

Hydrogels for Tissue Engineering

Introduction Organ failure is a major health issue. One of the best ways currently available to treat this type of ailment is organ transplantation; however, this solution is far from ideal. There is currently a severe shortage in donor organs. Of the over 79,000 people who were on the waiting list for an organ in the United States in the year 2002, over 6,000 of them died before they could receive one.¹ Even for those patients who are lucky enough to receive an organ, transplantation leaves much to be desired. On average, 15% of organ transplant recipients die within one year of their surgeries, and those who survive are destined to remain on immunosuppressant drugs for the rest of their lives.¹

Alternative therapies to transplantation include surgical reconstruction and the use of mechanical organ substitutes like kidney dialysis machines.² However, surgical reconstruction is difficult and can result in long term health problems, such as tumor development,² and machines cannot replace all of the functions of the diseased organ and thus cannot halt patient deterioration.² New, more effective, alternative therapies for organ failure are actively being sought. Tissue engineering is an emerging multidisciplinary field aimed at “develop[ing] biological substitutes that restore, maintain, or improve tissue function.”²

One method used in tissue engineering is the implantation of cells in a matrix. Cells can be harvested from another part of the patient’s body, from a human donor, or from a donor of a different species such as a cow or a pig.³ These cells are then incubated in the presence of a matrix in a process known as “seeding.” A matrix is a physical substance that maintains the structural integrity of the implant, provides a platform for the growth of new cells, and allows for the ingress of nutrients and the egress of wastes.³ The choice of material for the matrix is crucial, and several types of materials, including ceramics, steel, and polymers, have been tested.^{4,5} Polymers’ readily adaptable physical and chemical properties are quickly making them the material of choice for tissue engineering matrices.⁵

Hydrogels in particular are emerging as the polymeric material of choice for tissue engineering applications. A hydrogel is a hydrophilic polymeric network that can absorb many times its weight in water without dissolving.⁶ They were first suggested for use in biomedical applications by Wichterle and Lim in 1960, but their use did not become common in tissue engineering until almost 30 years later.⁷ Hydrogels can be made from either natural polymers such as poly(hyaluronic acid) and poly(sodium alginate) or synthetic polymers

such as poly(lactic acid), poly(N-isopropyl acrylamide), and poly(ethylene glycol) (Fig. 1).

Natural hydrogels most closely resemble the tissues they are designed to replace and are known to be biocompatible, but they are difficult to isolate from biological sources and suffer from

batch-to-batch variations. Synthetic hydrogels, on the other hand, can be reliably produced in large quantities and are amenable to many types of variations, but they are not always biocompatible.⁸

There are advantages and disadvantages involved in the use of hydrogels for tissue engineering. Their aqueous environments mimic those of cells in the body, they are porous for nutrient and waste diffusion, and they are usually biocompatible. On the other hand, they can be hard to handle, physically weak, and difficult to sterilize.⁹

Nevertheless, hydrogels are emerging as the material of choice for new tissue engineering applications. Some ways to synthesize hydrogels are depicted in Figure 2.⁹

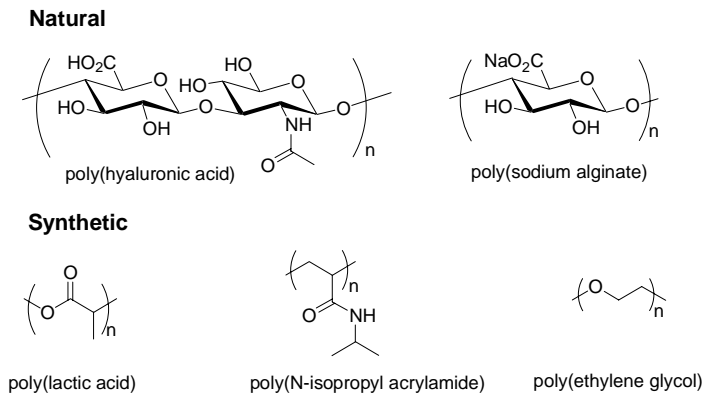


Figure 1. Structures of some polymers that can form hydrogels.

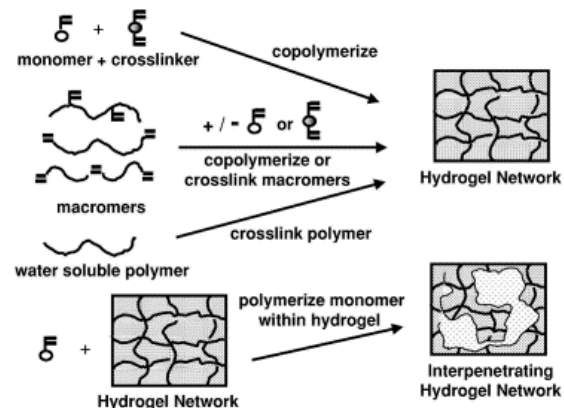


Figure 2. Methods of hydrogel synthesis.

Currently, there are three main obstacles facing scientists who wish to use hydrogels for tissue engineering. They are implant persistence, surgical issues, and cell attachment. Each of these obstacles, along with the strategies being employed to overcome them, will be discussed in turn.

Implant Persistence Once a cell-seeded hydrogel has been implanted, it needs to remain in the patient long enough to allow the cells to grow, but

can cause problems if allowed to stay too long. Such problems include: negative immunoresponses; weakening of the surrounding tissues; and a refusal to

integrate into the body. In some cases, surgical removal of the hydrogel implant is necessary, but it would be ideal if the implant would degrade naturally after serving its purpose. In order to achieve this goal, researchers have been

engineering biodegradability into hydrogels. This feature can be incorporated through the use of labile bonds in either the polymer backbone or crosslinkers. The most common choices for this task are esters, imines, and anhydrides, which are susceptible to hydrolysis. Less common are those hydrogels that have peptides as their labile segments. These hydrogels are degraded by enzymes in the patients' bodies.

It is not enough for the hydrogels to simply be biodegradable. The degradation must be controlled such that the hydrogel survives long enough to do its job. In the case of poly(anhydride) hydrogels, the hydrophobicity of the monomer unit can be altered to change the degradation time.¹⁰ As can be seen in Figure 3, the more hydrophobic the monomer, the slower the resulting hydrogel degrades.¹⁰ This variation in degradation rate with hydrophobicity can be accounted for by the degradation mechanism. Since the hydrogels degrade hydrolytically, it follows that the more water they have around them, or the more hydrophilic they are, the faster they will degrade.

Other poly(anhydride) macromers can be copolymerized to form hydrogels (Fig. 4).¹¹ These particular poly(anhydride) macromers are capped with methacrylate

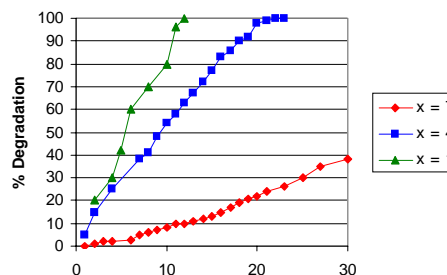
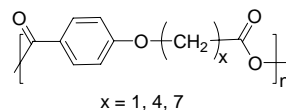


Figure 3. Degradation rate data of poly(anhydride) hydrogels.

endgroups that are used for crosslinking purposes. Again, the more hydrophobic

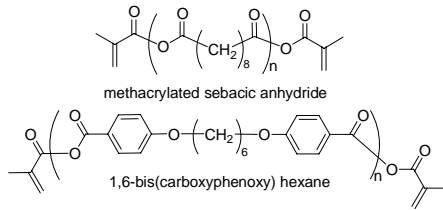


Figure 4. Structures of poly(anhydride) macromers.

also commonly used in tissue engineering studies.

Poly(ethylene glycol) (PEG) hydrogels have already been approved by the FDA for biomedical

use, and their known biocompatibility and

favorable physical properties make them very attractive for research in

this area. One drawback of PEG hydrogels is that they do not degrade

on any useful timescale. In order to remedy this situation, ester bonds have been

incorporated through the use of poly(lactide) (Fig. 5).¹² In these cases, the degradation

rate is controlled by changing the molecular weight of the PEG segment of the

macromer.¹³ PEG is very hydrophilic, and so the more PEG that is used per lactide

segment, the faster the resulting hydrogel will degrade. Also, longer PEG segments lead

to bigger spaces between crosslinks. Bigger spaces between crosslinks lead to a hydrogel

with larger pores, which in turn lead to more water incorporation and faster

degradation.¹³

Poly(ethylene glycol) hydrogels are

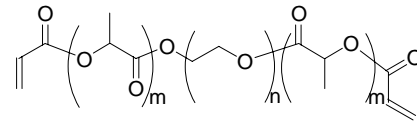


Figure 5. Structure of a PEG macromer modified by PLA.

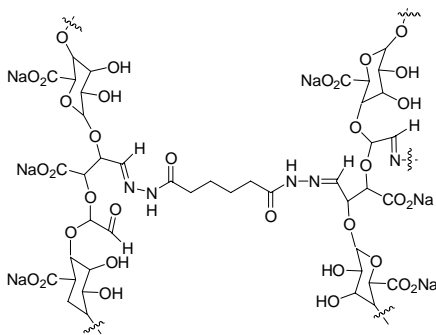


Figure 6. Crosslinking with a labile crosslinker.

Biodegradable hydrogels have been made from polymers that do not possess labile bonds in the backbone by using labile crosslinkers. This

option is used in the case of poly(aldehyde guluronate) hydrogels. This polymer is adapted

from a biological one through the oxidation of its alcohol groups to aldehydes prior to crosslinking

with adipic acid dihydrazide (Fig. 6).¹⁴ The

concentration of crosslinker used in the synthesis

of the hydrogel is a key factor in determining its

mechanical properties and degradation rate.

Higher concentrations of crosslinker lead to stiffer

of this type of hydrogel can be varied to change the degradation rate.¹⁴

In other cases, amide bonds provide the necessary labile elements that can be degraded by enzymes. The rate of degradation is controlled by the concentration of the required enzyme. Different peptide sequences can be employed in order to make the hydrogels susceptible to different kinds of enzymes.¹⁵ These types of biodegradable hydrogels are unlikely to find much use in clinical applications because of the inevitable patient-to-patient variation in enzyme levels. Such variations would make predictions of degradation time very inaccurate. For this reason, hydrolytically degradable hydrogels are much more likely to be pursued in the future.

Surgical Issues Implanting cell-seeded hydrogels presents two distinct problems for surgeons. First, the procedure often requires surgeons to make large incisions. Second, hydrogels cannot easily be cut into irregular shapes without the use of a mold. Implantation without major surgery and the ability to fabricate hydrogels with unique shapes are two goals that are being pursued through the fabrication of injectable hydrogels. Ideally, a liquid solution of polymer and cells could be injected into the patient and then made to set up into a gel. To accomplish this goal, scientists are seeking to: 1). exploit the temperature dependent phase transition between a solution and a gel with decreasing temperature; 2). alter the lower critical solution temperature (LCST); and 3). crosslink the polymers to form hydrogels *in vivo*.

Some hydrogels exhibit a phase transition from a gel to a solution upon heating. If this phase transition temperature is slightly above body temperature, a heated polymer-cell solution could be injected into the patient. Upon cooling to physiological temperatures, the polymer solution would set up into a gel, trapping the cells injected with it. Block copolymers of PEG with poly(lactide) (PLA) show such a phase transition upon heating. In this case, it is the hydrophobic packing of the PLA segments that drives the gelation.¹⁶ Increasing the molecular weight of the PLA segments allows the gelation event to take place at lower polymer concentrations, which is valuable in these situations.¹⁶ Interpenetrating networks (IPNs) have also been used in these types of applications. An IPN is a mixture of two or more polymers that are very closely physically mixed but have no covalent bonds between them. IPNs of poly(N-

acryloylglycinamide) (PAG) and poly(acrylic acid) (PAAc) form a gel at low temperatures due to hydrogen bonding between the two types of polymer.¹⁷ As the gel is heated, the hydrogen bonds are disrupted, causing dissolution of the IPN.¹⁷ Addition of urea to these IPNs lowers the phase transition temperature by destabilizing the hydrogen bonds; higher concentrations of urea lower the phase transition temperature more than lower concentrations (Fig. 7).¹⁷ Other types of IPN have been made which behave in a similar fashion.¹⁸

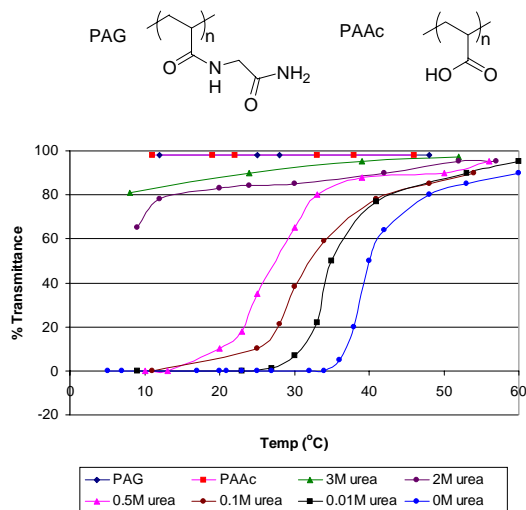


Figure 7. Phase transition data from PAG-PAAc IPNs.

These types of materials are not likely to be used in clinical settings because the high temperatures needed to keep the polymers in the solution phase may be detrimental to the seeded cells or the tissue surrounding the injection site.

A better option is to inject a room temperature polymer-cell solution, which would then set up into a gel upon warming to body temperature. Materials that exhibit a phase transition from a solution to a gel upon raising the temperature are said to have a

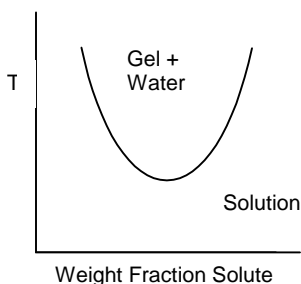


Figure 8. Generic phase diagram for materials that show an LCST.

lower critical solution temperature (LCST). A generic phase diagram of a material exhibiting an LCST is shown in Figure 8.¹⁹ In polymeric systems with an LCST, water can hydrogen bond to the polymer backbone. When the temperature is raised, those hydrogen bonds are disrupted, causing a collapse of the polymer accompanied by hydrophobic packing. The gain in entropy upon releasing bound water is the driving force for the phase transition. In order to take advantage of this phenomenon,

a delicate balance between hydrophobicity and hydrophilicity must be achieved. The polymer must be hydrophilic enough to allow water to hydrogen bond to the backbone while maintaining enough hydrophobicity to render hydrophobic packing a favorable event.

This balance between hydrophilicity and hydrophobicity can be exploited to alter the LCST. In acrylate based polymers, a pendant group can be changed to a more hydrophobic one in order to lower the LCST and to a more hydrophilic one to raise the LCST.¹⁹ This strategy works because the more hydrophobic groups discourage the hydrogen bonding of water in the first place, then they make hydrophobic packing more favorable such that lower temperatures are needed to trigger the phase transition. A more hydrophilic monomer would affect the LCST in an opposite fashion.¹⁹ Another way to change the LCST involves the copolymerization of monomers with different hydrophobicities. Poly(N-isopropylacrylamide) (PNIPAAm) hydrogels have an LCST around 31 °C and are thus attractive targets for this type of research. Copolymerization of NIPAAm with a more hydrophilic acrylate increases the LCST, but the phase transition becomes much broader in the process, spanning more than 5 °C (Fig. 9).²⁰ The loss of thermosensitivity is blamed on the change in the backbone structure of the polymer.^{20, 21}

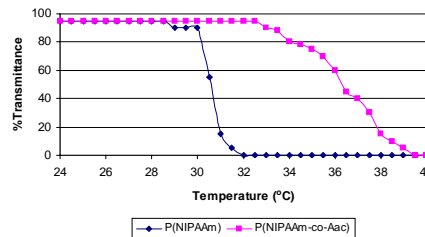
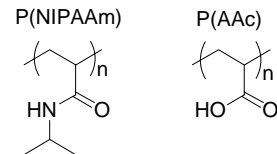


Figure 9. Thermosensitivity data for P(NIPAAm)-based hydrogels.

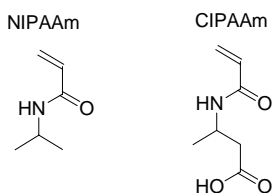


Figure 10. Comparison of NIPAAm to CIPAAm.

In order to maintain the thermosensitivity of PNIPAAm based hydrogels, new monomers that more closely resemble NIPAAm were synthesized (Fig. 10).²² The new monomers were then copolymerized with NIPAAm, and the phase transition was sharper than that seen with the original copolymers, but the expected jump in LCST was not seen.²³ Other studies were undertaken to attempt to raise the LCST of NIPAAm-based hydrogels while maintaining

thermosensitivity, but they were not successful.²⁴

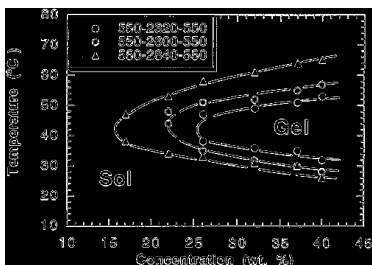


Figure 11. Phase diagram of PEG-PLGA-PEG block copolymers.

To avoid the issues surrounding PNIPAAm hydrogels, PEG-based hydrogels were then investigated to see if they could exhibit an LCST. In fact, both block and graft copolymers of PEG with poly(lactic acid-co-glycolic acid) (PLGA) displayed LCSTs.^{25, 26} In these cases, an increase in the length of the hydrophobic PLGA segments led to a decrease in the concentration necessary for gelation (Fig. 11).²⁶ Representative

photos of a hydrogel on either side of its LCST are shown in Figure 12.²⁷

Another strategy involves the *in vivo* crosslinking of a hydrogel. Methacrylated PEG was mixed with bovine cells and then injected into a mouse prior to exposing the mouse to ultraviolet light to crosslink the polymer.²⁸ This method, while intriguing, may not be useful for medical applications because of the intensity of the light used. In the *in vivo* studies, the light used was similar to that used in tanning beds, but the injection was made directly under the mouse's skin.²⁸ A deeper injection would require a much stronger light, which might cause damage to the injected cells and/or the surrounding tissue. Thus, success in the area of injectable hydrogels is most likely to come from those hydrogels that exhibit an LCST just below body temperature.

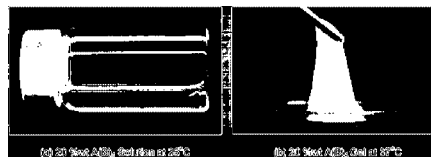


Figure 12. Photographs of a hydrogel above and below its LCST.

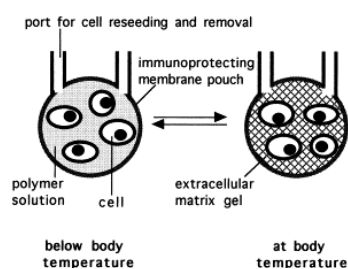


Figure 13. Schematic of an artificial pancreas.

encapsulation in the PNIPAAm hydrogel.³⁰ These experiments represent an initial step towards an artificial pancreas.

In a proof-of-principle experiment, Islets of Langerhans, found in the pancreas, were incubated in a PNIPAAm hydrogel prior to their injection into a membranous pouch. When the pouch was heated to 37 °C, the PNIPAAm set up into a gel, thus trapping the Islets of Langerhans (Fig. 13).^{29, 30} These cells continued to function normally, secreting insulin, even after

Cell Attachment In order for cells to grow and multiply, they require an extracellular matrix (ECM). Cells will secrete their own ECM if given enough time to develop, but in the initial stages of growth they depend on the ECM secreted by other cells. Therefore, in addition to providing the physical manifestation of a tissue engineering implant, the hydrogel must also serve as the ECM for the implanted cells. One of the critical functions of the ECM is to provide a surface upon which the cells can attach. Unfortunately, very few synthetic hydrogels display a natural tendency to adhere to cells. To ameliorate this problem, researchers have been using cell adhesion proteins in combination with hydrogels for tissue engineering applications.

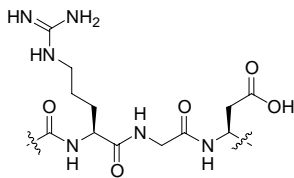


Figure 14. Structure of the RGD peptide fragment.

Cells have membrane bound receptors called integrins that bind to cell adhesion proteins.³¹ Mammals express many kinds of integrins, but almost all of them bind to the peptide sequence Arg-Gly-Asp (RGD) (Fig. 14).³¹ This peptide sequence can bind cells when attached to nonbiological substrates.³¹ Peptides containing the critical RGD sequence have been attached to polymers by condensing the N-terminus with an aldehyde functionality on the polymer backbone.³² Similar experiments conducted with poly(urethane) also led to polymer-peptide constructs that were shown to bind cells preferentially to those poly(urethanes) without peptide enhancement.³³ Other RGD enhanced hydrogels were synthesized from acrylate polymers.^{34,35} These hydrogels also displayed a better affinity for cells than did their non-peptide enhanced counterparts.

Outlook Despite the successes emerging from labs around the world, there is still a lot of work to be done in the field of tissue engineering.³⁶ This work will have to be done by researchers in a variety of different disciplines. Materials scientists and chemists will have to work together to develop a superior tissue engineering matrix. It will be necessary to combine the attributes of biodegradability, injectability, and cell affinity into one material that is also porous enough to allow diffusion of nutrients and wastes. Biologists and surgeons must work together to develop better surgical techniques for matrix implantation, improved bioreactors to allow for better cell growth, and the means

with which to allow cells to differentiate and become whole organs. In this era of multidisciplinary research, the future has never looked brighter for tissue engineering, and hydrogels figure to play an important role.

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