laboratory using a rat spinal cord injury model of partial cervical hemisection. These grafts resulted in long distance regeneration of axons from brainstem neurons and partial recovery of motor function.[8] Ongoing experiments with genetically modified fibroblasts are examining the effects of other growth factors, as well as adhesion molecules and growth-associated genes.

Conclusion

Transplantation of neural stem cells and precursor cells together with gene therapy offers great promise for spinal cord repair. Specific research goals include improving neuronal survival, promoting functional recovery through axonal regeneration, compensating for demyelination, and replacing lost cells.[9] Many issues will need to be resolved before stem cells can be considered for use in human subjects, but continued basic research on the properties of these cells and development of appropriate animal models of repair will pave the way for successful clinical application.

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