ELSEVIER

Reactive Polymers 25 (1995) 139–147

Reactive polymers

Photopolymerized hydrogel materials for drug delivery applications

Jennifer L. West [a], Jeffrey A. Hubbell [a,b,*]

[a] *Department of Biomedical Engineering, University of Texas, Austin, TX, USA*
[b] *Department of Chemical Engineering, University of Texas, Austin, TX, USA*

Accepted in revised form 21 November 1994

Abstract

A novel photopolymerized hydrogel material has been developed for use as a drug delivery vehicle for bioactive materials. The hydrogel precursor consists of polyethylene glycol copolymerized with an α-hydroxy acid and with acrylate termini at each end. The precursor is water-soluble and non-toxic. The precursor polymerization conditions are very mild, and polymerization can be carried out in direct contact with cells and tissues. The degradation rate and permeability of the hydrogel can be altered by changing the composition of the precursors, allowing use of this class of materials for a variety of applications. In vitro release of proteins and oligonucleotides is reported.

Keywords: Photopolymerized hydrogel; Drug delivery vehicle; Bioactive material; Water soluble; Non-toxic precursor

1. Introduction

Recent advances in biotechnology have resulted in the development of therapeutic proteins, peptides, and oligonucleotides, creating a need for suitable delivery vehicles for hydrophilic bioactive macromolecules. Matrices of hydrophobic polymers, such as ethylene–vinyl acetate copolymers and lactic acid–glycolic acid copolymers, containing powdered macromolecules, have been used for the sustained release of proteins, polysaccharides, and oligonucleotides [1], but it is sometimes difficult to homogeneously disperse hydrophilic materials within a hydrophobic polymer matrix, resulting in potentially unpredictable release profiles. Sustained systemic delivery of peptide drugs has been achieved with the use of polyethylene glycol derivatized, 'stealth' liposomes [2]. However, there still exists a need for a hydrophilic polymer which can be used for sustained release, locally or systemically, of bioactive macromolecules. In addition, the processing conditions used in the formation of the polymer matrix should be sufficiently mild to be carried out in the presence of biological materials: temperature and pH should be limited to near physiological ranges, organic solvents should be avoided, and chemical reactions which modify functional groups found on proteins should be minimized.

Our laboratory has developed a hydrogel material which may be suitable for delivery of proteins, peptides, and oligonucleotides [3]. The hydrogel is formed by photopolymerization of

* Corresponding author. Current address: California Institute of Technology, Division of Chemistry and Chemical Engineering 210-41, Pasadena, CA 91125, USA

SSDI 0923-1137(94)00096-4

an aqueous solution of a macromolecular precursor. Bioactive materials may be dissolved in the precursor solution to ensure homogeneous dispersion throughout the hydrogel matrix after photopolymerization. The precursor consists of a central polyethylene glycol (PEG) chain with oligomeric blocks of a hydrolyzable α-hydroxy acid, or other degradable moiety, on each side. The precursor is further capped at each end with a reactive acrylate unit to allow polymerization. Photoinitiation may be used to affect polymerization at low temperatures. The majority of the precursor is comprised of PEG, thus making the precursor highly water soluble. PEG is also noted for its excellent biocompatibility, since its highly hydrated, mobile, and non-ionic character render PEG relatively resistant to protein adsorption and cell deposition. The length of the PEG segment in the precursor determines the permeability of the hydrogel as well as its physical properties. The length and composition of the α-hydroxy acid segment regulate the degradation rate of the hydrogel. Gels made with glycolide degradable links degrade in days, lactide in weeks, and ϵ-caprolactone in months in vitro [3]. Degradation occurs via hydrolysis of the ester links in the oligomeric α-hydroxy acid segment and produces PEG, an α-hydroxy acid, and oligomers of acrylic acid, all of which present a low toxicological burden [3]. This versatile design of the precursor allows the synthesis of hydrogels appropriate for a wide variety of drug delivery applications. Release periods for proteins of vastly different sizes can be varied from hours to months, depending on the degradation rate and permeability of the releasing gel.

Photopolymerized PEG hydrogels can be implanted preformed or they may be formed by photopolymerizing the macromolecular precursor in situ. The photopolymerization process does not affect cell viability, even in cell layers in direct contact with the polymer [4]. The hydrogel has been utilized previously for cell encapsulation [4]. The hydrogel is intrinsically non-adherent to cells and tissues, but if formed in direct contact with a tissue, it will adhere strongly, presumably by interdigitation with the microscopic texture of the tissue. This technique makes it possible to localize the hydrogel at a specific site, thus allowing the use of smaller doses and possibly avoiding systemic side effects. The hydrogel remains adherent to the underlying tissue throughout the degradation process [5].

One hydrogel of this type, formed from an 8000-Da PEG chain with degradable lactic acid regions, has been used as a barrier material to prevent postsurgical adhesion formation in animal models [5–7]. Adhesion formation is a frequent surgical complication which may cause pain, bowel obstruction, and infertility. An aqueous solution of the precursor and photoinitiators can be applied to tissues that have been damaged by surgical manipulation and converted to a hydrogel by exposure to long wavelength ultraviolet light to form a conformal barrier over organs and tissues at risk of adhesion formation. This technique has been found to reduce adhesion formation by 87% in a rat model [5]. It may be possible to achieve greater adhesion reduction by locally releasing fibrinolytic agents, such as tissue plasminogen activator [8], from the hydrogel barrier.

This hydrogel material has also been used to prevent thrombosis and vessel narrowing following vascular injury [9]. A thin hydrogel barrier (<20 μm) can be formed on the inner wall of blood vessels by an interfacial photopolymerization technique, wherein the photoinitiator is first adsorbed to the vessel surface and then the vessel is filled with the hydrogel precursor and exposed to light [9]. The hydrogel barrier adheres firmly to the vessel wall during degradation. In studies evaluating response to balloon injury of the carotid artery in rabbits, application of the hydrogel barrier reduced thrombosis by approximately 97% and reduced intimal thickening, the cause of vessel narrowing, by approximately 80% [9]. In both of the cases where the hydrogel has been used as a mechanical barrier, prevention of postsurgical adhesions and inhibition of thrombosis and intimal thickening, incorporation of a pharmacological agent, such as

antisense oligonucleotides [10] or antibodies to platelet-derived growth factor [11], which would be locally released from the hydrogel barrier, could potentially yield even higher efficacy.

In the current study we report the initial characterization of the in vitro release of proteins and oligonucleotides from photopolymerized PEG hydrogels. The results shown display the versatility of this class of materials and suggest that they may be applicable for a wide range of drug delivery applications.

2. Materials and methods

Precursors were synthesized and characterized as described elsewhere [3]. Briefly, dihydroxy polyethylene glycol was reacted with D,L-lactide using stannous octoate as a catalyst. This polymer was then reacted with acryloyl chloride to add an acrylate unit at each end. Specifically, the following precursors were prepared: 10,000-Da PEG with 5 lactidyl units per end (10KL5), 10,000-Da PEG with no lactidyl units (10KDA), 8000-Da PEG with 5 lactidyl units per end (8KL5), 8000-Da PEG with 3 lactidyl units per end (8KL3), 6000-Da PEG with no lactidyl units (6KDA), and 400-Da PEG with no lactidyl units (0.4KDA). All of these precursors were acrylated at both termini. Precursors without lactidyl units formed non-degradable hydrogels. Precursors were stored under argon at 0°C until use. Precursor solutions were prepared in HEPES-buffered saline (pH 7.4, 10 mM) at a concentration of 23% w/v. A 600-mg/ml solution of the photoinitiator 2,2-dimethoxy,2-phenyl acetophenone (Aldrich) was prepared in *N*-vinyl pyrrolidone (Aldrich), and 1.5 μl of the photoinitiator solution was added to each 1 ml of the aqueous precursor solution for a final photoinitiator concentration of 900 ppm. Various proteins or oligonucleotides were added to the precursor solutions, as described below, and the precursor solutions were poured into disk-shaped molds and converted to hydrogel form by exposure to long wavelength ultraviolet light (70 mW/cm^2, Black-Ray) for 20 s. The hydrogel discs were incubated in HEPES-buffered saline at 37°C. Protein release was evaluated using the Pierce Protein Assay (Pierce, Rockford, IL). Oligonucleotide release was evaluated using UV spectroscopy (280 nm). All studies were performed in triplicate. Release profiles were constructed based on the amount of protein or oligonucleotide, or the enzymatic activity of protein, initially incorporated in the hydrogel samples.

Swelling of the hydrogel was evaluated using 10KL5 hydrogel disks. Disks were incubated in HEPES-buffered saline at 37° C. Disks were weighed at preset time intervals, and the percentage weight gain was computed.

Release of a protein with enzymatic activity was monitored using an activity assay to verify that protein activity was maintained following photopolymerization. The release of tissue plasminogen activator (tPA, MW = 68,000 Da, Genentech, South San Francisco, CA) from a 10KL5 hydrogel was examined. tPA was added to the precursor solution at a concentration of 1 mg/ml, and each hydrogel disk consisted of 0.25 ml of the precursor and had a diameter of 1 cm. Activity of tPA released was evaluated using a chromogenic substrate (S-2288, KABI Diagnostica, Stockholm, Sweden).

A series of proteins of increasing molecular weight was incorporated into 10KL5 hydrogel disks to evaluate the dependence of the release rate on the molecular weight of the entrapped drug. Insulin (MW = 6000 Da), lysozyme (MW = 14,300 Da), lactate dehydrogenase (MW = 36,500 Da), ovalbumin (MW = 45,000 Da), bovine serum albumin (BSA, MW = 66,000 Da), and immunoglobulin G (MW = 150,000 Da) were added to aliquots of a 10KL5 precursor solution at a concentration of 1 mg/ml, and each disk consisted of 0.25 ml precursor solution and had a diameter of 1 cm. All proteins were obtained from Sigma. In addition, BSA was added to solutions of the 8KL5 and 8KL3 precursors at a concentration of 1 mg/ml using 0.25 ml per hydrogel disk. This experiment was performed to determine the effect of slightly varying the

degradation rate while leaving all other properties unchanged.

Antisense oligonucleotides are a novel class of therapeutic agents that alter disease states by interfering with transcription and translation events. The release of anti-*rev* phosphorothioate deoxyribo-oligonucleotide (5′-TCG TCG CTG TCT CCG CTT CTT CPT GCC) was evaluated using degradable (10KL5) and non-degradable (10KDA) hydrogels to determine the amount of release attributable to diffusion with and without hydrogel degradation at this precursor molecular weight. The oligonucleotide was received as a gift from Lynx Therapeutics, Inc. The oligonucleotide was added to precursor solutions at a concentration of 1 mg/ml. An amount of 0.25 ml of precursor was used for each hydrogel disk (diameter = 1 cm). In a second study, 1 mg/ml of the anti-*rev* oligonucleotide was added to the non-degradable precursor solutions 6KDA and 0.4KDA to ascertain the effect of the PEG molecular weight on the permeability of the hydrogel.

3. Results

The release of tPA from photopolymerized hydrogels was examined to determine if the photopolymerization process caused loss of enzymatic activity. As shown in Fig. 1, tPA activity was highly preserved following photopolymerization and release. Release of tPA occurred over approximately a 5-day period using a 10KL5 hydrogel. The release rate over the first 24 h was somewhat greater than during later time periods. Fig. 2 shows a profile of the swelling of the hydrogel material from its initial state as polymerized from a 23% precursor solution. The period of high release rates seems to be correlated to the swelling of the hydrogel to its equilibrium state.

The release profiles of proteins varying in molecular weight from 6000 to 150,000 Da are summarized in Fig. 3. Insulin (MW = 6000 Da) was released very rapidly, being complete within approximately 2 days, while the 10KL5 hydrogel was impermeable to IgG (MW = 150,000 Da) over the time period examined. Insulin had an average release rate of approximately 47%/day, lysozyme of 41%/day, lactate dehydrogenase of 30%/day, ovalbumin of 28%/day, and bovine serum albumin of 21%/day. The average release rate was computed from the slope of the release profile from 0 to 80% release, except for IgG. Fig. 4 shows a plot of the molecular weight of the entrapped protein versus the average re-

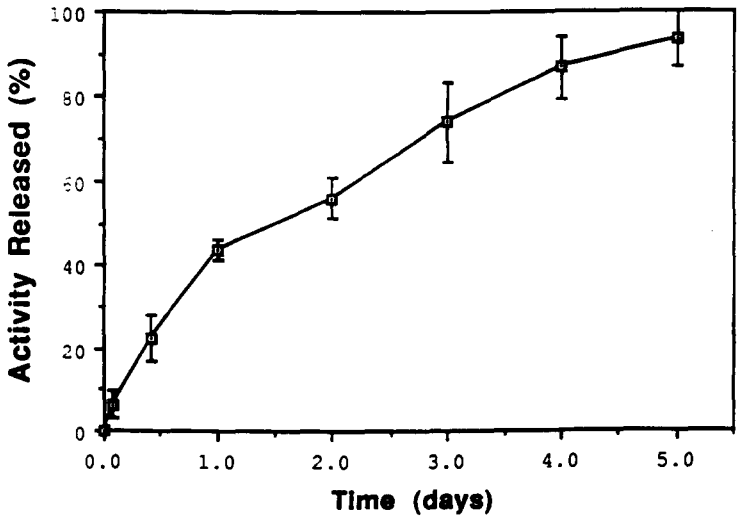

Fig. 1. Release of tPA from a degradable 10KL5 hydrogel. Activity was monitored using a chromogenic substrate assay. The photopolymerization process did not appear to reduce tPA activity. Data are means ± S.D.

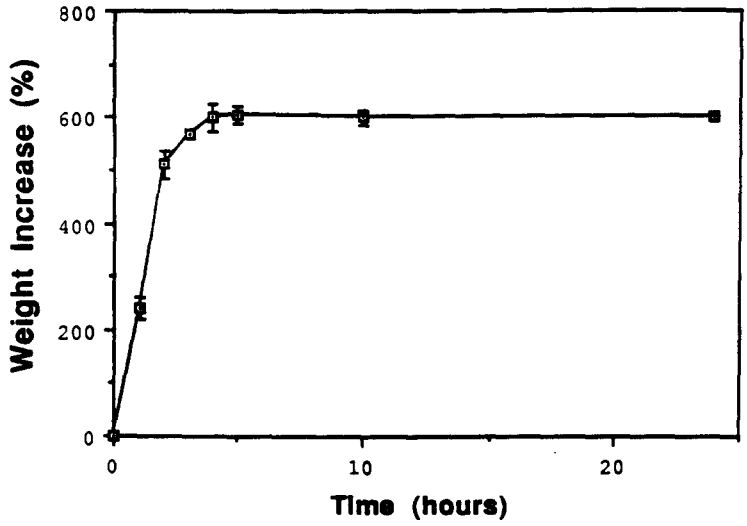

Fig. 2. Swelling of a 10KL5 hydrogel measured as the percentage weight gain over time in aqueous buffer. Data are means ± S.D.

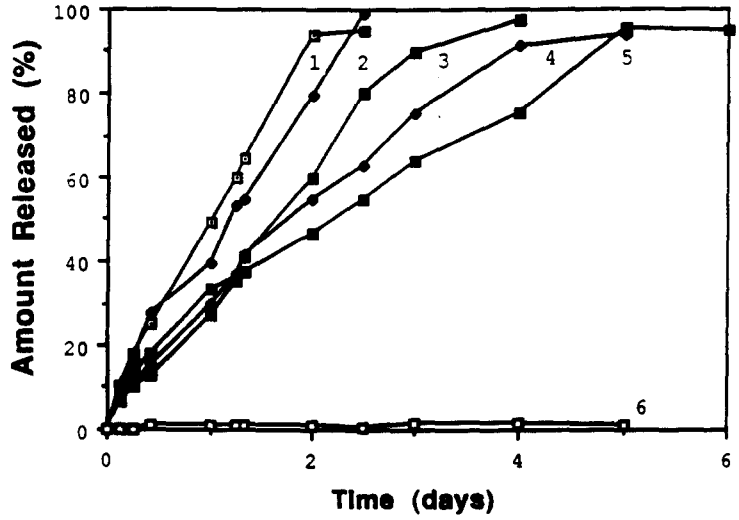

Fig. 3. A series of proteins with increasing molecular weights was released from 10KL5 hydrogels to determine the effect of the molecular weight of the permeant on the release rate. Curve 1, insulin, 6000 Da; curve 2, lysozyme, 14,300 Da; curve 3, lactate dehydrogenase, 36,500 Da; curve 4, ovalbumin, 45,000 Da; curve 5, bovine serum albumin, 66,000 Da; curve 6, immunoglobulin G, 150,000 Da.

lease rate: a linear relationship was found to exist ($R^2 = 0.98$).

BSA was released from two hydrogels with slightly different degradation rates, 8KL5 and 8KL3, as shown in Fig. 5. As expected, release from the 8KL3 hydrogel was essentially identical to release from the 8KL5 hydrogel for the first 24 h. Thereafter, release from the slower degrading 8KL3 hydrogel began lagging slightly behind release from the 8KL5 hydrogel. However, the difference in the amount released from each of the hydrogels at day 5 (when the difference was the greatest) was not significant ($P > 0.2$, Student's t-test).

Antisense oligonucleotides (anti-*rev*) were released from degradable and non-degradable hy-

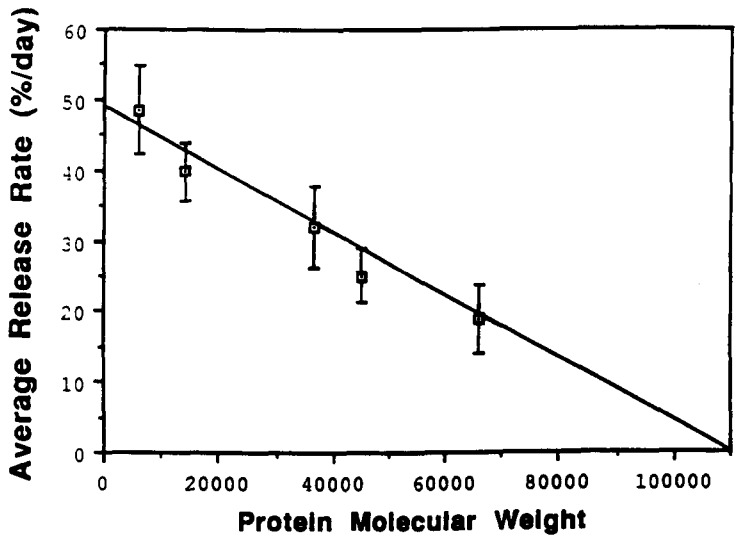

Fig. 4. The average release rates from the profiles in Fig. 3 were plotted against the molecular weights of the proteins. Data are means ± S.D.

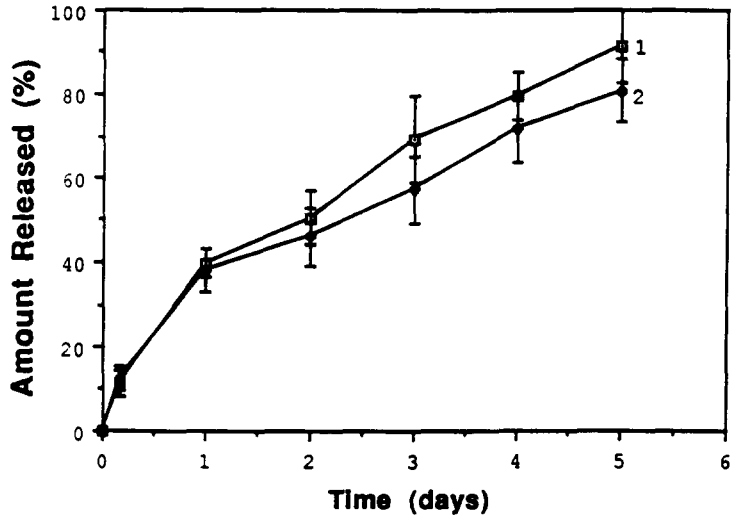

Fig. 5. Release of BSA from 8KL5 (curve 1) and 8KL3 (curve 2) hydrogels. These hydrogels have essentially the same permeability, but slightly different degradation rates. However, the difference in release rate observed was not statistically significant ($P > 0.2$).

drogels, 10KL5 and 10KDA, respectively, to determine the degree of release that resulted from diffusion of the oligonucleotide out of the matrix in the absence of degradation as compared to the increased diffusion due to hydrogel degradation. As shown in Fig. 6, release over the first 24 h appears to be largely due to diffusion, while after 24 h, very little release was seen from the non-degradable hydrogel. Release continued in the degradable gel as degradation augmented diffusion.

Fig. 7 shows the dependence of hydrogel permeability, altered by changing the molecular weight of the PEG-segment within the precursor, on the diffusional release. Non-degradable hydrogels were used so that diffusion could be

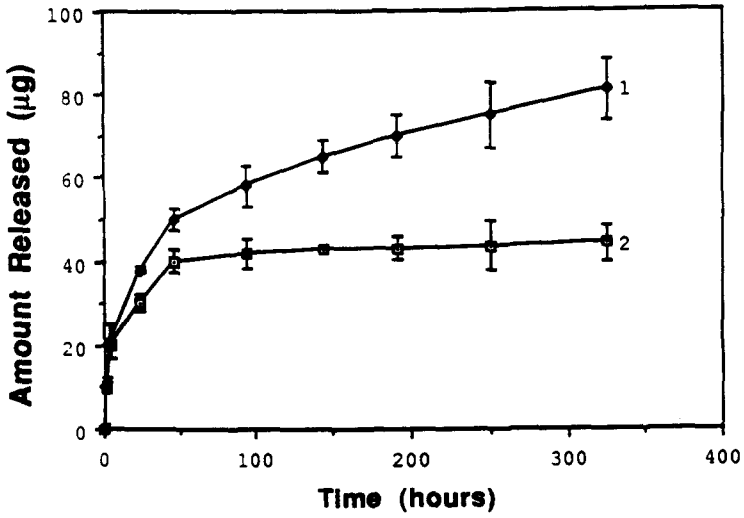

Fig. 6. Release of anti-*rev* mRNA from degradable 10KL5 (curve 1) and non-degradable 10KDA (curve 2) hydrogels. The majority of the release from 10KL5 is due to degradation of the hydrogel. Data are means ± S.D.

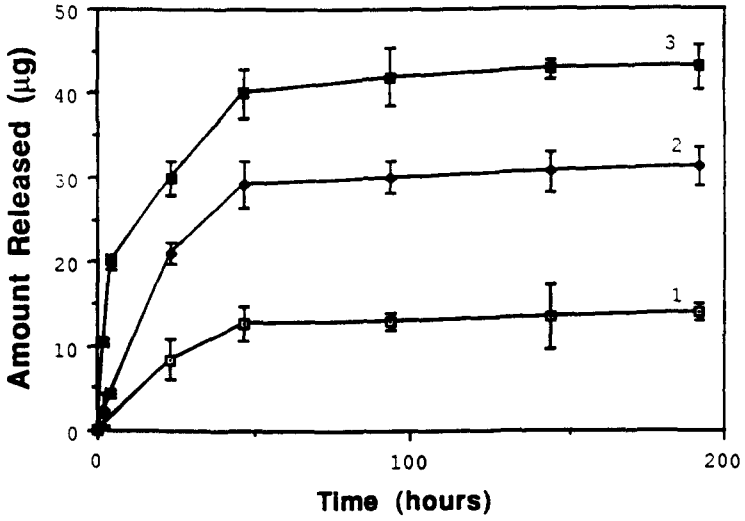

Fig. 7. Release of anti-*rev* mRNA from the following non-degradable hydrogels: 0.4KDA (curve 1); 6KDA (curve 2); and 10KDA (curve 3). The molecular weight of the PEG segment within the precursor was varied to examine the resultant changes in the hydrogel permeability.

examined independently of degradation. The higher the PEG molecular weight, the greater the permeability to the oligonucleotide, i.e., larger amounts of the oligonucleotide were able to diffuse out of the hydrogel matrix without the aid of degradation. However, only small amounts of the total entrapped oligonucleotide were able to diffuse out of the matrix.

4. Discussion

Hydrogels have been used extensively for controlled release applications, but most of these materials have been non-degradable polymers which release entrapped substances by diffusion and later require removal of the spent device. The photopolymerized hydrogel described here

is degradable, and as seen in Fig. 6, only a portion of the entrapped substance can be released by simple diffusion. Instead, release is regulated by degradation of the polymer. As the ester linkages in the oligomeric α-hydroxy acid segment are hydrolyzed, the pore sizes within the hydrogel matrix increase, and drug molecules are able to escape [3]. The fractional amount released by diffusion independently of degradation, depends on the molecular weight of the permeant and the molecular weight of the PEG chain in the hydrogel precursor. A small drug would require a low molecular weight PEG in the precursor to achieve release mediated by hydrogel degradation.

It is possible to control release rates of macromolecular drugs from these photopolymerized hydrogels through the design of the precursor. Release rates depend on both the permeability of the hydrogel prior to degradation and its degradation rate. Permeability depends on the crosslinking density, determined by the length of the PEG segment, and the degradation rate depends on both the length and composition of the α-hydroxy acid segment. Drug release from this type of hydrogel has been maintained for as long as 2 months in vitro [3]. Longer durations may be possible with precursors containing ϵ-caprolactone oligomers. Numerous variations on the acrylated PEG–co-α-hydroxy acid format can be synthesized, thus allowing one to essentially tailor the hydrogel to the requirements of a specific drug delivery application.

In the current study, in vitro release of proteins and oligonucleotides was evaluated to determine some of the parameters which must be understood before such tailoring for specific applications can be accomplished. Fig. 1 shows the release of tPA form a 10KL5 hydrogel, evaluated in terms of activity released. The initial release rate was slightly faster than release at later times. This period was correlated with the swelling of the hydrogel matrix to its equilibrium state, and it appears that the increased water flux during this time may be responsible for enhanced diffusion of the entrapped protein. A previous study [3] examined the release of proteins from hydrogels which were completely impermeable to the entrapped protein prior to degradation. In this case, the swelling effect was not observed, but instead there was a lag period with low release rates, and release increased at later times as the hydrogel degraded and became more permeable. It should also be noted that the photopolymerization process did not appear to adversely affect the activity of tPA, as all of the initial enzymatic activity was released from the gel.

Fig. 3 summarizes the release profiles of different molecular weight proteins, ranging from 6000 to 150,000 Da, from a 10KL5 hydrogel. A linear relationship was found between the molecular weight of the entrapped protein and the average release rate, as shown in Fig. 4, so it should be possible to predict the release rate of other proteins from the 10KL5 hydrogel. Similar plots will have to be constructed for other photopolymerized PEG hydrogels.

Fig. 5 shows the effect of slight variations in the degradation rate of the hydrogel, achieved by altering the length of the polylactidyl segment. Even the greatest difference in the amount of release, seen at day 5, was not statistically significant ($P > 0.2$). This suggests that the synthesis conditions do not need to be tightly controlled, as only large variations in the length of the α-hydroxy acid segment are able to significantly affect release rates. The composition of the α-hydroxy acid is probably a more effective means of altering the degradation rate.

As can be seen in Figs. 6 and 7, the hydrogel precursor must be matched to the molecular weight of the drug if drug release is to be controlled by the degradation of the hydrogel. For the 27mer antisense oligonucleotide to *rev*, approximately 11% was released passively (without degradation) from a hydrogel with a 10,000-Da PEG, 8% from a 6000-Da PEG, and only 3% from a 400-Da PEG. Thus, if the rapid initial release observed in Fig. 6 is to be reduced, the molecular weight of the PEG in the precursor must be reduced.

Perhaps a unique advantage of the photopolymerized PEG-based hydrogels described herein

over previously available materials is that they may be formed in situ. Hydrogels which are formed in contact with tissue adhere firmly to the underlying tissue [5], effectively localizing the hydrogel and the drug release. This feature may allow one to form a hydrogel upon a specific site, such as a tumor or the surface of a damaged blood vessel, and release the entrapped drug precisely where it is required. Localized drug release often allows one to use smaller doses and may help to minimize side effects of certain drugs. Future studies will compare the effect of localized release of protein drugs to systemic release in vivo using photopolymerized, biodegradable PEG hydrogels.

Acknowledgements

The technical assistance of Valerie Virta is gratefully acknowledged. Funding was provided by Focal Inc., Cambridge, MA.

References

[1] R. Langer and J. Folkman, Nature, 263 (1976) 797.
[2] D. Needham, K. Hristova, T.J. McIntosh, M. Dewhirst, N. Wu and D.D. Lasic, J. Liposome Res., 2 (1992) 411.
[3] A.S. Sawhney, C.P. Pathak and J.A. Hubbell, Macromolecules, 26 (1993) 581.
[4] C.P. Pathak, A.S. Sawhney and J.A. Hubbell, J. Am. Chem. Soc., 114 (1992) 8311.
[5] J.L. Hill-West, S.M. Chowdhury, A.S. Sawhney, C.P. Pathak, R.C. Dunn and J.A. Hubbell, J. Reprod. Med., submitted.
[6] J.L. Hill-West, S.M. Chowdhury, A.S. Sawhney, C.P. Pathak, R.C. Dunn and J.A. Hubbell, Obstet. Gynecol., 83 (1994) 59.
[7] J.L. Hill-West, S.M. Chowdhury, R.C. Dunn and J.A. Hubbell, Fertil. Steril., in press.
[8] K.J. Doody, R.C. Dunn and V.C. Buttram, Fertil. Steril., 51 (1989) 509.
[9] J.L. Hill-West, S.M. Chowdhury, M.J. Slepian and J.A. Hubbell, Proc. Natl. Acad. Sci. U.S.A., 91 (1994) 5967.
[10] M. Simons, E.R. Edelman, J.L. DeKeyser, R. Langer and R.D. Rosenberg, Nature, 359 (1992) 57.
[11] G.A. Ferns, E.W. Raines, K.H. Sprugel, A.S. Motani, M.A. Reidy and R. Ross, Science, 253 (1991) 1129.