Biomaterials in Drug Delivery and Tissue Engineering: One Laboratory's Experience

ROBERT LANGER

Department of Chemical Engineering, Massachusetts Institute of Technology, 45 Carlton Street, Building E25, Room 342, Cambridge, Massachusetts 02139-4307

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ABSTRACT

This Account reviews our laboratory's research in biomaterials. In one area, drug delivery, we discuss the development of materials that are capable of releasing macromolecules such as proteins and peptides, intelligent delivery systems based on magnetism or microchip technology, new degradable materials such as polyanhydrides, and noninvasive approaches for delivering molecules through the skin and lungs. A second area, tissue engineering, is also discussed. New polymer systems for creating cartilage, blood vessels, nerves, and other tissues are examined.

Introduction

Biomaterials have an enormous impact on human health care. Applications include medical devices, diagnostics, sensors, drug delivery systems, and tissue engineering (for examples, see refs 1−5). Here we discuss our laboratory's research in two of these areas: drug delivery systems and tissue engineering.

Drug Delivery

Our drug delivery research started in 1974. Our first investigations were aimed at studying whether large molecules, such as peptides or proteins, could be released slowly from biocompatible polymers. At that time, such a question was purely of academic interest and was motivated by our desire to create a 30-day *in vivo* bioassay for angiogenesis inhibitors and stimulators.[6] Delivering large molecules orally would be difficult since enzymes usually destroy them as they pass through the gastrointestinal tract; such molecules also cannot be easily absorbed through the intestine because of their size. At that time it was also believed that macromolecules were too large to pass through the skin transdermally. In addition, when such molecules were injected, they were rapidly destroyed by the body. They often had half-lives of 20−25 min or less.[7]

Robert Langer is the Germeshausen Professor of Chemical and Biomedical Engineering at MIT. He has received honorary doctorates from ETH (Switzerland) in 1996 and the Technion (Israel) in 1997. Dr. Langer has written 620 articles, 400 abstracts, 368 patents, and edited 12 books. He has received over 70 major awards, including the Gairdner Foundation International Award, the Lemelson-MIT prize, and the American Chemical Society's (ACS) Polymer Chemistry and Applied Polymer Science Awards and Creative Polymer Chemistry Award (ACS, Polymer Division). He has been elected to the Institute of Medicine of the National Academy of Sciences, the National Academy of Engineering, and the National Academy of Sciences.

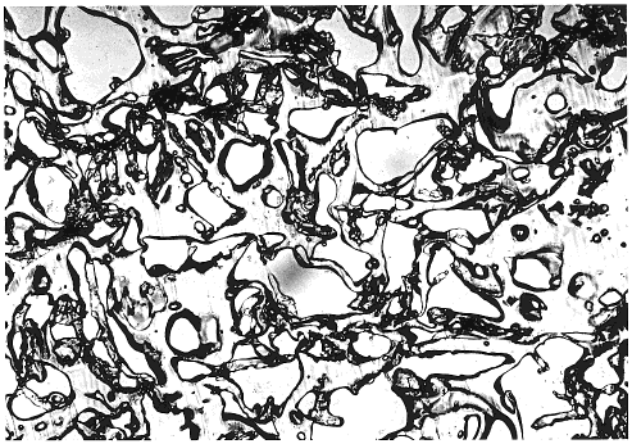

FIGURE 1. Five-micrometer cross section of ethylene−vinyl acetate system capable of releasing macromolecules.

In the 1970s, the field of genetic engineering emerged, making production of significant quantities of large-molecular-weight drugs possible. However, these drugs sometimes faced serious delivery problems. Large molecules needed a way to be delivered in unaltered form and yet be protected from harm. However, in the 1970s, it was generally believed that large molecules could not be slowly released from biocompatible polymers.[8]

Against this background, our research group began studying this problem. We utilized hydrophobic polymers, like ethylene−vinyl acetate[9] or lactic−glycolic acid copolymers,[10] dissolved them in certain solvents (like methylene chloride), and mixed them with proteins (often at low temperatures). Then, depending on the fabrication procedure, these polymers could be made into microparticles or other physical forms. These microparticles could slowly release proteins ranging from lysozyme (14 000 Da) to catalase (250 000 Da) for over 100 days. Sometimes the microparticles displayed a burst effect wherein a large portion of the drug was released in the first day. However, by controlling implant geometry, molecules could slowly be released at a constant rate.[11]

To determine how large molecules could be slowly released from these seemingly impenetrable polymers, we employed a cryomicrotome to cut thin sections through polymer matrices. This helped to elucidate the polymer microstructure. One model polymer studied was ethylene−vinyl acetate copolymer. When no drug or protein was placed in the polymer matrix, no pores were found, and molecules of 300 Da or greater were unable to diffuse from one side of a thin (5 μm) polymer matrix section to the other. However, if a protein was placed in a polymer matrix and sectioned, a phase separation was observed. When these systems were released for a year and then thin sections were cut, pores were left behind in place of the proteins that were originally there (Figure 1). The pores were created by this phase separation. In observing these pore structures by scanning electron microscopy, we found that the pores were large enough for molecules, even of several million daltons molecular weight, to pass through. However, the pores had tight constrictions and

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were very tortuous, slowing the net rate of molecular movement out of the matrix. Using approaches such as controlling polymer molecular weight or composition[13] or protein particle size and concentration,[14] the pore structures could be predicted and even tailor-made to achieve different release rates.

The area of research for slow release of peptides and proteins is beginning to have a clinical impact. In 1989, the U.S. Food and Drug Administration (FDA) approved the first system to slowly release a peptide. These are polymer microspheres that slowly release luteinizing hormone releasing hormone (LHRH) analogues, which are of about 1200 Da molecular weight.[15] This is the most widely used system for treating advanced prostate cancer, endometriosis, and precocious puberty. This molecule, if given orally or injected in unencapsulated form, is rapidly destroyed. However, when placed in a polymer matrix, release occurs for 4 months. Soon, a 1-month delivery system for a protein, human growth hormone,[16] should be available.

Increasing the Rate. The above studies involve using materials to deliver drugs at constant or decreasing release rates. However, increases in release rate might be useful in diseases like diabetes, for example. To examine this, we began developing magnetic polymeric composites. Our thought was to use an elastic material such as ethylene−vinyl acetate copolymer as the polymer matrix and add magnetic beads and powdered drug to it. Without an external magnetic field, drug is released by slow diffusion through pores as described above. However, when an oscillating magnetic field is activated, these pores are compressed, and more drug is released.[17] Eventually, by designing a triggering system in the form of a special wristwatch-like device that could be programmed or connected to a biosensor, release could be activated on demand.

To test this idea, an oscillating magnetic field was designed from two Plexiglas disks placed vertically on top of each other and separated by several inches. A motor was attached to the bottom disk. The top disk was stationary, and the bottom one, which contained magnets, rotated. When exposed to the oscillating magnetic field, the polymer−magnet composites displayed release rates up to 30 times higher than those without the magnetic field. Release rate could be controlled by magnetic field strength and frequency[18] (Figure 2).

To test this process *in vivo*, we designed a polymer system with a 2-year supply of insulin and a small magnet. These systems were implanted subcutaneously in diabetic rats, and the same rotating device was used. The rats were placed in small cages on the stationary top disk, while the bottom disk rotated, giving the rats 20-min exposures to the oscillating magnetic field. Blood sugars were lowered to a near-normal level, when the magnetic field was applied. Four control groups exposed showed no such effect.[19]

More recently, a microchip was designed to deliver drugs[20] (Figure 3). The microchip is made of silicon and contains multiple (up to 1000) drug reservoirs, each

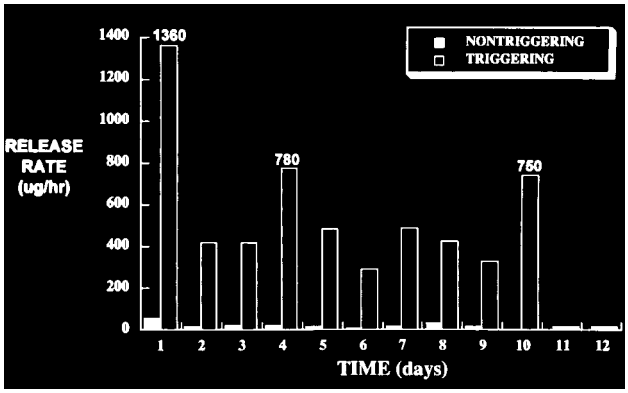

FIGURE 2. Release rates of a polymer−magnetic system releasing albumin in the absence and in the presence of an external magnetic field.

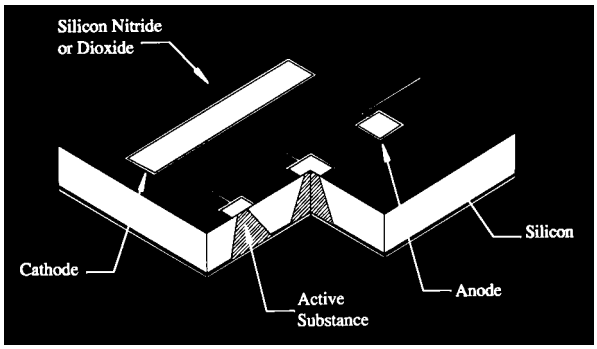

FIGURE 3. Cutaway section of a drug delivery microchip (prototype device).

covered with a thin gold film. The chip is made using photolithography, chemical vapor deposition, and reactive ion etching; the reservoirs are filled via inkjet printing or microinjection. By applying approximately 1 V selectively to any individual reservoir, the gold was dissolved electrochemically, thereby releasing entrapped drug. Proof of principle release studies have been conducted with single or multiple model drugs. This microchip can potentially be regulated by remote control, or eventually a biosensor might be attached to create a smart delivery system.

The area of pulsatile drug delivery has attracted the attention of a number of laboratories. A variety of triggering mechanisms including pH, temperature, and specific molecules are under study (for examples, see refs 21−25).

New Biomaterials. In most cases, materials used in medicine were not designed for medical purposes. Usually, these materials were commodity objects utilized by physicians as they attempted to solve medical problems. While this approach has enabled progress, it has also created problems.[26] To develop improved materials, we and others have begun asking what is desirable in biomaterials from an engineering, chemical, and biological standpoint, and then synthesizing these materials. One example involves designing better materials for drug delivery.

We approached this as an engineering design problem, asking how one would design a polymer to degrade optimally for controlled drug delivery. Ideally, one would

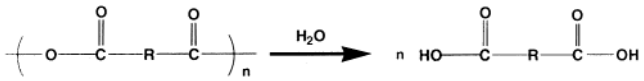

FIGURE 4. Polyanhydrides and their breakdown products.

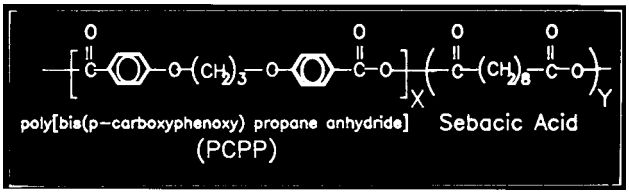

FIGURE 5. Structure of the polyanhydride composed of carboxyphenoxypropane and sebacic acid.

like to have the property of surface erosion, analogous to how a soap bar dissolves, as opposed to bulk erosion, whereby a bar of soap, over time, appears like a sponge.[26] To design such a polymer, we asked various design questions. First, what should cause polymer degradation—enzymes or water? Water should be superior from a reproducibility standpoint because enzyme levels may vary as the cellular response around the polymer changes over time and from person to person. In contrast, everyone has excess water. The next question is, if water is the reactant, what should be the nature of the monomers? We decided the monomers should be hydrophobic to exclude water from the polymer matrix interior, theoretically limiting erosion to the matrix surface. We next asked how to make polymers dissolve at a fast enough rate. One approach would be to connect the monomers with highly water reactive bonds. We hypothesized that anhydride bonds would serve this purpose. Thus, one way to cause surface erosion would be to synthesize hydrophobic polyanhydrides (Figure 4). We next selected monomers hypothesized to be nontoxic. In one example we used a hydrophobic monomer, carboxyphenoxypropane (CPP), and a slightly less hydrophobic monomer, sebacic acid (SA) (Figure 5). To make these polymers, numerous polymer chemistry challenges had to be addressed, such as synthesis of high-molecular-weight polyanhydrides. However, by optimizing the time and temperature of the polymerization reaction and using appropriate catalysts, polyanhydrides with molecular weights up to 250 000 were synthesized.[27] From an erosion standpoint, using 100% CPP and no SA, making a very hydrophobic polyanhydride, about 8% of the polymer matrix dissolves in 14 weeks. A millimeter-thick disk will take about 3 or 4 years to dissolve. However, if we add as a co-monomer 15% sebacic acid, the polymer matrix dissolves faster; with 79% sebacic acid, the polymer matrix completely dissolved in 2 weeks (Figure 6). Thus, by specifying the monomer ratio, these polymers can be made to last for essentially whatever length of time is desired. If drug is uniformly distributed inside such a polymer matrix, generally at 10 wt % or less, it will be released at the same rate at which the polymer dissolves.[28]

To apply this technology to clinical problems, we began developing a better way to treat glioblastoma multiforme, a uniformly fatal form of brain cancer. Untreated, the median life expectancy is 4 weeks. Surgery changes that

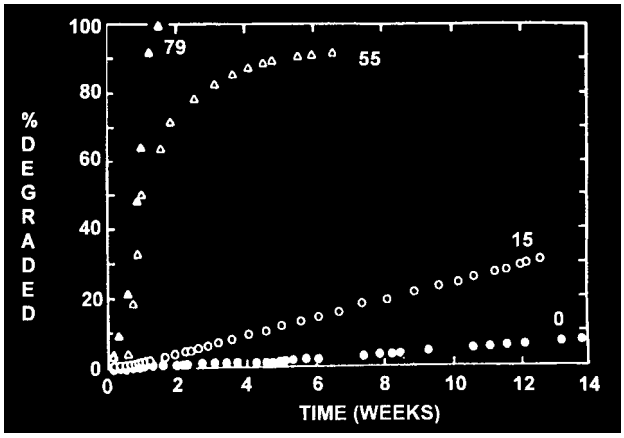

FIGURE 6. Erosion rates of 1-mm-thick disks of carboxyphenoxypropane–sebacic acid polyanhydride copolymers with different percentages of sebacic acid.

to 16 weeks; surgery and radiation to 40 weeks; and surgery, radiation, and chemotherapy to 50 weeks. Furthermore, patients often are subjected to an undesirable quality of life in which they may experience repeated surgeries and are given one of the most toxic anticancer drugs, carmustine (BCNU). Typically taken intravenously, the drug is transported throughout the body, causing damage to the liver, kidneys, and spleen. Our objective was to develop a localized therapy that might provide a new way to treat patients without these side effects.

Our goal was to enable the surgeons to operate, removing as much tumor as possible, and then before closing the patient, adding polymer–BCNU disks. We desired a polymer that was biodegradable so that it would not remain in the brain over long time periods, and one that was surface eroding because the drug is toxic. From animal studies we wanted the polymer to last 4 weeks. Based on the principles described earlier, we synthesized such polymers. We placed BCNU—which normally has a half-life of 12 min—in the polymer matrix. Once in the polymer matrix, the drug lasts as long as the polymer does because it is protected from degradation. The surgeons inserted this delivery system in the brain where it was needed and did not cause damage to various organs.

Initially, we and our collaborators experimented with the polymer in *in vitro* assays using mammalian cells to examine toxicity.[29] Then safety studies were done in rats,[30] rabbits,[31] and monkeys.[32] In all cases the polymers were safe. Then human clinical trials began. In these studies, the disks, which are about the size of dimes, were placed into the brains of patients who were being operated on for cancer.

The initial human studies examined safety.[33] Blood tests, MRI scans, and CAT scans showed no toxicity. Subsequent studies addressed efficacy.[34] One study showed that after a year, 63% of patients were alive in the treated group and 19% in the control group. After 2 years, 31% of patients were alive in the treated group and 6% in the controls.[35] The FDA recently approved this treatment. This marks the first time in over 20 years that a new brain cancer treatment was approved and the first time ever that

controlled release polymer-based chemotherapy was approved.[36]

Ultrasound Enhanced Delivery Drugs. Transdermal delivery has grown in the past 20 years from the point where no transdermal products existed to a $2 billion a year business.[37] However, the successful use of transdermal delivery has been restricted to low-molecular-weight, highly lipophilic drugs such as nicotine or nitroglycerin.[38] To extend transdermal delivery beyond such drugs, transport enhancement is needed. Investigators have studied chemical, electrical, and ultrasound approaches; we discuss here our efforts in the latter area.

In early studies, we found ultrasound could enhance molecular transport through polymer matrices;[39] we wondered whether transfer of molecules through human skin might also be aided by ultrasound. We found using mannitol, inulin, and physostigmine on rats and guinea pigs that ultrasound caused enhanced drug transport through skin (termed "sonophoresis").[40] Other laboratories subsequently studied this approach for different drugs but received differing results.[41−44] The extent of transport was drug dependent, but no fundamental understanding of the physical chemical nature of the drugs or the optimal ultrasound conditions to achieve transdermal transport existed.

To address this issue, we began studies to understand the mechanism for the enhanced movement of molecules through skin. The major resistance to transdermal transport is the outermost skin layer, the stratum corneum (which is dead skin), which is composed of lipids and keratinocytes. Human cadaver skin was used as a model system. We examined four possible release mechanisms for ultrasound-assisted transdermal transport: (1) temperature, (2) convection through hair follicles, (3) mechanical oscillation of lipid bilayers, and (4) cavitation effects on bilayers. Measurements showed that there was no temperature change due to sonophoresis. The role of convection was eliminated by studying charged drugs and finding no enhancement. Sonophoresis was found to be inversely proportional to ultrasound frequency, suggesting that mechanical effects were not responsible. If cavitation were the mechanism, we hypothesized there should be a threshold of activity at a certain frequency. We found that above 2.5 MHz—the cavitation threshold under the conditions studied—enhancement disappeared. At 1 MHz, a 13-fold increased flux was observed for a model drug, estradiol, while at 3 MHz there was no effect. A second set of experiments, in which gases were evacuated from skin, also eliminated the effect, providing additional support for the cavitation mechanism. Finally, placing the skin under high pressure (30 atm) reduced the effect. All these studies suggested that the mechanism consists of disordering of the skin's lipid bilayers by cavitation. A simple model was then developed:

Enhancement rate is equal to $f[D^1/D^b]$, where D^1 is the diffusivity through disordered lipids, D^b is the diffusivity through normal lipid bilayers, and f is the fraction of bilayers disordered. These terms could be independently estimated or measured. This equation was used to predict the transdermal transport of different drugs[45] (Figure 7).

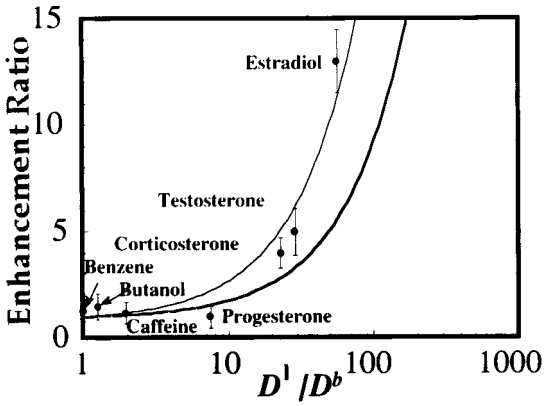

FIGURE 7. Model predictions and experimental observations of the enhancement rate, which is the rate of fluxes in the presence and in the absence of ultrasound, as a function of drug diffusion coefficients.

Since cavitation was inversely proportional to ultrasound frequency, we next reduced the frequency to 20 kHz to examine the enhancement for different drugs using human cadaver skin. For some drugs, over a 1000-fold enhancement was observed.[46] Furthermore, many compounds, even proteins, showed enhanced transdermal flux at lower frequency. Some examples include insulin, interferon, and erythropoeitin. The levels of insulin and interferon delivered by sonophoresis were in the therapeutic range when tested on human cadaver skin.[47] Further testing using diabetic rats showed that insulin delivered this way lowered blood sugar levels to normal within 30 min.[47] Histology established that the treatment did not damage skin, and water permeability experiments demonstrated that the skin resumed normal penetration within 2 h.[46] Initial tests on patients showed no negative effects.[48]

We also examined whether diagnostic samples could be *removed* by this technique. By positioning a reservoir between a rat's skin and an ultrasound transducer, interstitial fluid was extracted and theophylline, glucose, cholesterol, urea, and calcium could be measured. Studies using the Clark grid test showed that this was an acceptable measurement route for examining sugar levels in human diabetics.[48] In studies to date, we used an ordinary laboratory sonicator. Future studies will examine the possibility of designing a small portable sonicator.

Porous Aerosols for Inhalation Therapy. Aerosols are widely used for lung delivery.[49−51] However, conventional aerosol treatment, e.g. for asthmatics, is inefficient. Aerosol particles are small, generally on the order of 3−4 μm, and generally have a density of 1 g/cm^3. Because they are so small and therefore have such a high surface area, they tend to aggregate, making aerosolization inefficient. In many cases, only 5−10% of the medication is utilized. To address this, novel aerosolizers are being designed. This has led to improvements of up to about 10−20% of the medication being utilized. In addition to low efficiency, a patient has to frequently take aerosols because, once in

the lung, the bulk of the aerosolized drug is destroyed by macrophages, via phagocytosis.

Rather than focus on aerosolizer design, we redesigned the aerosols themselves. Conventional aerosols—either liquids or dry powders—are not only small, but they generally have a density of approximately 1 g/cm^3. We hypothesized that larger but lower density aerosols could be an improvement. The aerodynamics of such a particle should be better, so the drugs could be transported deeper into the lung. There should be less aggregation because of less total aerosol surface area. Furthermore, the macrophages should have a more difficult time phagocytosizing these larger particles. The particles were produced by spray-drying or emulsion techniques. Depending on the drug and conditions used, the large porous particles improved drug bioavailability from 5−10% to 60−95%. *In vivo* studies in rats showed that insulin bioavailability was 86%, and blood sugar levels remained low for 4 days from a single inhalation.[52] In preliminary human studies, using estradiol and albuterol as model drugs, a single inhalation was shown to be safe and effective for up to 3 days.

Tissue Engineering

Tissue engineering represents another important area of biomaterials. The possibility of creating new tissues is important because organ transplants are severely hampered by donor shortages. For example, approximately 30 000 patients in the United States die annually of liver failure, with less than 3000 transplants available. Because of this tragedy, scientists have been researching various strategies to solve the donor shortage problem. These include xenotransplants and cell transplants.[53,54]

We proposed a cell transplantation strategy called neomorphogenesis. Our concept was that cells are inherently intelligent. When isolated dissociated cells are injected randomly *in vivo*, they generally do not form tissues, but when they are placed close enough together, they tend to form tissue structures. For example, isolated mammary epithelial cells, placed close together, even outside the body, form structures that make milk.[55] Endothelial cells placed close together *in vitro* form capillary tubes.[56]

Our objective was to create polymer fibers close enough together in a scaffold that allowed the cells to (1) configure themselves together in desired structures and (2) be nourished, proliferate, and function. We also wanted the polymers to be biodegradable so that they could not cause potential problems years later. Thus, we wanted to engineer a new organ *in situ* by placing functional dissociated cells onto biodegradable polymers and culture them outside the body. When placed into the host, that is, the patient, vascularization (if needed) and tissue growth would theoretically occur[57] (Figure 8).

We considered the ideal polymer requirements were biodegradability, biocompatibility, and proper physical attributes so that the scaffold could be made into whatever form desired. Depending on the tissues desired, we often used lactic−glycolic acid copolymer as a scaffold on the

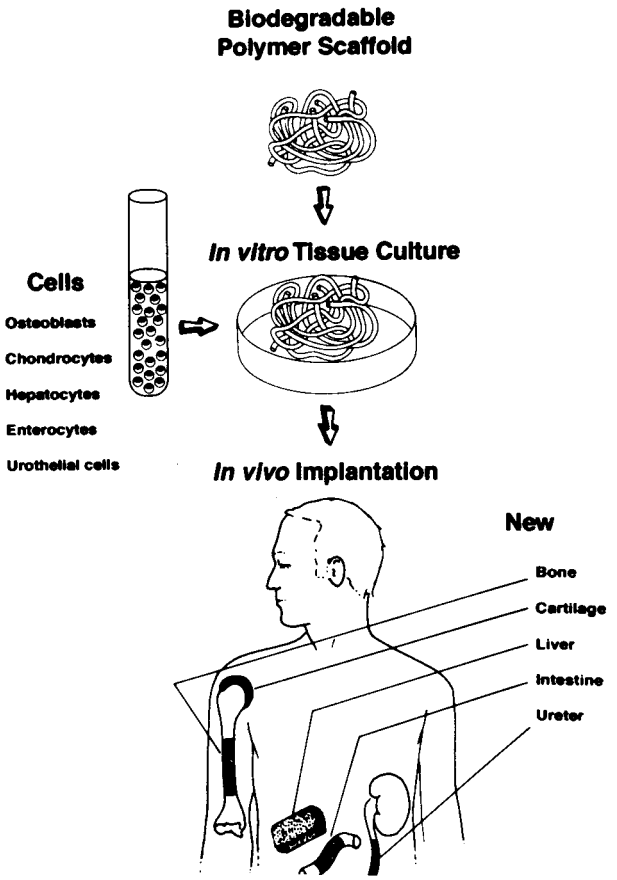

FIGURE 8. Schematic diagram of tissue engineering process.

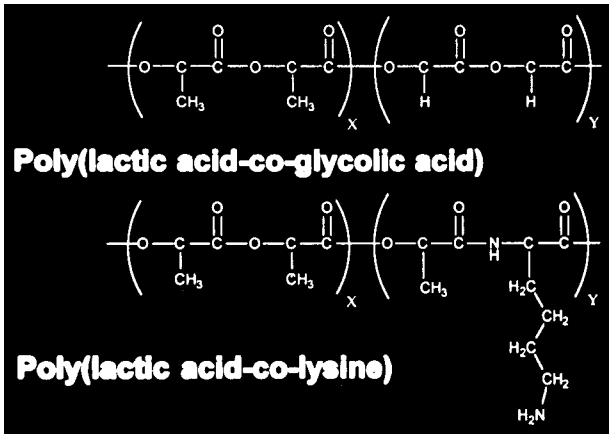

FIGURE 9. Comparison of the structures of some polymers used in tissue engineering.

basis of its safety and mechanical properties.[58] However, in some cases, copolymers of lactic acid and glycolic acid have too little affinity for certain cells. Thus we synthesized polymers with the ability to attach ligands such as small peptides, which possess specific cell adhesion sites. To achieve this, we synthesized a copolymer of lactic acid and lysine, to have pendant amino groups on the polymeric backbone (Figure 9). By adding a carbodiimide in a DMSO and dichloromethane cosolvent, appropriate peptides could be dissolved and then attached via the amino groups in lysine, giving a polymer to which amino acid sequences such as arginine−glycine−asparagine

(RGD) could be attached.[59] Such sequences can help guide cell behavior.[60]

The polymers must then be manufactured into scaffolds that have a very large surface area per unit volume to get the desired cell density. Techniques such as incorporating water-soluble salts into polymer matrices followed by leaching can lead to foams.[61] Several approaches were developed to create fibrous structures in specific anatomic shapes.[62,63]

Examples of Engineered Tissues. We have attempted to engineer a variety of tissues. One example is cartilage. Current approaches involve transplants which have donor limitation problems as well as difficulties in shaping the tissue (because of cartilage's mechanical properties), or prostheses which suffer from inflammation or loosening at the host−device interface. Our objective was to engineer cartilage from polymers and chondrocytes.

In theory, we proposed to remove a small sample of cartilage cells from a patient by minimally invasive procedures and grow the cells on the polymer scaffold, creating "explants". The explants are then placed in an animal, where they occupy the precise dimensions of the polymer scaffold onto which the cells were originally placed.

To examine a plastic surgery application, we extracted cartilage cells from an animal and multiplied them in a bioreactor.[63] This helped address the donor shortage problem. By making a mold of a polymer in the specific shape desired and then placing cartilage cells on the polymer matrix, the polymer cell scaffold was grafted onto mice and rabbits in that specific anatomic shape.[64]

Recently, clinical trials began. The first patient was a 12-year-old boy. He had a deformed chest, with no ribcage and hence no protection to his heart if he were to be struck in the chest. Right over the heart, cartilage cells were implanted on a polymer scaffold that was created in the shape of a ribcage. This created new cartilage and offered protection of the heart.

In another case, modified polymer tubes were seeded with urothelial cells and generated a replacement for an incomplete urethra.[66] Urinary valves have also been bioengineered to replace damaged valves that allow urine backflow, by local injection of a cell−polymer matrix. The reverse problem, incontinence, which affects 1−3 million people, 85% women, can be similarly treated by augmenting the weakened urologic tissue.[67]

To engineer blood vessels, 3-mm-diameter polymer tubes were cultured with two types of cells—smooth muscle and endothelial cells. By connecting the tubes to a pump, nutrient medium flowed through them *in vitro* in a bioreactor. To do this successfully, it was important to use pulsatile radial stress in the bioreactor, mimicking a beating heart. This enabled the cells to produce more collagen and hence be stronger than vessels grown in static culture. The synthesized vessels were similar to normal blood vessels: 50% collagen, high rupture strength, and ability to be sutured. When transplanted into pigs, the vessels retained the ability to allow blood flow for 1 month—the study duration.[68] To make replacement heart or pulmonary valves, two cell types, endothelial cells and fibroblasts, were also employed. After 6 months, the synthetic valves were functional in lambs.[69]

In another example, an electrically conducting polymer−polypyrrole (PP) was synthesized as a substrate for nerve regrowth. It was used first in *in vitro* studies in which PC-12 (a nerve-like cell) or Schwann cells were grown. Interestingly, while minimal growth or attachment of such cells was observed on polylactic acid or polylactic−glycolic acid (PLGA) films, neurite extension was observed on PP films, and neurite outgrowth doubled when an electric stimulus (100 mV for 2 h) was passed through the film. Initial *in vivo* studies showed these polymers were biocompatible.[70] When used as a nerve guide, in a sciatic nerve regeneration rat model, they display a density and topology of nerve fibers similar to those of native nerve.

In a final example, this same general approach has been used to create skin for patients with burns or skin ulcers.[71] In this case, neonatal dermal fibroblasts are placed on PLGA scaffolds and grown into sheets to create skin.

Conclusion

The above examples review of some of our research efforts in biomaterials. There are numerous challenges ahead in such research. Many laboratories are conducting studies in these areas. With chemists and materials scientists working together with clinicians and engineers, new solutions to important medical problems will hopefully be found.

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