Journal of Cellular Biochemistry 45:340–345 (1991)

Biocompatible Controlled Release Polymers for Delivery of Polypeptides and Growth Factors

Robert Langer and Marsha Moses

Department of Chemical Engineering and Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 (R.L.); Department of Surgery, The Children's Hospital Medical Center, Boston, Massachusetts 02115 (R.L., M.M.)

Abstract The development of biocompatible, controlled release systems for macromolecules has provided the opportunity for researchers and clinicians to target and deliver, on site, biologically active factors. This advance has also facilitated the purification and characterization of a number of important biomolecules. These systems include controlled release delivery systems which release proteins through porous polymer matrices, degradable polymeric delivery systems, and modulated polymer release systems. These areas of research will be reviewed with regards to their design, release kinetics, and biocompatibilities. The utilization of these systems to release such biologically important polypeptides as growth factors (e.g., fibroblast growth factor, epidermal growth factor, transforming growth factor-B) as well as a number of important inhibitory factors (e.g., nitrosoureas, angiogenesis inhibitors) in both in vivo and in vitro studies will be discussed.

Key words: controlled release, sustained release, drug delivery, proteins, polymer

Controlled release systems are generally composed of polymers and release their contents continuously over long time periods—from days to years. Over the past decade there has been increasing attention devoted to the development of controlled release systems for drugs, pesticides, nutrients, agricultural products, and fragrances. However, nearly all of the systems that have been developed have not been capable of slowly releasing drugs of large molecular weight (MW > 600) such as proteins, e.g., growth factors. In fact, up until 1976 it was a fairly common conception in the field of controlled release that effective systems could not be developed for macromolecules [1]. However, after several years of effort we discovered an approach that permitted the continuous release of biologically active macromolecules as large as 2,000,000 daltons from normally impermeable, yet biocompatible, polymers for over 100 days [2]. In this paper we review three areas of our research: 1) systems that release large molecules through porous polymer matrices, 2) novel degradable polymeric delivery systems, and 3) pulsatile controlled release polymer systems. Finally, we discuss studies on using delivery systems to release growth factors and other tissue inducing factors.

Received September 20, 1990; accepted November 16, 1990.

CONTROLLED DELIVERY SYSTEMS THAT RELEASE PROTEINS BY DIFFUSION THROUGH PORES

While short term release of proteins can be achieved using gels such as polyacrylamide or highly porous membranes such as millipore filters, diffusion is generally too rapid to be of value. The first approach that permitted controlled release of large molecules from biocompatible polymers was based on the discovery that mixtures of solid proteins and hydrophobic polymers could release the proteins for hundreds of days. The earliest methods to prepare these controlled release systems involved dissolving the polymer in an appropriate solvent and adding the macromolecule (protein) in powder form [2]. The resulting mixture can be cast in a mold and dried. When the pellets are placed in water, they release the molecules trapped within the polymer matrix. Subsequently, methods of forming these polymer macromolecule delivery systems which require no solvent at all, enable microspheres to be formed, or permit micro-

gram quantities to be released were developed [for review, see 3].

A number of polymer systems were examined both for tissue biocompatibility and release kinetics. Polymers that function best include nondegradable ethylene-vinyl acetate copolymers or degradable lactic/glycolic acid copolymers. Certain hydrogels like polyhydroxyethylmethacrylate or polyvinylalcohol also worked effectively, but released proteins for shorter time periods. Polysaccharides and polynucleotides can also be released from these polymers [2].

The release mechanism in these systems involves movement of the polypeptide through a complex porous path in the polymer matrix. If the polymer erodes, this will increase the porosity which will also affect the release rate. There are a number of design parameters that can be used to control release rates; these include protein particle size and loading, protein solubility and molecular weight, polymer composition and molecular weight, and the dimensions and shape of the matrix. Recently, several controlled release systems for delivering peptides have been introduced clinically. These systems are composed of lactic/glycolic acid copolymer and leuprolide acetate in the form of injectable rods or microspheres. These systems generally last 30 days and are being used for the treatment of prostate cancer. Other lactic/glycolic acid copolymer systems for releasing similar drugs are also under evaluation for treating endometriosis and other conditions.

DEGRADABLE POLYMERS

Degradable controlled release systems have an advantage over other systems in obviating the need to surgically remove the drug depleted device. In many cases, however, the release is augmented by diffusion through the matrix, rendering the process difficult to control—particularly if the matrix is hydrophilic and thereby absorbs water, promoting degradation in the interior of the matrix. To maximize control over the release process, it is desirable to have a polymeric system which degrades only from the surface and deters the permeation of the drug molecules. Achieving such a heterogeneous degradation requires the rate of hydrolytic degradation on the polymer matrix surface to be much faster than the rate of water penetration into the bulk. With this in mind, we proposed that an ideal polymer would have a hydrophobic backbone, but with a water labile linkage. Many classes of polymers, including polyesters, polyamides, polyurethanes, polyorthoesters, and polyacrylonitriles, have been studied for controlled delivery applications, but only polyorthoesters erode from surface and then only if additives were included in the matrix. In designing a biodegradable system that would erode in a controlled heterogeneous manner without requiring any additives, we have suggested that, due to the high lability of the anhydride linkage, polyanhydrides may be a promising candidate.

In a number of studies, a model polyanhydride—a copolymer of carbophenoxy propane (CPP) and sebacic acid (SD)—was used. We discovered that 1 mm thick discs of polyCPP will completely erode in over 3 years in aqueous media. The degradation rates can be enhanced by copolymerization with sebacic acid (SA). An increase of 800 times was observed when the sebacic acid concentration reached 80%. By altering the CPP/SA ratio, nearly any degradation rate between 1 day and 3 years can be achieved [4].

The release behavior of drugs incorporated into these polymers depends on both the polymer and the formulation procedure (solvent casting, compression molding, injection molding). Release of proteins can be achieved using this approach, but it is important to use formulation methods that involve minimal heating [5].

While no polyanhydride-protein combination has yet been used clinically, polyanhydrides (releasing smaller molecules) have already begun to be used in medicine. In 1985, we began a collaboration with a neurosurgery group headed by Dr. Henry Brem at Johns Hopkins to explore the possibility of implanting polyanhydride discs containing the nitrosourea, BCNU, for brain cancer following surgery. Surface erosion would be critical in the use of such drugs, for if bulk erosion occurred uncontrolled amounts of this potentially toxic substance could be released during breakup of the matrix. The Hopkins group extended our safety studies and received Institutional Review Board (IRB) approval to conduct human clinical trials with polyanhydrides (Duke, Northwestern, UCLA, and U. Alabama also received IRB approval for this purpose). In 1987, the FDA approved these polyanhydrides for human clinical trials. Safety studies have shown these polymers to be nontoxic and patient life time has been extended

beyond conventional drug treatment. In 1989, phase 3 clinical trials in 32 U.S. and Canadian hospitals were initiated. At this writing, over 100 patients have been treated with the polyanhydride-BCNU combination [5].

MODULATED RELEASE SYSTEMS

We have also developed several polymeric systems capable of delivering drugs at increased rates on demand. The first system consists of drug powder dispersed within a polymeric matrix (generally ethylene vinyl acetate copolymer, EVAc) together with magnetic beads. Release rates are controlled by an oscillating external magnetic field, which is generated by a device that rotates permanent magnets beneath the vials. By placing small plastic cages containing animals on the top of the device, it can also be used for in vivo studies. Polymer matrices containing drug and magnets can release up to 30 times more drug when exposed to the magnetic field compared to baseline release, and release rates return to normal when the magnetic field is discontinued. The response time to peak release rate is nearly immediate. The magnetically controlled implant does not cause inflammation in vivo. This was confirmed by the lack of edema, cellular infiltrate, or neovascularization as judged by gross and histologic examination in animals. A variety of proteins including insulin and albumin have been released using this magnetic approach [6,7].

We also discovered that ultrasound could affect the release of substances from polymers. The ultrasound system has a potential advantage over many other systems in that no additional substance (e.g., magnetic bead) is required in the polymeric matrix. Furthermore, in the case of ultrasound the polymer can be injected since it can be made into microspheres, and since it can be erodible there is no need for surgical removal. The application of ultrasound in humans, both for diagnostic and therapeutic purposes, has been extensively studied and is considered a safe practice.

Enhanced (up to 20 times baseline) polymer erosion and drug release were observed when bioerodible samples were exposed to ultrasound. The system's response to the ultrasonic triggering was also rapid (within 2 min) and reversible. This approach has been shown to be effective *both in vitro and in vivo* [8].

We have also developed an approach for feedback control of polypeptides incorporated within polymeric drug delivery systems. This approach is based on the observation that changes in pH can cause dramatic shifts in the solubility of polypeptide drugs; solubility is one of the prime determinants of release rate in any diffusion, dissolution, or osmotic controlled release system. The system components involve an external trigger molecule and a polymer-bound enzyme that, in the presence of the trigger molecule, will cause acid or base to form. To test this concept, we used insulin as a drug and diabetic rats as the animal model. We chose to adapt an ethylene-vinyl acetate (EVAc) polymeric insulin delivery system capable of treating diabetic rats for over 100 days. To establish feedback we utilized the fact that insulin solubility is pH dependent and that, in the presence of glucose oxidase, glucose is converted to gluconic acid. Thus, when this enzyme is incorporated within a controlled release polymer matrix, external glucose should theoretically reduce the pH in the polymer microenvironment. Since the isoelectric point of insulin is 5.3, when the polymer is exposed to the physiological pH of 7.4 a decrease in insulin solubility and release rate is expected. This undesired effect is overcome by using a modified insulin which contains more basic groups and thus has a higher isoelectric point. Tri-lysyl insulin with an isoelectric point of 7.4 was synthesized for this purpose. The feasibility of this enzyme mediated feedback mechanism was investigated by three sets of experiments: 1) the effect of glucose on the pH in the microenvironment of the polymer, 2) the effect of glucose on insulin release in vitro, and 3) the effect of glucose on insulin release in vivo. These experiments demonstrated that changes in external glucose altered the local pH inside the polymer matrix and, in turn, enhanced insulin release rates both in vitro and in vivo [9].

CONTROLLED RELEASE OF GROWTH FACTORS

Controlled release systems are particularly suited to the delivery of bioactive growth factors which are available in microgram or smaller amounts. As the foundation for controlled release of polypeptides became established, these techniques began to be applied by scientists to release different factors. One such report was the work of Gospodarowicz and coworkers [10] who employed the porous ethylene-vinyl acetate copolymer (EVAc) system [2] to provide sustained release of fibroblast growth factor (FGF)

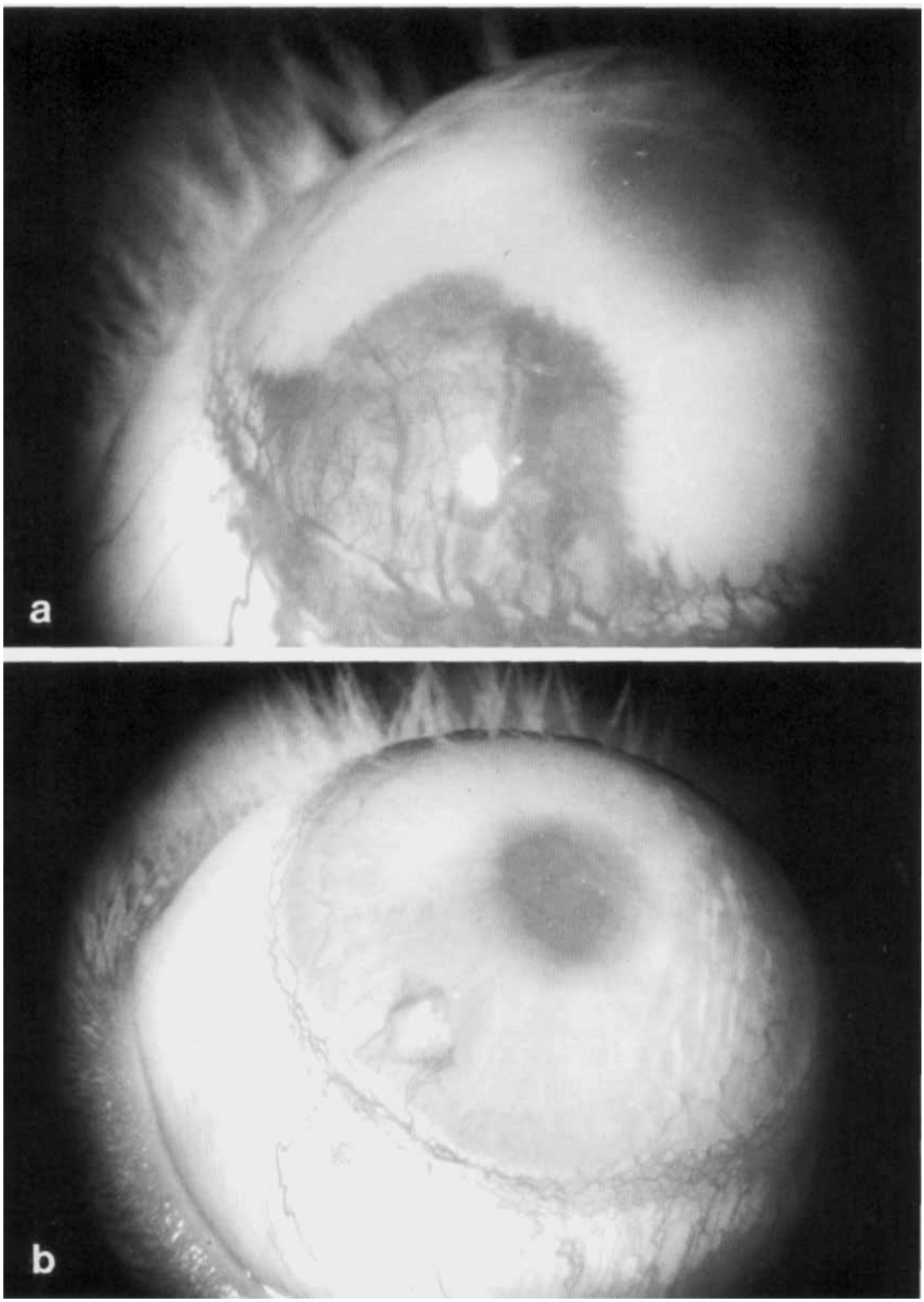

Fig. 1. Inhibition of tumor-derived angiogenesis by a cartilage-derived angiogenesis inhibitor in the rabbit corneal pocket assay. **a:** Control eye implanted with empty EVAc polymer pellet juxtaposed between limbus of eye and V2 carcinoma implant. **b:** Test eye implanted with EVAc polymer pellet impregnated with cartilage-derived angiogenesis inhibitor juxtaposed between limbus of eye and V2 carcinoma implant.

or epidermal growth factor (EGF); using this approach, these investigators showed these factors induced neovascularization in the rabbit cornea [10]. Polverini and co-workers [11] used a similar approach (in this case using polyhydroxyethylmethacrylate) to study the in vivo effects of a macrophage induced growth factor; at Johns Hopkins, the EVAc systems have been used in a number of studies as an integral part of in vivo bioassays to follow the purification of certain growth factors [12] and inhibitors [13] in the eye. These controlled release polymers (EVAc) have also been used in bioassays to aid in the isolation of factors from wound fluid [14], in developmental studies [15], to release human follicular fluid [16], to release placental factors [17], and to release tumor inhibitors [18] including tissue-derived inhibitors of tumor-induced neovascularization (Fig. 1) [19] and other inhibitors of neovascularization [20]. Other applications include the release of tumor extracts [21], the release of limb regenerating factors [22], the release of growth factors directly into cell culture systems [23], the in vivo release of endotoxins [20], and the release of transforming growth factor (TGF-β) [24].

The polyanhydride systems have also been used to release growth factors. For example, Lucas et al. have released osteogenic proteins from these polymers and used them to produce ectopic cartilage or bone in animal models [25]. Other studies have shown that these polymers can deliver angiogenesis inhibitors using a rabbit eye model [26].

CONCLUDING REMARKS

Controlled release polymers may offer a number of potential advantages when compared to present methods of administering low molecular weight drugs. While all of these potential advantages are relevant for macromolecules, one that is particularly important is that in the absence of some protective vehicle, almost all macromolecules are degraded relatively quickly in vivo and, thus, may be ideal candidates for controlled release (in contrast to small molecules many of which are long-lived [e.g., digoxin]). In addition, development of long-term controlled delivery systems for large molecules may eventually be more important than for small molecules because the option of a series of oral doses over time is often not possible (because large molecules are often degraded by enzymes or poorly absorbed when taken orally). In fact, for molecules which have very short lives (consider certain growth hormones), there may be no delivery system alternative (other than many, many shots, frequent administration through passages like the nose or rectum (if the molecule is small enough), or a bulky external pump) to a controlled release system.

Finally, two trends in pharmaceutical research may make controlled release delivery systems for macromolecules even more important in the future. The first of these is the possibility that naturally occurring macromolecular substances produced by the body (e.g., endorphins, enkephalins, luteinizing hormone releasing hormone, interferon) may be used as drugs. The second of these is genetic engineering which can now, for the first time, permit the development of sizeable quantities of macromolecular drugs such as human or animal growth hormones. These two trends may, in time, permit a whole new arsenal of macromolecular drugs to be developed which are not currently available. However, effective delivery systems for such substances have never been designed, and we believe that the delivery systems discussed in this paper will have an ever increasing impact—first on the testing, and second on the eventual use of these molecules.

REFERENCES

1. Stannett VT, Koros WJ, Paul DR, Lonsdale HK, Baker RW: Adv Polym Sci 32:71, 1979.
2. Langer R, Folkman, J: Nature 263:797, 1976.
3. Langer R, Brown L, Edelman E: Drug and Enzyme Targeting, Meth in Enzym 112:399, 1985.
4. Leong KW, Brott BC, Langer R: J Biomed Mat Res 19:941, 1985.
5. Chasin M, Domb A, Ron E, Mathiowitz E, Leong K, Laurencin C, Brem H, Grossman B, Langer R: In Langer R, Chasin M (eds): "Biodegradable Polymers for Drug Delivery." New York: Marcel Dekker Inc., 1990, pp 43–70.
6. Hsieh DST, Langer R, Folkman J: Proc Natl Acad Sci 78:1863, 1981.
7. Edelman E, Brown L, Langer R: J Biomed Mat Sci 21:339, 1987.
8. Kost J, Leong K, Langer R: Proc Nat Acad Sci 86:7663, 1989.
9. Ghodsian FF, Brown L, Mathiowitz E, Brandenburg D, Langer R: Proc Nat Acad Sci 85:2403, 1988.
10. Gospodarowicz P, Bialecki H, Thakral TK: Exp Eye Res 28:501, 1979.
11. Polverini P, Cotran R, Gimbrone M, Unanue E: Nature 269:904, 1977.
12. Glaser BM, D'Amore P: J Cell Biol 84:298, 1980.

13. Lutty GA, Thompson DC, Gallup JY, Mello RJ, Patz A, Fenseleau A: Inv Opthal 24:52, 1983.
14. Banda MJ, Knighton DR, Hunt TK, Werb Z: Proc Natl Acad Sci 79:773, 1982.
15. Silberstein GB, Daniel CQ: Developmental Biol 93:272, 1982.
16. Frederic JL, Shimanuki T, Dizerega GS: Science 224: 389, 1984.
17. Burgos H: Eur J Clin Inv 13:289, 1983.
18. Gross J, Azizkhan RS, Biswas C, Bruns R, Hsieh D, Folkman J: Proc Natl Acad Sci 78:1176, 1981.
19. Moses MA, Sudhalter J, Langer R: Science 248:1408, 1990.
20. Folkman J, Weisz PB, Jouillié MM, Li WW, Ewing WQ: Science 243:1490, 1989.
21. Brent DA, Parith I, Cuatrescasas P: Science 236:843, 1987.
22. Pliskin ME: Transplantation 29:255, 1980.
23. Murray JB, Brown L, Langer R, Klagsbrun M: In Vitro 19:748, 1983.
24. Silberstein, GB, Daniel CW: Science 237:291, 1987.
25. Lucas PA, Laurencin C, Syftestad GT, Domb A, Goldberg VM, Caplan AI, Langer R: J Biomed Mat Res 24:901, 1990.
26. Langer R, Lund D, Leong K, Folkman J: J Controlled Release 2:331, 1985.