Article

Advances in Biomedical Hydrogels

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Introduction

Hydrogels are cross-linked networks of polymers swollen by water (usually 30% or more by weight, although no rigid limit exists). Because of the water, the polymer chains are further apart than in the bulk polymer. Diffusion is thereby enhanced, meaning that transport of important substances such as oxygen, proteins, and carbohydrates through the material is improved. Hydrogels are usually soft and compliant, allowing good physical compatibility with biological tissue. Many hydrophilic polymers used in hydrogels such as polyethylene oxide are very biocompatible, and present a low immunological profile. However, the physical properties of hydrogels are poor, and they cannot be used directly in areas of high stress such as joints.

To form a gel the polymer chains need to be chemically or physically cross-linked. Chemical cross-linking often provides better mechanical strength; however, the polymer is then slow or impossible to biodegrade. Physically cross-linked networks rely on changes in crystallinity, solvation or hydrogen bonding to link chains together, and in principle are easier to degrade when needed. The first biomedical application of hydrogels was in the area of soft contact lenses, where their high oxygen permeability proved a great advantage over the previous generation of hard polymer lenses.[1] This was followed by uses in drug release, tissue culture, and wound healing. Each area requires a different set of conditions with its own challenges to overcome.

Hydrogels can be synthesized from either natural or synthetic polymers. The latter offer more opportunities for modification, fine-tuning and control of structure. However, for applications inside the body they can suffer from much larger regulatory barriers for approval, and have to go through longer processes to demonstrate lack of toxicity, not only of the polymer itself, but also its breakdown products and any residual monomers. Many synthetic polymers cannot be degraded by the body and thus have to be excreted whole through the kidneys if possible. However, this is only possible if the polymer has a molecular weight of under 50,000.[2] Poly(ethylene oxide) (PEO) and poly(vinyl pyrrolidone) (PVP) are examples of the few water soluble synthetic polymers that are widely used in human medical applications. Most naturally occurring polymers possess low toxicity and are biodegraded into harmless substances. Proteins and polysaccharides such as chitosan, dextran, cellulose derivatives and hyaluronic acid can be easily gelled, and are often the first choice for clinical products. It is also common to modify the natural polymer to allow chemical cross-linking. Common natural and synthetic polymers used in biomedical hydrogels are shown in Fig. 1. Hydrogels for external application (e.g., wound patches) are less stringently controlled, as the potential toxicity concerns are less.

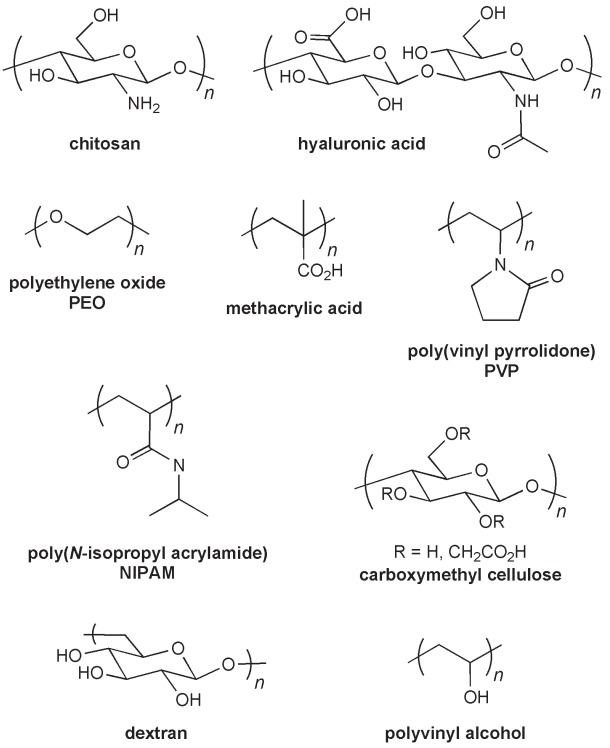

Fig. 1. Commonly used polymers in biomedical hydrogels.

Tissue Culture

Hydrogels are attractive in the area of tissue culture, where the goal is to grow replacement organs and parts for implantation into the body. Here, the hydrogel is attempting to replicate the extracellular matrix (ECM) that developing cells would usually encounter in the body. While the diffusion rate of small molecules such as oxygen through thin hydrogel constructs is good, there may still be problems with mass transport of larger molecules.[3] Extra porosity is then required, and it may be useful to employ such techniques as controlled microchannels,[4-6] embedded oxygen-carrying regions within the construct,[7] or nanoparticles to disrupt the gel structure.[8]

Apart from the high diffusion rates, another useful aspect of hydrogels is their low stiffness, as it has been found that certain stem cell lines can be adversely affected on contact with more rigid, denser materials.[9] In fact, the stiffness of the matrix can be used to control the differentiation of the stem cells. Using mesenchymal stem cells, it was found that soft matrices promote neuron-type cells, intermediate modulus matrices give myelinic cells, and hard matrices that mimic collagenous bone prove osteogenic.[10] The high crosslink density of hydrogels means that large objects such as cells might not be able to move or multiply through the gel. This is a potentially serious problem when using hydrogels in cell culture and has to be designed around carefully. In a popular approach, the gel is designed to be cleaved by the cells as they grow. This is often done through the use of protein segments in the

polymer that can be degraded by exoproteases released by the cells. As an example, a matrix metalloproteinase which is released by human mesenchymal stem cells was used to cleave specific peptide linkers within the gel during tissue growth.[11] Another simpler approach is to use materials such as polycaprolactone which can naturally hydrolytically degrade.[12] The challenge is that these materials are more hydrophobic and may not always integrate well with hydrogels. A second problem is that the rate of gel breakdown is determined by the macromolecular structure rather than the growing cells. However, hydrogels are soft and easily deformed, and so may be able to accommodate a large amount of cell division, even without degradation. It was found that growth rates of rabbit-derived mesenchymal stem cells were actually better in a nondegradable gel than in a degradable gel,[13] though the different chemical makeup of each gel precluded any firm conclusions.

If the gel is to be used for *in vivo* tissue growth, then biodegradability is also very important. In some cases the cell culture-hydrogel mixture is injected or placed directly into the desired body site. This is best suited when the new structure to be formed is simple, or needs to be firmly attached to surrounding tissue, as in the case of cartilage repair. Otherwise, the desired tissue can be grown *in vitro*, and transplanted at an advanced stage of development. Interpenetrating networks (where a new monomer is polymerized around an existing polymer scaffold) can offer some advantages in strength and functionality. Thus, for instance, a polyester provides the structural support, and an uncrosslinked monomer, such as polyvinyl alcohol, functions to attract the water.[14] An interpenetrating network of agarose and PEO for cartilage tissue engineering gave four times the strength of either monomer alone.[15]

As well as just providing support and nutrient flow, the hydrogel should also play a part in modulating the cell signaling events that control ECM production, cell proliferation and migration. Growth factors such as IGF can be incorporated to encourage differential cell proliferation.[16,17] For the growth of neural stem cells, gels containing bound factors such as interferon-γ were shown to improve differentiation in 2D and 3D-cultures.[18,19] The chemical functionality of the gel can also be used to differentiate potential cell lines. A PEO hydrogel containing either phosphate groups or t-butyl groups was found to exert osteogenic and adipogenic preferences to mesenchymal stem cells, respectively.[20]

Wound Healing and Surgery

The use of hydrogels in wound healing dates back to the late 1970s. By absorbing and retaining wound exudates along with foreign bodies, such as bacteria, within its network structure the gel can improve the wound outcome.[21] The optimal dressing should be able to retain and create a moist environment, allow for effective oxygen circulation to aid regenerating cells and tissues, and possess a low bacterial load.[22] Depending on their monomeric components, a hydrogel may possess inherent antimicrobial activity or have a known antimicrobial agent incorporated into the hydrogel mixture. Hydrogels can be applied as an amorphous gel, as an elastic, solid sheet, or as a film, according to the type of injury.

Synthetic and natural polymers may be mixed and/or derivatized to optimize the functionality of the hydrogel. For example, PVP is a well characterized polymer with good biocompatibility, but by itself does not exhibit good swelling properties.[23,24] However, when PVP is combined with polysaccharides such as carboxymethylcellulose (CMC),[25] carboxymethylchitosan,[26] or agar, its swelling properties improve. Chemical modifications of hyaluronic acid has been extensively considered for use in numerous surgical applications and wound healing.[27] Natural polysaccharides, in addition to their nontoxicity, are also advantageous owing to their biodegradability and relative abundance.[25]

During the past twenty years the Food and Drug Administration (FDA) in the US has approved more than 40 hydrogel products. The FDA defines a hydrogel as a wound and burn dressing that is available in sterile or nonsterile form, is intended to cover a wound, to absorb wound exudate, to control bleeding or fluid loss, and to protect against abrasion, friction, desiccation, and contamination.[28] One example, ProGel, is used as a surgical sealant in standard visceral pleural closures of visible air leaks incurred during resectioning of lung tissue. It consists of a solution of human serum albumin and a synthetic cross-linking component of polyethylene glycol (PEG) that is functionalized with succinate groups. Upon mixing a clear, flexible hydrogel is formed,[29] which can reduce the incidence of post-surgical air leaks from 86% to 65%.

Adhesions are a well-known problem occurring after surgery. These are essentially unwanted internal scarring which can cause such problems as dangerous obstructions or pressure on nerves, and lead to problems such as pain, infertility or even death. Confluent Spraygel is a chemically cross-linked PEO material that has proved to dramatically decrease pelvic adhesions. It works by physically separating the tissues during healing, preventing them from adhering together.[30] Other gel materials used for this purpose include hyaluronic acid (Sepragel) and polypropylene-polyethylene block polymers (FloGel). Their affect may be more than just a physical one, as some materials have been shown to make adhesions worse even with separation of tissues.[31] Adhesions are also a great problem in sinus surgery, and a chitosan-dextran aldehyde gel developed in Otago has been found to be extremely good at preventing adhesions and infections, and controlling bleeding (Fig. 2).[32] While the physical separation effect is also important, it is believed that the excess of carbonyl groups in the product has a marked biochemical impact on the migration and proliferation of fibroblasts that initiate the adhesions.[33] It also shows good antimicrobial properties.

Drug Delivery

Hydrogels have a fast, controllable diffusion rate, and so are attractive substrates for certain types of drug release applications.[34] This could be in the form of a film for surface delivery, a depot for implantation, or as nanoparticles for blood circulation. Many hydrogel wound-healing products have antibiotics or anti-inflammatory agents incorporated in them, which represents the simplest approach. The rate of diffusion of larger molecules can be

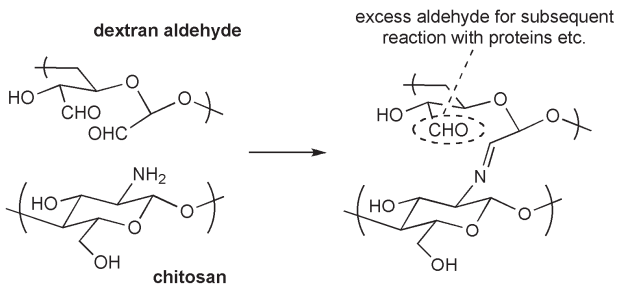

Fig. 2. Cross-linking of a chitosan-dextran aldehyde gel with excess aldehyde functionality for protein conjugation.[33]

effectively controlled through changes in the effective cross-link density.[35] As well, hydrogels can be used to model the processes involved in the transportation of molecules across membranes, which is very important in drug delivery processes.[36]

It has been tempting to investigate the use of nanomaterials owing to their ability to be delivered *via* the blood stream. Nature has already done this with ferritin, which helps store and circulate iron in the blood. As the vasculature around tumour cells are often more porous than normal cells,[37] it is also seen as a particularly useful way of targeting cancerous tissue. The problem with using simple nanogels as carriers for drug delivery is that normally they would release their payload too readily and non-selectively by simple diffusion. Hence, a major focus has been on 'smart' materials that will only release their contents with an external trigger. In one example, a nanogel was crosslinked with dithiol groups (Fig. 3). Upon reduction of the crosslinks with glutathione (as might happen inside the cell), release of the payload was found to be much more rapid.[38] This was shown to be a viable technique for the delivery of hydrophobic drugs, such as dioxyrubin, into cells *in vitro*.

It was noticed that some microorganisms found in the colon are able to reductively cleave azo-groups in small molecules. This proved useful in the design of a supramolecular hydrogel that could release its payload upon breakage of the azo-linker, and subsequent disruption of the gel structure.[39,40] This idea could then lead to materials that are able to selectively target certain groups of cells or microbes based on selective reactions. In a similar vein, disulphide cross-linked nanogels were found to release a variety of model drugs, including Doxorubicin, upon triggering with glutathione.[41]

Transport of large molecules is difficult across the blood brain barrier, and nanogels have been considered as possible delivery mechanisms. A cross-linked poly(ethylene glycol) and polyethylenimine nanogel was able to selectively incorporate negatively charged oligonucleotides and deliver them into bovine endothelial cells in an *in vitro* study, and a 15-fold improvement in delivery *in vivo* in mice.[42] Similar particles were able to successfully encapsulate cytotoxic 5-fluoroadenine arabinoside, and protect against enzymatic degradation as well as giving controlled release and delivery.[43]

Future Directions

There are several key challenges for hydrogels ahead. It is clear that simple matrixes can be designed to host cells and incorporate bioactives, with many successes to date. However, signalling process in the body that are important in healing and growing new tissue have only been crudely approximated in current materials. Targeting for precise drug delivery is still in its infancy. One area that is ripe for exploitation is integrating electrical conduction into the gel. In this way, it may be possible to stimulate neurons, providing triggers for *in vivo* drug release or act as sensors.[44]

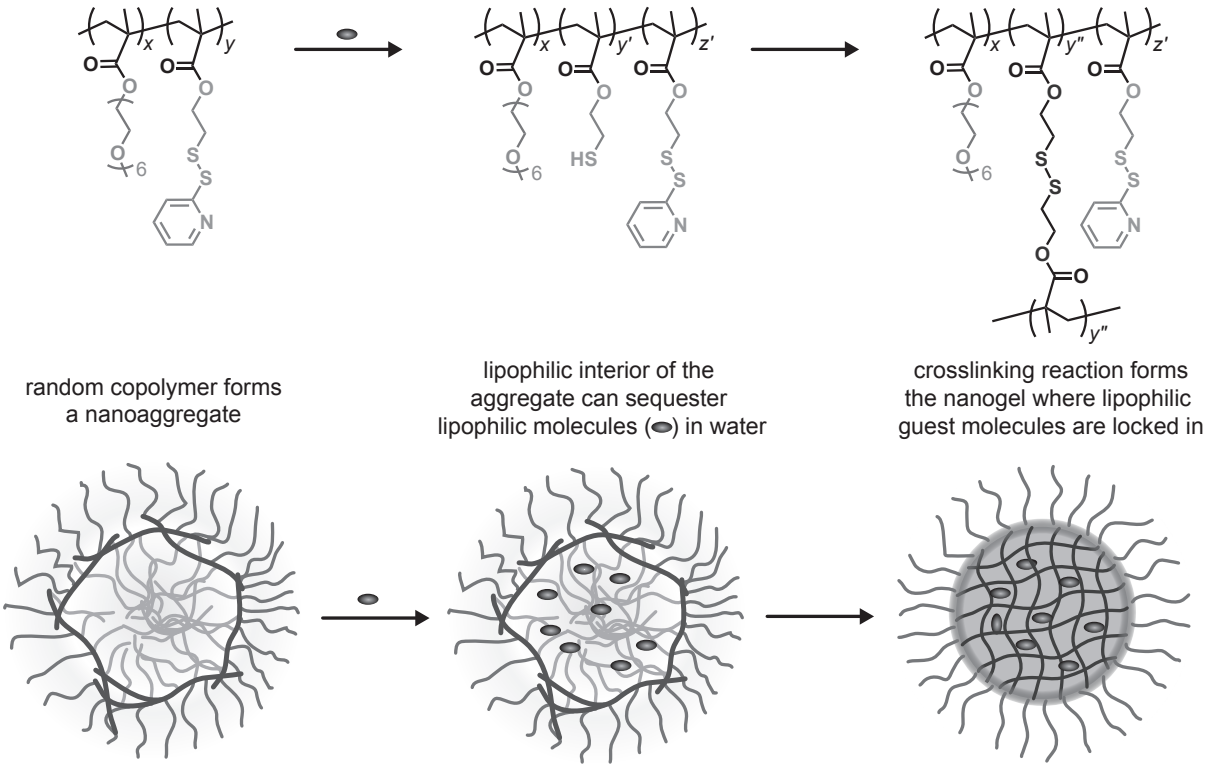

Fig. 3. The oxidative crosslinking of nanogel particles results in the trapping of lipophilic molecules or drugs, which can be released at under more reducing conditions, such as the inside of a cell (adapted from ref. 38).

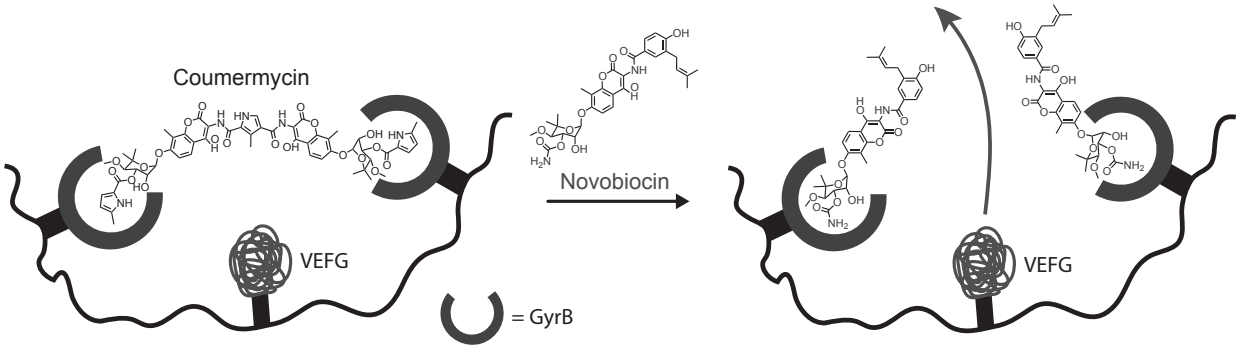

Fig. 4. *Left:* Cross-linking of gel with coumermycin. *Right:* Release of payload (VEGF) can be triggered by addition of novobiocin (adapted from ref. 46).

Taking a leaf from the world of viruses, the ideal hydrogel drug delivery platform would consist of small, stable nanogel particles that would selectively target the desired cells (such as liver, pancreas, tumours, etc.), presumably via surface functionality. The particle would enter the cell and then degrade rapidly owing to some trigger (change in pH, redox or presence of enzymes) and deliver its payload. The residual polymer material would then be rapidly degraded to non-toxic products and be metabolised or excreted. While advances have been made in all three areas, no present system can claim to solve all three in a cost-effective, safe and efficacious manner. Although there has been much work in this general area of nanoparticle drug delivery, most of the nanoparticles referred to in the literature could not strictly be described as hydrogels as they lack the high internal water content. However, there is much cross-over in ideas and techniques between the two fields.

In a good example of advanced nanoparticle drug design, polylactide-copolymer nanoparticles were decorated with fluoropyrimidine RNA aptamers that were specifically recognized by prostate cancer cells. The particles were loaded with the drug docetaxel, and they showed greater efficacy and reduced toxicity in mice models as opposed to the drug alone.[45] Another useful advance would to be to control the release of drugs via an external trigger which could be chemical, thermal, radiation, etc. While many gels can be broken apart by changes in pH or ionic strength, it is difficult to achieve more selective targeting. In an advance towards this goal, a dimeric binder – coumermycin – was used to cross-link a gel. Upon addition of a competitive monomeric binder novobiocin, the gel broke up and was able to release entrapped molecules (Fig. 4).[46,47]

The key challenge in tissue engineering is to be able to modulate the spatial and temporal environment of growing stem cells to allow for more complex differentiation, with the ultimate aim of growing complete organs *in vitro*. Even in something as seemingly simple as cartilage, it will be important to mimic the natural layered structure to ensure good strength and biocompatibility.[48] One approach is organ printing, where cells are deposited into structures from the top down using mechanical devices such as a gel matrix.[49] It is unlikely that small vascular can be printed, so this will rely on normal developmental signals. Another approach would be to use 3-D photolithography to pattern a gel with different areas, and seeded with a stem cell line capable of differentiation in the different environments. Two-dimensional photopatterning of gels is well known,[50] but much less work has been done in three dimensions. Polizotti and co-workers used orthogonal chemistry and a photo-ene coupling to pattern PEG hydrogels with well-defined biomolecules,[51] although this was not tested on cells. Later work by Anseth's team showed that cell migration can be influenced in a solid gel by 3-D patterning techniques based on controlled photodegradation.[52]

It is clear that there are still great challenges in growing functional organs *in vitro*, including delivery of nutrients and control of growth and differentiation. However, the advances over the last few years in patterning means that within the next ten years harvesting whole organs may indeed be practical.

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