of cancer metastasis that mimics the lodging of a single tumor cell in a capillary bed would facilitate the development of antimetastatic drugs.

The increasingly intimate combination of engineering and biology offers the prospect of sophisticated physiological in vitro models of many different human tissues. These physiological surrogates will ultimately allow major advances in prevention, diagnosis, and molecular treatment of diseases that are currently considered potential targets for tissue engineering. Ultimately, this may result in a greater emphasis on treating different target diseases, such as trauma and congenital defects, with engineered tissue.

With a scientific foundation firmly established, we now need a robust infusion of biology-based engineering analysis and design to move the tissue-engineering field from an era of phenomenological observation and serendipity to one of commercially viable products that will improve the lives of millions of patients.

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VIEWPOINT

Third-Generation Biomedical Materials

Larry L. Hench* and Julia M. Polak*

Whereas second-generation biomaterials were designed to be either resorbable or bioactive, the next generation of biomaterials is combining these two properties, with the aim of developing materials that, once implanted, will help the body heal itself.

Initially, the choice of biomedical materials for use in the body was dependent on those already available off the shelf. Until an understanding of the immune system developed, many of the materials selected proved to be either pathogenic or toxic. During the 1960s and 1970s a first generation of materials was developed for use inside the human body. The goal of all early biomaterials was to "achieve a suitable combination of physical properties to match those of the replaced tissue with a minimal toxic response in the host" (*1*). In 1980 there were more than 50 implanted devices (prostheses) in clinical use made from 40 different materials (*1*), and some 2 to 3 million prosthetic parts were implanted in patients in the United States annually. A common feature of most of the materials was their biological "inertness." The principle underlying the bulk of biomaterials development was to reduce to a minimum the immune response to the foreign body, and this is still valid 21 years later. Tens of millions of individuals have had their quality of life enhanced for 5 to 25 years by use of implants made from such "inert" biomaterials.

Department of Materials and the Tissue Engineering Centre, Imperial College of Science, Technology and Medicine, University of London, Prince Consort Road, London SW7 2BP, UK.

*To whom correspondence should be addressed. E-mail: l.hench@ic.ac.uk and julia.polak@ic.ac.uk

Second-Generation Biomaterials

The field of biomaterials began to shift in emphasis from achieving exclusively a bioin-

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ert tissue response to instead producing bioactive components that could elicit a controlled action and reaction in the physiological environment (*2*). The mechanism of bonding of bioactive glasses (composed of Na_2O-CaO-P_2O_5-SiO_2) to living tissue (*3*) was shown to involve a sequence of 11 reaction steps (*4*). The first five steps occurred on the surface of the material and involved rapid ion exchange of Na^+ with H^+ and H_3O^+ followed by a polycondensation reaction of surface silanols to create a high–surface area silica gel, which provided a large number of sites for heterogeneous nucleation and crystallization of a biologically reactive hydroxycarbonate apatite (HCA) layer equivalent to the inorganic mineral phase of bone. The growing HCA layer on the surface of the material provided an ideal environment for six cellular reaction steps that included colonization by osteoblasts (the cells that make bone), followed by proliferation and differentiation of the cells to form new bone that had a mechanically strong bond to the implant surface.

By the mid-1980s bioactive materials had reached clinical use in a variety of orthopedic and dental applications, including various compositions of bioactive glasses, ceramics, glass-ceramics, and composites. Synthetic hydroxyapatite (HA) ceramics began to be routinely used as porous implants, powders, and coatings on metallic prostheses to provide bioactive fixation (*5*, *6*). The presence of sparingly soluble HA coatings led to a tissue response (termed osteoconduction) in which bone grew along the coating and formed a mechanically strong interface. Bioactive glasses and glass-ceramics were used as middle-ear prostheses to restore the ossicular chain and treat conductive hearing loss and as oral implants to preserve the alveolar ridge from the bone resorption that follows tooth extraction (*7*). A mechanically strong and tough bioactive glass-ceramic was used for replacement of vertebrae in patients with spinal tumors (*8*). By the 1990s bioactive composites, such as HA particles in a polyethylene matrix, had become important in the repair and replacement of bones in the middle ear (*9*).

Another advance in this second generation was the development of resorbable biomaterials that exhibited clinically relevant controlled chemical breakdown and resorption. In this manner, the interface problem is resolved, because the foreign material is ultimately replaced by regenerating tissues, and ultimately there is no discernible difference between the implant site and the host tissue (*1*). An example of this is the biodegradable suture, in which the polymer composed of polylactic (PLA) and polyglycolic (PGA) acids hydrolyticly decomposes into CO_2 and H_2O. By 1984 clinical use of resorbable polymers as sutures was routine. Resorbable fracture fixation plates and screws in orthopedics and controlled-release drug-delivery systems were in their infancy (*10*).

The clinical success of bioinert, bioactive, and resorbable implants has been a vital response to the medical needs of a rapidly aging population. However, survival analyses of skeletal prostheses (*7*, *11*) and artificial heart valves (*12*) show that a third to half of prostheses fail within 10 to 25 years, and patients require revision surgery. Twenty years of research has had only small effects on failure rates (*7*), and continuing this path of trial-and-error experiments that require use of many animals and human clinical trials is prohibitively expensive. Improvements of first- and second-generation biomaterials are limited in part because all man-made biomaterials used for repair or restoration of the body represent a compromise (*1*). Living tissues can respond to changing physiological loads or biochemical stimuli, but synthetic materials cannot. This limits the lifetime of artificial body parts. It also signals that we have reached a limit to our current medical paradigm that emphasizes replacement of tissues. It is time to consider a shift toward a more biologically based method for the repair and regeneration of tissues.

Third-Generation Biomaterials: Cell- and Gene-Activating Materials

Third-generation biomaterials are being designed to stimulate specific cellular responses at the molecular level. The separate concepts of bioactive materials and resorbable materials have converged; bioactive materials are being made resorbable and resorbable polymers are being made bioactive. Molecular modifications of resorbable polymer systems elicit specific interactions with cell integrins and thereby direct cell proliferation, differentiation, and extracellular matrix production and organization. Third-generation bioactive glasses and macroporous foams are being designed to activate genes that stimulate regeneration of living tissues. Two alternative routes of repair are now available with the use of these tailored biomaterials.

Tissue engineering. Progenitor cells are seeded onto modified resorbable scaffolds. The cells grow outside the body and become differentiated and mimic naturally occurring tissues. These tissue-engineered constructs are then implanted into the patients to replace diseased or damaged tissues. With time the scaffolds are resorbed and replaced by host tissues that include a viable blood supply and nerves. The living tissue-engineered constructs adapt to the physiological environment and should provide long-lasting repair. Clinical applications include repair of articular cartilage, skin, and the vascular system, although stability of the repaired tissues needs improvement.

In situ tissue regeneration. This approach involves the use of biomaterials in the form of powders, solutions, or doped microparticles to stimulate local tissue repair. Bioactive materials release chemicals in the form of ionic dissolution products, or growth factors such as bone morphogenic protein (BMP), at controlled rates, by diffusion or network breakdown, that activate the cells in contact with the stimuli. The cells produce additional growth factors that in turn stimulate multiple generations of growing cells to self-assemble into the required tissues in situ along the biochemical and biomechanical gradients that are present.

For example, when a particulate of bioactive glass is used to fill a bone defect there is rapid regeneration of bone that matches the architecture and mechanical properties of bone at the site of repair. Both osteoconduction and osteoproduction (*13*) occur as a consequence of rapid reactions on a bioactive glass surface (*2*, *4*). The surface reactions release critical concentrations of soluble Si, Ca, P, and Na ions that give rise to both intracellular and extracellular responses at the interface of the glass with its cellular environment.

Genetic Control and Activation

Rapid repair of bone requires differentiation as well as proliferation of osteoblasts. A syn-

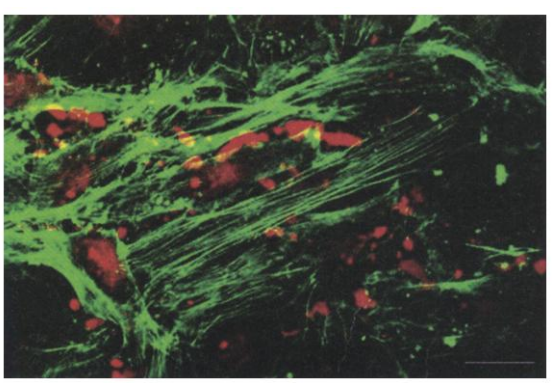

Fig. 1. Confocal micrograph of primary human osteoblasts cultured on a 45S5 monolith for 48 hours. Cells were stained with phalloidin (green) for F-actin and propidium iodide (red) for nuclei. A high level of actin organization can be observed with parallel stress fibers. Bar, 10 μm. [Image supplied by J. Gough, Imperial College]

chronized sequence of genes must be activated in the osteoblasts so that they undergo cell division and then synthesize an extracellular matrix that is capable of mineralizing to become bone. Recent research shows that there is genetic control of the cellular response of osteoblasts to bioactive glasses. Seven families of genes are upregulated within 48 hours of the exposure of primary human osteoblasts to the ionic dissolution products of bioactive glasses (*14*). The activated genes express numerous proteins that influence all aspects of differentiation and proliferation of osteoblasts: (i) transcription factors and cell-cycle regulators; (ii) signal transduction molecules; (iii) proteins involved in DNA synthesis, repair, and recombination; (iv) growth factors and cytokines that influence the inflammatory response to the material; (v) cell-surface antigens and receptors; (vi) extracellular-matrix components; and (vii) apoptosis regulators.

Use of the dissolution products of resorbable bioactive gel-glasses to stimulate cellular repair at a molecular level offers promise for creating scaffolds for bone tissue engineering. Under appropriate culture conditions, differentiating embryonic stem (ES) cells can be induced to form bone nodules (*15*); these structures are multilayers of cells embedded in a mineralized extracellular matrix that contains type I collagen and osteocalcin. When a standard osteoblast differentiation medium is conditioned by exposure to resorbable bioactive gel-glasses for 24 hours and then applied to differentiating ES cells, a dose-dependent increase in the numbers of bone nodules formed compared with control cultures is seen (*16*). Bioactive scaffolds have been made that release optimal concentrations of the ionic dissolution products as they resorb in the presence of adherent human osteoblasts (Figs. 1 and 2). Molecular modifications of the three-dimensional resorbable inorganic scaffolds by chemisorption of surfactant proteins have been made to enhance attachment and proliferation of lung cells (*17*, *18*).

Molecularly Tailored Resorbable Polymers

Third-generation biomaterials that involve molecular tailoring of resorbable polymers for specific cellular responses show great promise. By immobilizing specific proteins, peptides, and other biomolecules onto a material it is possible to mimic the extracellular matrix (ECM) environment (*19*) and provide a multifunctional cell-adhesive surface (*20*–*22*). Cell-specific recognition factors can be incorporated onto the resorbable polymer surface, including the adhesive protein fibronectin or functional domains of ECM components (*23*). Polymer surfaces can be tailored with proteins that influence interactions with endothelium (*24*), synaptic development (*25*), and neurite stimulation (*26*).

Cell transplants offer promise for treatment of neurological disorders, such as Parkinson's disease. However, to restore function within degenerating regions of the central nervous system, transplanted cells must differentiate and extend axons that form synaptic contacts within the host tissue. Creating a local environment for transplanted brain cells that enhances this regenerative process is essential. A third-generation biomaterial shows promise for achieving this microenvironment. Mahoney and Saltzman (*27*) have developed a technique that creates local and sustained levels of insoluble and soluble molecules directly at the site of cell transplantation in the brain. PLA/PGA copolymers were used to incorporate nerve growth factor (NGF) and release it at controlled rates. Cells were assembled with the cell-adhesive/controlled-release microparticles to form transplantable neo-tissues. NGF delivery by way of the synthetic microenvironment increased levels of NGF-induced biological activity over the course of 21 days in vivo. A similar approach with molecularly tailored polymers has been used to enhance directional regeneration of nerves (*26*).

Implications for the Future

A cellular and molecular basis for development of third-generation biomaterials provides the scientific foundation for molecular design of scaffolds for tissue engineering and for in situ tissue regeneration and repair, with minimally invasive surgery. There are important economic advantages to each of these new approaches that may aid in solving the problems of caring for an aging population. It should be feasible to design a new generation of gene-activating biomaterials tailored for specific patients and disease states. Tissue-engineered constructs based on a patient's own cells may be produced that can be used to select an optimal pharmaceutical treatment. Perhaps of even more importance is the possibility that bioactive stimuli can be used to activate genes in a preventive treatment to maintain the health of tissues as they age. Only a few years ago this concept would have seemed unimaginable. But we need to remember that only 30 years ago the concept of a material that would not be rejected by living tissues also seemed unimaginable.

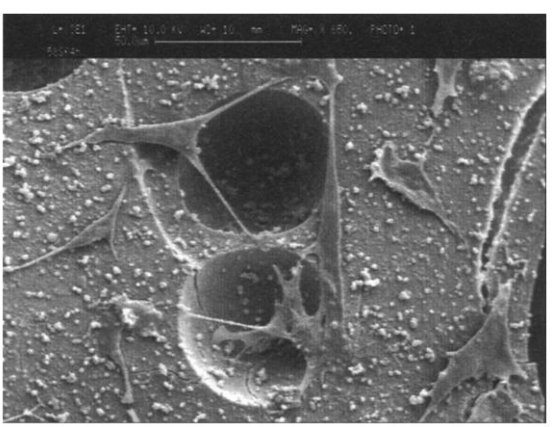

Fig. 2. Scanning electron micrograph of primary human osteoblasts cultured on porous 58S foam for 24 hours. Cells can be observed bridging smaller pores with fine cellular projections. In addition, calcium phosphate crystals can be observed covering the material surface and within the porous structure. Bar, 50 μm. [Image supplied by J. Gough, Imperial College]

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