5 Hydrogels in Cancer Drug Delivery Systems

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1. INTRODUCTION TO HYDROGELS

Hydrogels have played a vital role in the development of controlled-release drug delivery systems. A hydrogel (also called an aquagel) is a three-dimensional (3-D) network of hydrophilic polymers swollen in water *(1)*. The 3-D polymer network of a hydrogel is maintained in the form of elastic solid in the sense that there exists a remembered reference configuration to which the system returns even after being deformed for a very long time. By definition, hydrogels usually contain water at least 10% of the total weight. The term hydrogel implies that the material is already swollen in water. Dried hydrogels (or xerogels) absorb water to swell, and the size of the swollen gel depends on how much water is absorbed. A hydrogel swells for the same reason that an analogous linear polymer dissolves in water to form an ordinary polymer solution. The extent of swelling is usually measured by the swelling ratio, which is the volume (or weight) of the swollen gel divided by the volume (or weight) of the xerogel. If the weight of absorbed water exceeds 95% of the total weight, a hydrogel is often called a superabsorbent. Thus, 20 g of fully swollen superabsorbent will have 1 g or less of polymer network and 19 g or more of water (i.e., the swelling ratio is more than 20). The swelling ratio of many hydrogels can easily reach greater than 100. Despite such a large quantity of water, highly swollen hydrogels still maintain solid forms.

1.1. Preparation of Hydrogels

Hydrogels can be divided into chemical and physical gels depending on the nature of the crosslinking. Figure 1 shows chemical and physical gels. Chemical gels are those that have covalently crosslinked networks. Thus, chemical gels will not dissolve in water or other

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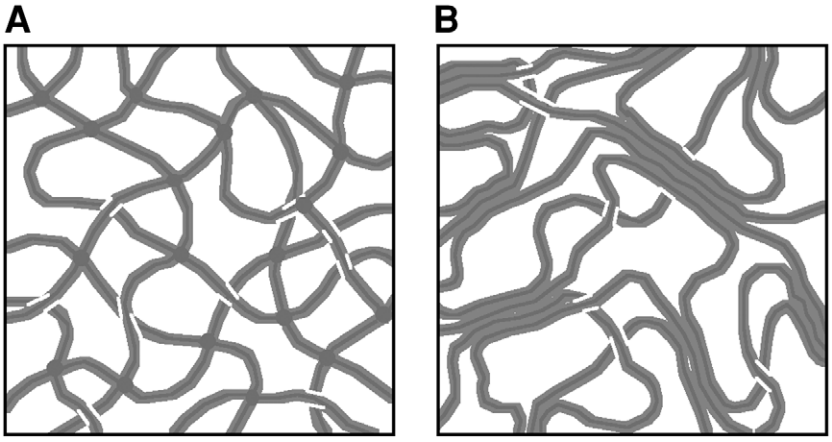


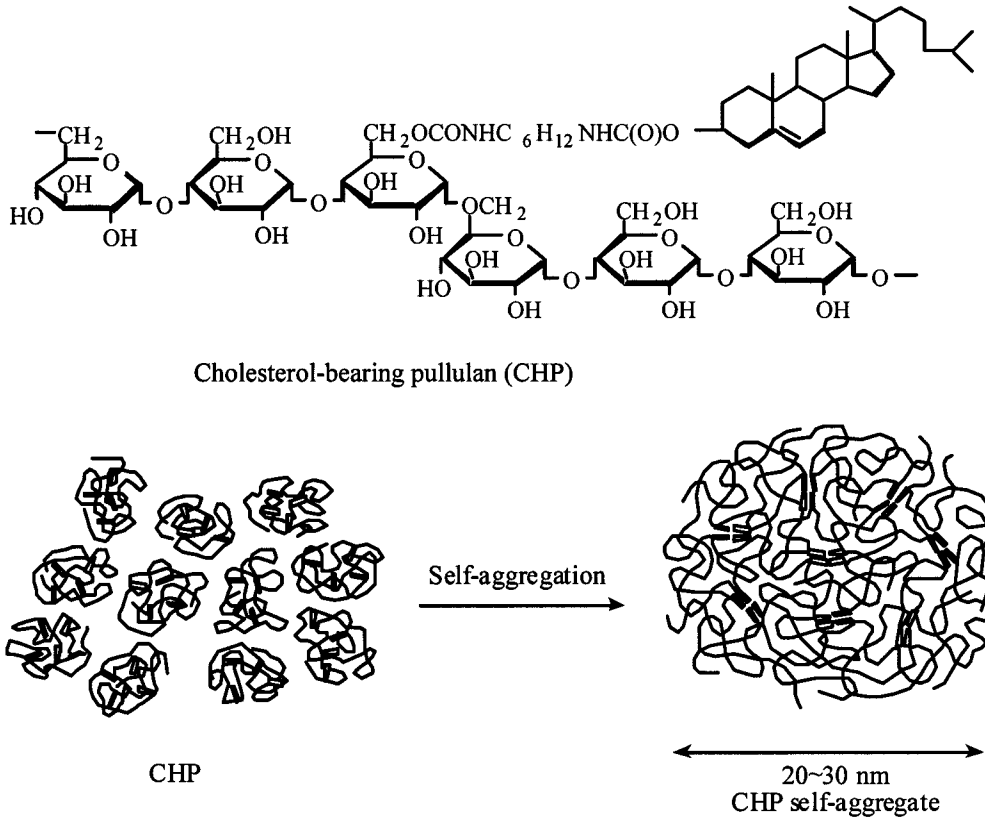
Fig. 1. Chemical **(A)** and physical **(B)** gels. In physical gels, a substantial fraction of a polymer chain is involved in the formation of stable contacts between polymer chains. Association of certain linear segments of long polymer molecules form extended "junction zones," which is distinguished from well-defined point crosslinks of chemical gels.

organic solvent unless covalent crosslinks are cleaved. Chemical gels can be prepared by two different approaches. First, chemical gels can be made by polymerizing water-soluble monomers in the presence of bi- or multifunctional crosslinking agents (i.e., by crosslinking polymerization). Second, chemical gels can be prepared by crosslinking water-soluble polymer molecules using typical organic chemical reactions that involve functional groups of the polymers. Physical gels (also called physical networks, association networks, or pseudogels) are the continuous, disordered 3-D networks formed by associative forces capable of forming noncovalent crosslinks. The point covalent crosslinks found in chemical gels are replaced by weaker and potentially more reversible forms of chain–chain interactions. These interactions include hydrogen bonding, ionic association, hydrophobic interaction, stereocomplex formation, and solvent complexation.

A weak and noncovalent molecular association is sometimes more than sufficient to result in a supramolecular assembly. For example, pullulan, which was partly substituted by cholesterol moieties (i.e., cholesterol-bearing pullulan, or CHP), formed monodispersive nanoparticles (20–30 nm) as shown in Fig. 2 *(2)*. The CHP self-aggregate can be regarded as a hydrogel, in which microdomains provided noncovalent crosslinking points arising from the association of hydrophobic cholesterol moieties. One of the advantages of this type of physical gel nanoparticles is that they can form complex various hydrophobic substances such as adriamycin, and even various soluble proteins and enzymes. Physical and biochemical stability of insulin, for example, is known to be drastically increased upon complexation *(2,3)*. When a 3-D structure of a chemical gel is formed, the network extends from one end to the other and occupies the entire reaction vessel. For this reason, the hydrogel formed is essentially one molecule, no matter how large the hydrogel is. Thus, there is no concept of molecular weight for hydrogels. Hydrogels can be prepared in various sizes and shapes, depending on the application.

1.1.1. Monomers Used for Making Hydrogels

Any monomers that become hydrophilic polymers can be used to make hydrogels. Table 1 lists some of the monomers and crosslinkers commonly used to prepare hydro-



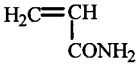
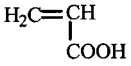
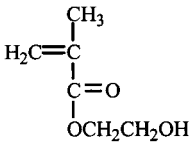
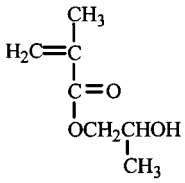
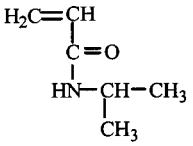
AU: Pls. provide permission to reproduce Fig. 2

Fig. 2. Chemical structure of cholesterol-bearing pullulan (CHP) and schematic representation of self-aggregation of CHP into a hydrogel nanoparticle. From *ref (2).*

gels. The monomers shown in Table 1 are all vinyl monomers, since they are most widely used in preparation of hydrogels. Most monomers in Table 1 are hydrophilic and highly water-soluble. Some monomers are not freely water-soluble. For example, hydroxyethyl methacrylate (HEMA) is not hydrophilic enough to be soluble in water, but a poly(hydroxyethyl methacrylate) (polyHEMA) matrix, whether crosslinked or not, takes up sufficient amount of water to be called a hydrogel. PolyHEMA does not dissolve in water even in the absence of crosslinking. To form a crosslinked network, a crosslinking agent is added to a monomer solution, and the mixture is polymerized using an initiator. Any combination of monomer and crosslinker in Table 1 can be used to form hydrogels. More than one type of monomer can be used to form hydrogels. It is quite common to use two different types of monomers, and in this case, the obtained polymer is known as a copolymer instead of a homopolymer. For example, if acrylic acid and HEMA are used as monomers, the obtained hydrogel is known as crosslinked poly(acrylic acid-co-HEMA). Vinyl monomers are polymerized by free radical polymerization using an initiator. Commonly used initiators are azo initiators (e.g., azobisisobutyronitrile), peroxide (e.g., benzoyl peroxide), persulfate (ammonium persulfate), and redox initiators (e.g., ammonium persulfate and tetramethylethylenediamine). The monomer concentration is adjusted by diluting with suitable solvents, usually water. The monomer mixture containing a crosslinking agent and an initiator can be dispersed in organic solvent to form hydrogels in droplets. Hydrogels can also be prepared by

Table 1
Examples of Monomers, Crosslinkers, and Initiators Frequently Used for Preparation of Hydrogels by Free Radical Polymerization

1. Monomers

Acrylamide		Methacrylic acid
Acrylic acid		Methyl methacrylate
Hydroxyethyl methacrylate (HEMA)		Monomethyl itaconate
Hydroxypropyl methacrylate (HPMA)		Vinylpyrrolidone
N-isopropylacrylamide (NIPAM)		

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mixing with other types of polymers. The concentrations of monomer and crosslinking agent affect the mesh size of the polymer network and thus, the release property of the loaded drugs from a hydrogel matrix. The drug release rate from collagen-poly(HEMA) hydrogels is known to be controlled by adjusting the crosslinking density of the hydrogels. Crosslinked hydrogels released methotrexate (MTX) at a slower rate than an uncrosslinked hydrogel *(4)*.

1.1.2. Polymers Used for Making Hydrogels

Hydrophilic polymers can be crosslinked, either by chemical reaction or by physical associations, to form hydrogels. Hydrophilic polymers include not only synthetic polymers, but also natural polymers such as proteins and polysaccharides. Commonly used proteins are collagen, gelatin, fibrin, and mucin. Widely used polysaccharides are agarose, alginate, carrageenan, cellulose derivatives, chitosan, chondroitin sulfate, dextran, guar gum, heparin, hyaluronic acid, pectin, and starch. To make a

Table 1
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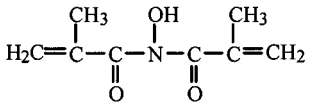
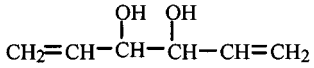
2. Crosslinkers

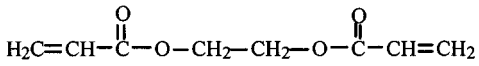
Dimethacryloyl hydroxylamine



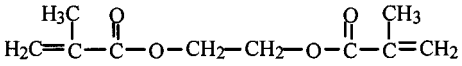
Divinyl glycol (1,5-hexadiene-3,4-diol)



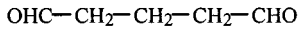
Ethylene diacrylate



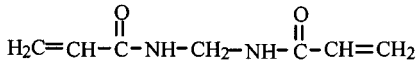
Ethylene glycol dimethacrylate (EGDMA) or ethylene dimethacrylate (EDMA)



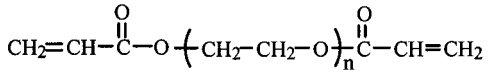
Glutaraldehyde



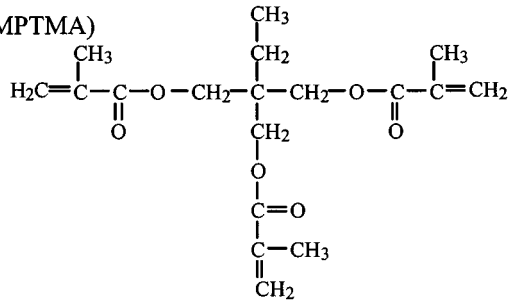
N,N'-methylene-bisacrylamide (BIS)



PEG diacrylate



Trimethylolpropane trimethacrylate (TMPTMA)



crosslinked network from polymer molecules, the polymers have to possess chemically active functional groups for crosslinking. Thus, polymers with carboxyl groups, such as acrylic acid, or with amine groups, such as chitosan, can be easily crosslinked to form hydrogels. Polymers with hydroxyl groups can also be easily crosslinked.

1.2. Drug Loading into Hydrogels and Release from Hydrogels

Anticancer drugs and imaging agents, such as X-ray contrast or radiopaque materials, can be loaded into hydrogels by a number of methods. Drugs can be added to the monomer solution before crosslinking polymerization or to the polymer solution before crosslinking reaction. In this case, relatively large concentrations of drugs can be added, but the prepared hydrogels may have to be purified to remove residual initiators, monomers, and crosslinkers, although their concentrations may be small. However, the washing step may remove the loaded drugs as well. Ara-C was added to the mixture of monomers (HEMA and vinylpyrrolidone) and a crosslinking agent (EGDMA) at the concentration of 34% (v/v). The solution was then polymerized to obtain an optically transparent hydrogel, indicating complete solubility of ara-C in the matrix *(5)*. The same approach was used to load ara-C into poly(HEMA) hydrogel crosslinked with EGDMA *(6,7)*. Prepared hydrogels can be dried for storage. The prepared hydrogel was cut into disks which contain ara-C from 5 to 25 mg/disk. Release of ara-C from disks was varied from 1 d to 16 d by adjusting the concentration of the crosslinking agent used *(5)*.

The drug can be loaded into hydrogels after they are purified. In this case, the concentration of the loaded drug will be rather limited because the drug loading is limited by the concentration of the drug in the loading solution. 5-FU, MTX, and ara-C were loaded into poly(HEMA) hydrogels by immersing the hydrogels into aqueous solutions saturated with drug molecules *(8,9)*. Since purified hydrogels are used in this approach, the prepared drug-loaded hydrogels are ready to use. 5-FU was also loaded into hydrogels of poly(acrylamide-co-monomethyl itaconate) and poly(acrylamide-co-monopropyl itaconate). Sodium salt of 5-FU has a solubility of 65 mg/mL, which is five times higher than that of 5-FU (13 mg/mL). Thus, in order to trap the maximum amount of 5-FU in the xerogel (dried hydrogel) disk, aqueous solutions of 5-FU neutralized with NaOH were used instead of water in the feed mixture of polymerization *(10,11)*. Adriamycin (ADR) was loaded into CHP aggregates by simply mixing ADR with CHP suspensions. ADR formed complexes with hydrophobic cholesterol moieties of CHP. ADR was spontaneously dissociated from the complex as a function of time. Less than 30% of complexed ADR was released even after 7 d in phosphate buffered saline (PBS, pH 7.4) at 25°C. The dissociation significantly increased as the medium temperature increased to 37°C and/or decreased to pH 5.9 or 3.7. Approximately 20% of the loaded ADR was released at pH 7.4, whereas more than 50% was released at lower pH readings in 24 h (Fig. 3). The enhanced dissociation of ADR from the complex at lower pH is expected to be caused by the increase in its water solubility in an acidic medium. The chemical stability of ADR was largely improved by the complexation. The in vitro cytotoxicity of ADR was also diminished by the complexation. The diminished cytotoxicity of the CHP-ADR complex would be ascribed to either retarded release of ADR from the complex or decreased cell internalization of the complex *(2,3)*.

If both polymer chains and drug molecules have chemically active groups, drug molecules can be covalently attached to the polymer chains. The immobilized drug molecules are released by chemical or enzymatic dissociation from polymer chains.

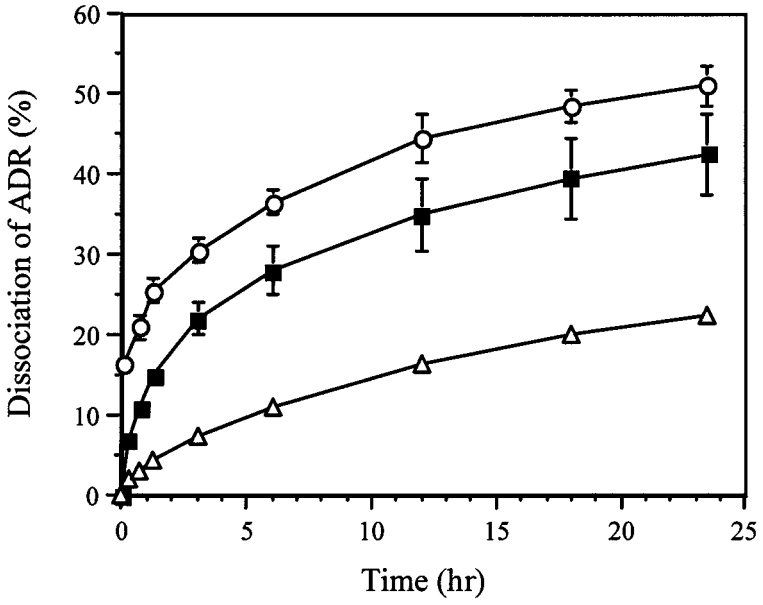


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Fig. 3. Dissociation of adriamycin (ADR) from CHP-ADR complex at 37°C and at pH 3.7 (○), 5.9 (■), and 7.4 (□). The concentrations of ADR and CHP were $3.6\times10^{-6}M$ and $4.1\times10^{-8}M$, respectively. From *ref. (2).*

One of the advantages of this approach is that the drug-polymer conjugates can be purified without losing the grafted drug molecules.

1.3. Swelling Kinetics

As it is preferred to prepare final hydrogel dosage forms in the dried state (i.e., xerogels) for long-term storage before in vivo applications, the swelling kinetics of the xerogels also contribute significantly to controlling the drug release kinetics. During the drying process of hydrogels, water evaporates from a gel and the surface tension of water causes collapse of polymer chains and thus shrinking of the hydrogel body to only a small fraction of its swollen size. The physical state of xerogels is known to be glassy. Water absorption into the glassy polymer occurs by diffusion, which is a very slow process, leading to very slow swelling. This slow swelling property is used to slowly release loaded drug molecules.

If water is removed without collapsing the polymer network either by lyophilization (i.e., freeze drying) or by extraction with organic solvents, then a xerogel is porous. The pore size is typically less than 10 μm. When bubbles of air (or nitrogen or carbon dioxide) are introduced during hydrogel formation, the formed hydrogel contains very large pores of approx 100 μm even in the dried state. These hydrogels are called superporous hydrogels *(12).* Superporous hydrogels absorb water through the interconnected pores forming open channels (i.e., by capillary action); the water absorption is very fast and swelling can be completed in a matter of minutes instead of hours for the glassy xerogels. Swelling ratios can be as high as a few hundred. The rapid and large swelling properties can be highly useful in certain applications, such as endovascular chemoembolization.

2. HYDROGELS IN ANTICANCER THERAPY

2.1. Endovascular Chemoembolization

Blocking the blood vessel feeding a tumor and thus starving the tumor of blood and oxygen is an effective way of treating cancer. In addition to antiangiogenesis therapy, endovascular chemoembolization is another means of blocking the blood supply to a tumor. Endovascular chemoembolization is the method of simultaneously administering into the blood vessel of the tumor tissue the vascular occlusion materials and antitumor agents that block the supply of nutrients to the tumor tissue as well as contribute cytotoxic action of the anticancer agents. Blocking of the artery decreases the blood flow rate, thereby increasing the dwell time and the concentration of anticancer agents in the tumor tissue. Arterial chemoembolization with microencapsulated drugs has been used clinically since 1978 *(13).* This mode of treatment can be applied to a variety of tumor lesions with remarkable therapeutic effect and minimal systemic cytotoxicity. Vascular occlusion in chemoembolization has been accomplished by using different embolizing agents as listed in Table 2. All materials in Table 2, except Lipiodol®, are hydrogels in a microparticulate form. Degradable starch microspheres (Spherex®, Pharmacia, Sweden) without anticancer drug have been frequently used for embolization *(14).* Starch microspheres, which were first used for scintigraphic imaging in the diagnosis of lung emboli, are currently used for transient occlusion of blood flow *(14).* The most important feature of degradable starch microspheres is that the degradation time can be regulated by means of the degree of crosslinking to suit various organs and applications. Degradable starch microspheres have been delivered to the liver, kidney, and mesenterium without harm. Poly(HEMA) microparticles grafted with MTX were also used for chemoembolization *(15).* Unlike starch microspheres, poly(HEMA) microparticles are not degradable. Although many materials were used for the purpose of embolization and drug reservoir, there was no effort to control the drug release. Adverse effects of anticancer agents are frequent, involving more than 60% of patients, although they are often transitory. The adverse effects were caused by rapid release of drug from embolization materials. Thus, it is necessary to use embolizing materials with the ability to control the drug release rate.

When injected intraarterially into the target organ, microparticles of a suitable size (e.g., 500 μm) become trapped in arteriolae. The hydrogel microparticles can release anticancer drugs locally for extended periods of time. The locally released drug from microspheres can cross the capillary walls and enter the cells of the target organ within the time of circulatory arrest. This targeted delivery of anticancer drugs would reduce the systemic concentration significantly *(22,23).* This regional cancer chemotherapy can strongly increase the efficiency of the drug, while limiting the toxic effects. One of the advantages of this approach is that chemotherapy can be combined with embolization. Hydrogel microparticles can swell to block blood vessels and thus the supply of blood to tumors. For this particular application, fast-swelling superporous hydrogels are more useful than conventional hydrogels. For biodegradable hydrogels, the occlusion of the blood vessels can be transient until all drug is released. Permanent occlusion for tumor necrosis can be achieved using nondegradable hydrogel microparticles. Endovascular embolization before surgery (i.e., preoperative embolization) is thought to reduce the risk of hemorrhage and to decrease the release of tumor cells into the blood stream during surgical removal of solid tumors *(24).* Successful emboli materials

Table 2
Examples of Materials Used in Chemoembolization

Name	*Component*	*Size*	*References*
Gelfoam®	Gelatin	90 μm in diameter	*16,17*
Ivalon®	Poly(vinyl alcohol)	150~250 μm in diameter	*18*
Spherex®	Starch	45 μm in diameter	*19*
Angiostat®	Microfibrillar collagen (Crosslinked)	5 μm × 75 μm (diameter × length)	*20*
Albumin microspheres	Albumin + chitosan (glutaraldehyde crosslinked)		*21*
Lipiodol®	Iodised poppyseed oil	25 μm in diameter	*18*

are expected to be nontoxic, nonantigenic, hydrophilic, thrombogenic, chemically stable, and radiopaque. At present, there is no standard emboli material that meets all the required properties. Rapid advances in polymer chemistry, however, are expected to produce ideal emboli materials in the near future. In addition to endovascular embolization, microparticles can also be employed for intratumoral, subcutaneous, extravascular, and intravascular administration.

Chemoembolization using different microparticles has been used for metastatic colorectal carcinoma of the liver and hepatocellular carcinoma *(18,20).* Although there was an increase in the mean survival time in many cases, there was no statistical significance for most of materials used. This is mainly due to the use of inadequate embolization materials. For this approach to work effectively, hydrogel microparticles should swell rapidly to a size large enough to block the blood vessel. Currently, there are no hydrogels that can swell rapidly in blood, especially when they are dried. Recent development of superporous hydrogels that swell extremely fast in aqueous solution *(12)* provides an approach to develop effective chemoembolization or embolization materials.

2.2. Intratumoral Administration

Higher anticancer concentrations in tumors during the course of a fractionated irradiation treatment are known to increase therapeutic efficacy *(25).* One way of achieving localized high concentrations is to implant a rod-shaped hydrogel in the center of subcutaneous tumors. The drug enhancement ratio for the group of mice treated with intratumoral hydrogel rods was higher than those for other groups where drug in solution was administered intraperitoneally or intratumorally. In this approach, the release kinetics of cisplatin from the implanted hydrogel rods was important. If the drug release was too slow, the drug distribution within tumors became inhomogeneous, resulting in low therapeutic effect *(26).* The highest response, which showed a delay of tumor growth for 55 d, was obtained with a hydrogel formulation that released 56% of cisplatin in 4 d with 14% of water uptake. Figure 4 shows in vitro release profiles of platinum [i.e., *cis*-diamminedichloroplatinum(II) or cisplatin] from polyether hydrogels. The water uptakes of three different polyether hydrogel rods (1.5 mm diameter × 5 mm length) containing 10% (w/w) cisplatin were 4%, 14%, and 40% (w/w). The polyether hydrogel rods, which absorbed only 4% of water, are by definition not hydrogels. Polyether hydrogel rods released only 10% of cisplatin in 4 d and 17% in 11 d.

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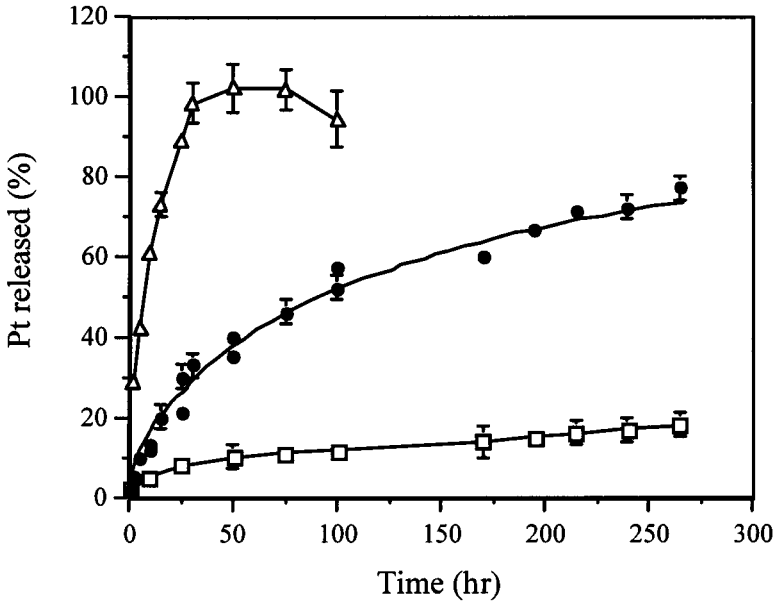

Fig. 4. In vitro release of Pt(or cisplatin) from polyether hydrogel rods with water uptake of 4% (□), 14% (●), and 40% (□). The hydrogel rods contained 10% (w/w) cisplatin. From *ref. (27).*

The 14%-hydrogel released 56% and 75% of the incorporated cisplatin in 4 d and 11 d, respectively. The 40%-hydrogel released almost 90% on day 1 and the remaining drug was released on day 2. The absolute amount of the drug released from hydrogels increased with increasing the payload (i.e., loading amount), but the cumulative fractional release decreased with increasing the payload *(27).* The intratumoral implants have a therapeutic advantage over systemic therapy. Implantation of hydrogel rods, which release cisplatin during the period of a fractionated radiotherapy, was shown to be an effective method of administering the drug. Such treatment may be useful in patients with inoperable pelvic or head-and-neck tumors in which hydrogel rods could be implanted under ultrasound guidance *(25).*

Complexes of hydrogels with radiotherapeutic agents were also used to maintain high concentrations of therapeutic agents *(28).* Chitosan is soluble under acidic conditions, but becomes gel under basic conditions. The Holmium-166-chitosan complex solution becomes a gel upon administration into the body. Higher radioactivity at the administration site was obtained with the administration of the complex than that of Holmium-166 alone *(29).*

Hydrogels can also be used to deliver α-interferon. Ocular inserts were made of hybrid polymers of maleic anhydride-alkyl vinyl ether copolymers and human serum albumin *(30).* α-Interferon was loaded into transparent, flexible, and coherent hydrogel films by a low temperature casting procedure. The ocular inserts exhibited a gel-like behavior with a strong morphological stability even at a fairly high level of water uptake. The water uptake into the inserts showed that about 90% of the equilibrium swelling was observed after 10–12 h. The swelling ratio was more than 30 and the insert diameter was increased from 3 mm to 10–12 mm, while maintaining the shape and integrity of disk-like inserts *(30).* The most hydrophilic matrix, based on the

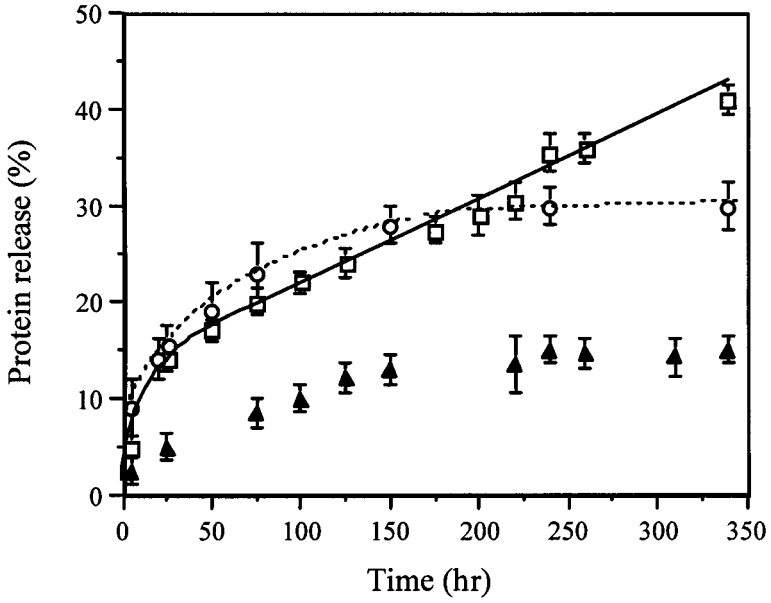

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Fig. 5. Kinetic profiles of the protein release of inserts based on partial esters of poly(maleic anhydride-alt-alkyl vinyl ether)s in PBS at 37°C. Samples MP1m (○), MP1b (□), and MP3m (□). From *ref. (30).*

methyl ester of poly(MAn-alt-Peg3VE), showed the lowest hydration, probably owing to the stronger interactions occurring between the hydrophilic portions of the polymer and the protein. The percent released protein was proportional to polymer hydrophobicity (*see* Fig. 5). Initial-burst release was observed during the first 18 h, and it was more pronounced for the esters of copolymer based on maleic anhydride and mono-*O*-methyloligoethyleneglycol vinyl ether. The initial-burst release was followed by an almost constant release for the next 10–15 d. During this period, the percent releases of the protein virtual load were 40%, 30%, and 15%, respectively, for the inserts based on butyl ester of poly(MAn-alt-Peg1VE) (MP1b in Fig. 5), methyl ester of poly(MAn-alt-Peg1VE) (MP1m in Fig. 5), and methyl ester of poly(MAn-alt-Peg3VE) (MP3m in Fig. 5) *(30).* The more hydrophobic hydrogels released more proteins. Again, this may be because of the interaction of hydrophilic polymer chains with proteins, resulting in an increase of effective crosslinking density. The study indicated that erosion of hydrogel matrices also contributed to the initial-burst releases. This was supported by kinetic measurements of weight losses of hydrogel inserts, which showed rapid weight loss in the first several hours *(30).*

2.3. Implantation of Hydrogels

One of the most effective ways for treating cancer could be delivering high concentrations of anticancer drugs to the cancerous lesions for periods long enough to kill all of the cancer cells. Anticancer drugs can be infused directly into the artery supplying blood to the neoplastic tissue. While this approach achieves targeted introduction of anticancer drugs into tumor tissues, its effect is only short-term unless the catheter is left in the vessel for a long time *(15).* A pronounced therapeutic effect usually requires

AU: Pls. provide permission to reproduce Fig. 6

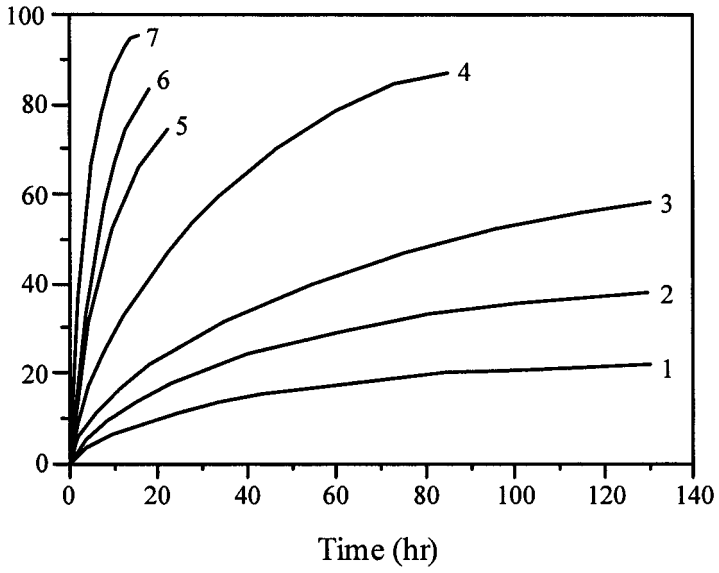
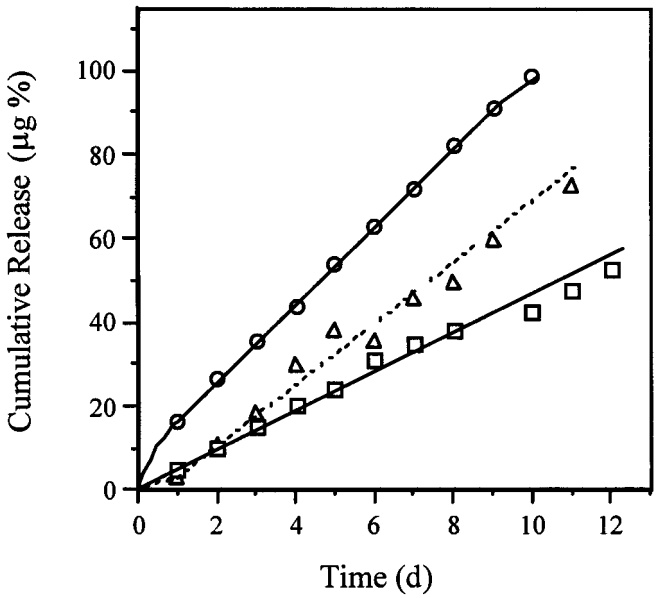


Fig. 6. Release profiles of narciclasin from polyHEMA matrices containing different concentration (v/v) of TMPTMA or MPEG. 1, HEMA:TMPTMA (80:20); 2, HEMA:TMPTMA (85:15); 3, HEMA:TMPTMA (90:10); 4, HEMA; 5, HEMA:MPEG (90:10); 6, HEMA:MPEG (75:25); and 7, HEMA:MPEG (50:50). From *ref. (32).*

that the procedures must be repeated many times. In addition, such treatment may still be accompanied by the same toxic side effects as conventional treatments. An alternative approach may be local delivery of anticancer agents from controlled-release devices, such as drug-loaded hydrogels. Various hydrogels have been used for subcutaneous delivery of anticancer agents. Examples are narciclasine-containing poly(HEMA) *(31,32),* cytarabine (ara-C)-containing α, β-polyasparthydrazide hydrogel *(33),* and 5-fluorouracil-containing poly(acrylamide-co-monomethyl itaconate) or poly(acrylamide-co-monopropyl itaconate) *(10,11).*

The drug release rate from hydrogel implants can be controlled by adjusting crosslinking density and/or by adding water-soluble components. Figure 6 shows examples of narciclasin release from polyHEMA implants. PolyHEMA forms hydrogels even in the absence of a crosslinking agent, but addition of a crosslinking agent delays the drug release. The addition of TMPTMA, a crosslinking agent shown in Table 1, to the narciclasin-HEMA mixture significantly delayed the drug release. In the absence of a crosslinking agent *(Fig. 6, line 4),* more than 80% of the drug was released in 3 d. On the other hand, when the TMPTMA concentration was 20% (v/v) (Fig. 6, line 1), only about 20% of the drug was released even after several days. The addition of poly(ethylene glycol) methyl ether (MPEG), a water-soluble component, resulted in release of most of the drug within a day (Fig. 6, lines 5–7).

Hydrogels swell to a large extent in aqueous solution and this effect tends to result in mechanically weak structures that may limit their pharmaceutical and medical applications *(34).* The mechanical strength of hydrogels can be improved by making an interpenetrating network with collagen. Collagen-poly(HEMA) hydrogel pellets were loaded with drugs, such as 5-fluorouracil, mitomycin C, bleomycin A2 *(35,36),* and



AU: Pls. provide permission to reproduce Fig. 7

Fig. 7. In vitro cumulative release of 5-fluorouracil (○), mitomycin C (□), and bleomycin A2 (□) from collagen-poly(HEMA) hydrogel matrices. From *ref. (35).*

camptothecin derivatives *(37).* The drug-loaded collagen-poly(HEMA) hydrogel pellets were subcutaneously implanted into rats with solid tumor fibrosarcoma *(37,38)* or into mice *(37).* In both cases, the drug was released at zero-order rate for more than several days. The main advantage of using implantable hydrogels is that the long-term delivery of anticancer agents can eliminate daily administration of the drugs and reduce the potential side effects *(33).* Figure 7 shows the in vitro cumulative drug release profiles of 5-fluorouracil, mitomycin C, bleomycin A2 from the hydrated collagen-poly(HEMA) hydrogel pellets (10 mm diameter × 3 mm thick) in phosphate buffer at 37°C and pH 7.4 *(35).* As shown in Fig. 7, the release profiles were different. The release of 5-fluorouracil indicated burst release followed by the zero-order release, whereas that of bleomycin showed the lag-time effect before reaching the steady state. No burst release or lag time was observed with mitomycin C. The burst release of 5-fluorouracil is most likely owing to migration of the drug to the surface during drying. The molecular weights of 5-fluorouracil, mitomycin C, and bleomycin A2 are 130, 334, and 1400, respectively *(35).* The highest molecular weight of bleomycin A2 may be responsible for the observed lag time. The hydrogel may have to swell before allowing diffusion of large molecules such as bleomycin A2. Because all three drugs showed the zero-order release at steady state, the release rate decreased in the order of 5-fluorouracil > bleomycin A2 > mitomycin C. The slow release of mitomycin C may be owing to interaction with collagen in the hydrogel pellets *(35).*

Copolymers of N-(2-hydroxypropyl)methacrylamide and N,O-dimethacryloyl hydroxylamine were used to prepare hydrolytically degradable hydrogels for the release of doxorubicin and polymer-doxorubicin conjugates *(39,40).* D-galactosamine was attached to the polymer-doxorubicin conjugate as a targeting moiety to hepatocytes. When the hydrogel was implanted intraperitoneally into DBA2 mice, 35% of the

targeted conjugates accumulated in the liver, whereas only 2% of the control conjugates were found in the liver 48 h after implantation.

Albumin microparticular (100–500 m) hydrogels were prepared by crosslinking albumin with polyethylene glycol disuccinate *(41,42)*. Anticancer agents and diagnostic agents were covalently attached in stoichiometric quantities. The hydrogel microparticles effectively reduced the risk of local tumor recurrence in a rat model when implanted locally after surgical tumor removal. The albumin microparticles were degraded by proteases released from macrophages. Typically, 1-mL samples that were implanted into paraspinal muscles of rats were completely absorbed within 4 wk and its constituents were metabolized.

2.4. Peroral and Oral Administration of Hydrogels

α, β-Polyasparthydrazide microparticles have been used for peroral administration of anticancer agents *(43)*. α, β-Polyasparthydrazide is a linear polymer and a promising plasma expander and drug carrier with interesting properties such as water-solubility, and absence of toxicity and antigenicity *(44)*. A different crosslinking degree was obtained by varying the ratio of crosslinking agent/polymer that influenced the swelling behavior of the gel. A 5-fluorouracil was incorporated into the matrices during the crosslinking reaction, and in vitro release studies were performed in simulated gastric juice (pH 1.1) and in pH 7.4 buffer solution. The dried hydrogel samples were ground, and the particles obtained were analyzed by sieving on a mechanical shaker to obtain sizes ranging 20–90 μm. The prepared hydrogels were chemically stable in the dissolution media. The observed data demonstrated the potential application of these new matrices for peroral administration of anticancer agents *(43)*.

One of the salient features of the gastrointestinal (GI) tract is the large pH change from stomach to intestine. Quite often, such a pH change is exploited for targeted delivery of drugs either to the stomach or to the intestine. A semi-interpenetrating polymer network of poly(vinylpyrrolidone-co-acrylic acid) and poly(ethylene glycol) containing 5-fluorouracil was prepared *(45)*. Poly(vinylpyrrolidone-co-poly[ethylene glycol]) containing 5-fluorouracil was also synthesized using poly(ethylene glycol diacrylate) *(46)*. Because these dosage forms were able to release the entrapped drug for periods of days/weeks, their clinical applicability is highly limited as the GI transit time is only several hours or less. The hydrogel formulation for the delivery of anticancer agent in the GI tract requires an effective platform that maintains the delivery module at the area of tumors.

2.5. Topical Applications

Gel-forming hydrophilic polymers are commonly used to prepare semisolid dosage forms, such as dermatological, ophthalmic, dental, rectal, vaginal, and nasal hydrogels. These are especially useful for application of therapeutic agents to mucous membranes and ulcerated tissues because their high water content reduces irritation *(47)*. Carbopol® hydrogels, which are loosely crosslinked poly(acrylic acid), were used to formulate topical delivery systems for treatment of multiple actinic keratoses and superficial basal cell carcinoma with 5-fluorouracil *(47,48)*. Mycosis fungoides, the most common type of cutaneous T-cell lymphoma, progresses in three clinical phases: the premycotic, mycotic, and tumor stages. Treatment with chemotherapy and radiotherapy in the earlier stages can result in cure of mycosis fungoides. Topically treated

hydrogels in sheet form (Nu-Gel® Wound Dressing, Johnson & Johnson Medical, Inc., Arlington, TX) can absorb exudate, contain odor, and reduce pain upon dressing removal from patients in the tumor stage of mycosis fungoides *(49)*. Hydrogel sheets have also been used to deliver recombinant interferon α2c and interferon β for treatment of condylomata acuminata *(50,51)*.

2.6. Rectal Applications

Eudispert® hydrogels were used for rectal delivery of hydrophilic 5-fluorouracil in rats *(52)*. The addition of capric acid or linolenic acid to the hydrogel increased the permeability of 5-fluorouracil through the rectal membranes. Eudispert hydrogels with capric acid may be a useful preparation for increasing the maximum plasma level and improving the absolute bioavailability of 5-fluorouracil after rectal administration.

3. APPLICATIONS OF HYDROGELS IN THE CANCER-RELATED AREA

3.1. Assessment of Tumor Cell-Induced Angiogenesis

Hydrogels were used to develop a quantitative assay system for in vivo evaluation of angiogenesis induced by human tumor cells in mice *(53,54)*. The human epidermoid carcinoma A431 cells cultured on microcarriers were microencapsulated with agarose hydrogel to isolate them from the immune system of the C57BL/6 mice after subcutaneous dorsal midline implantation. When A431 cell-containing microcapsules (diameter, 300 μm) were subcutaneously injected into mice, notable angiogenesis was observed at the site of implantation. The extent of angiogenesis was quantitated by measuring the hemoglobin content in the implanted site using a mouse hemoglobin (mHb) enzyme-linked immunosorbent assay system. This type of simple system allows quantitative evaluation of angiogenesis in mice induced by xenogeneic cells such as human tumor cells. This may be useful in testing antiangiogenic properties of various agents using human tumor cells. There are many hydrogel systems that can be used to microencapsulate cells. Alginate has been commonly used to encapsulate cells and proteins in various sizes and shapes.

3.2. Removal of Adriamycin From Blood

Anthracyclines, such as adriamycin, generally possess a long plasma half-life that might produce serious toxicity to myeloproliferative and cardiac cells. In patients with impaired liver function or biliary obstruction, cytotoxic blood levels tend to be maintained for excessive periods with resultant severe, and potentially lethal, acute toxicity. Acrylic hydrogel-coated activated charcoal was used for hemoperfusion of beagle dogs 4 h after an intravenous bolus of adriamycin (2.5 mg/kg) *(55–57)*. Throughout the 3-h hemoperfusion period, the extraction of adriamycin averaged 43%, which was a 20-fold increase in total body elimination of adriamycin. The extended hemoperfusion would have resulted in reduction of tissue concentrations of adriamycin. The role of the hydrogel coating was to increase blood compatibility. Hemoperfusion using hydrogel-coated activated charcoal may be useful in reducing blood levels of adriamycin in cases of accidental overdose or in patients with hepatic disease.

3.3. Solid-Phase Radioimmunoassay

A sensitive, rapid method for the measurement of MTX in biologic fluids has been developed using hydrogel-based, solid-phase radioimmunoassay. Rabbit antimethotrex-

ate antisera were added to hydroxyethylmethacrylate monomer before polymerization. The resultant hydrogel was lyophilized, ground to fine powder, and aliquoted into 3-mL syringes fitted with a fritted filter disk *(58)*. A dose-response curve expressing percent bound MTX versus antiserum concentration allowed measurement of drug concentrations less than 1 ng/mL. The controlled entrapment of antiserum into a hydrogel matrix was shown to be simple, inexpensive, and stable. The porosity of the hydrogels, which is related to the utility of the hydrogel as a solid phase, can be easily controlled by the concentration of crosslinking agent.

3.4. Hydrogels as a Culture Medium

Agar has been commonly used as the supporting gel for testing of antimicrobial susceptibility. The results of such testing are known to be influenced by both the nutrient milieu and the supporting gel *(59)*. Agar, obtained from red seaweed, is a complex mixture of neutral and acidic polysaccharides with variable quantities of lipids, metallic cations, and other unknown substances. Some of the components in agar may antagonize or boost certain antimicrobial or anticancer agents. Synthetic hydrogels with well-defined amino acid medium may yield reproducible solid medium without potential antagonistic or booster effects of some components of agar. Such a medium could be used as a reference medium for testing anticancer effects of various drugs.

4. FUTURE HYDROGEL TECHNOLOGIES

Hydrogels possess many properties useful for controlled drug delivery. Because of very high water content, hydrogels are known to be biocompatible, however, most polymers used in hydrogel synthesis are not degradable in the body. Thus, to avoid manual removal of hydrogel matrices after all drug is released, the use of biodegradable hydrogels is preferred. One way of preparing biodegradable hydrogels is to use proteins and polysaccharides. Currently available biodegradable polymers, such as poly(lactic acid) or poly(glycolic acid), are not water-soluble and cannot be used for making hydrogels. Synthesis of new biodegradable, hydrophilic polymers is needed. Another property that will make hydrogels even more useful is improved mechanical strength. Because of the absorption of large amounts of water, hydrogels are usually weak and may not be able to withstand pressures occurring in the body. Currently, hydrogels can be made to swell rapidly with large swelling ratios by making interconnected pores inside the hydrogels *(12)*. Such superporous hydrogels can be effectively used for chemoembolization, and the high mechanical strength of such hydrogels would make them more useful. Certain types of anticancer drugs have high molecular weights. For example, many angiogenesis inhibitors *(60,61)* are peptides or proteins. Delivery of peptide and protein drugs can be easily achieved using macro (or super) porous hydrogels and/or biodegradable hydrogels.

It is only 40 yr since the first synthetic hydrogels were proposed for bioapplications *(62)*. During this relatively short time period, remarkable advances have been made in the development of hydrogels with numerous properties. Hydrogels that respond *(i.e., either expand, shrink, or degrade)* to changes in environmental factors, such as temperature, pH, or salt concentration, are known as smart hydrogels *(63)*. Poly(acrylic acid) hydrogels respond to changes in environmental pH or salt concentration, while poly(N-isopropylacrylamide) hydrogels respond to temperature changes. These smart hydro-

gels can be used to target delivery of anticancer agents by exploiting small changes in pH naturally occurring in the body as well as artificial changes in local temperatures. Further advances in hydrogel research will undoubtedly result in hydrogels with new properties ideal for anticancer therapy.

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