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Hydrogels for biomedical applications☆

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ABSTRACT

This article reviews the composition and synthesis of hydrogels, the character of their absorbed water, and permeation of solutes within their swollen matrices. The most important properties of hydrogels relevant to their biomedical applications are also identified, especially for use of hydrogels as drug and cell carriers, and as tissue engineering matrices.

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1. Introduction

Since the pioneering work of Wichterle and Lim in 1960 on crosslinked HEMA hydrogels [1], and because of their hydrophilic character and potential to be biocompatible, hydrogels have been of great interest to biomaterial scientists for many years [2–9]. The important and influential work of Lim and Sun in 1980 [10] demonstrated the successful application of calcium alginate microcapsules for cell encapsulation. Later in the 1980s, Yannas and coworkers [11] incorporated natural polymers such as collagen and shark cartilage into hydrogels for use as artificial burn dressings. Hydrogels based on both natural and synthetic polymers have continued to be of interest for encapsulation of cells [12–15] and most recently such hydrogels have become especially attractive to the new field of 'tissue engineering' as matrices for repairing and regenerating a wide variety of tissues and organs [16–41].

Hydrogels are hydrophilic polymer networks which may absorb from 10–20% (an arbitrary lower limit) up to thousands of times their dry weight in water. Hydrogels may be chemically stable or they may degrade and eventually disintegrate and dissolve. They are called 'reversible', or 'physical' gels when the networks are held together by molecular entanglements, and/or secondary forces including ionic, H-bonding or hydrophobic forces [42,43]. Physical hydrogels are not homogeneous, since clusters of molecular entanglements, or hydrophobically- or ionically-associated domains, can create inhomogeneities. Free chain ends or chain loops also represent transient network defects in physical gels.

Abbreviations: CD, cyclodextrin; DX, *p*-dioxanone; EG, ethylene glycol; EGDMA, ethylene glycol dimethacrylate; HA, hyaluronic acid; HEMA, hydroxyethyl methacrylate; IPN, inter-penetrating network; MBAAm, methylene-bis-acrylamide; P(…), poly(…); PAAc, poly(acrylic acid); PAAm, polyacrylamide; PAGE, polyacrylamide gel electrophoresis; PAN, polyacrylonitrile; PBO, poly(butylene oxide); PCL, polycaprolactone; PEG, poly(ethylene glycol); PEI, poly(ethylene imine); PEO, poly(ethylene oxide); PEMA, poly(ethyl methacrylate); PF, propylene fumarate; PGEMA, poly(glucosylethyl methacrylate); PHB, poly(hydroxy butyrate); PHEMA, poly(hydroxyethyl methacrylate); PHPMA, poly(hydroxypropyl methacrylamide); PLA, poly(lactic acid); PLGA, poly(lactic-co-glycolic acid); PMMA, poly(methyl methacrylate); PNIPAAm, poly(*N*-isopropyl acrylamide); PNVP, poly(*N*-vinyl pyrrolidone); PPO, poly(propylene oxide); PVA, poly(vinyl alcohol); PVAc, poly(vinyl acetate); PVamine, poly(vinyl amine).

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When a polyelectrolyte is combined with a multivalent ion of the opposite charge, it may form a physical hydrogel known as an 'ionotropic' hydrogel. Calcium alginate is an example of this type of hydrogel. Further, when polyelectrolytes of opposite charges are mixed, they may gel or precipitate depending on their concentrations, the ionic strength, and pH of the solution. The products of such ion-crosslinked systems are known as complex coacervates, polyion complexes, or polyelectrolyte complexes. For example, the calcium alginate capsules of Lim and Sun [10] were coated with a complex coacervate of alginate–poly(L-lysine) (PLL) in order to stabilize the capsule. Complex coacervates and polyion complex hydrogels have become attractive as tissue engineering matrices. Sometimes, physical gels can form from biospecific recognitions, such as Conconavalin A with a polymeric sugar [44], or avidin with a polymeric biotin [45]. All of these interactions are reversible, and can be disrupted by changes in physical conditions such as ionic strength, pH, temperature, application of stress, or addition of specific solutes that compete with the polymeric ligand for the affinity site on the protein.

Hydrogels are called 'permanent' or 'chemical' gels when they are covalently-crosslinked networks. The synthetic hydrogels of Wichterle and Lim [1] were based on copolymerization of HEMA with the crosslinker EGDMA (see Abbreviations for definitions of acronyms). Chemical hydrogels may also be generated by crosslinking of water-soluble polymers, or by conversion of hydrophobic polymers to hydrophilic polymers plus crosslinking to form a network. Sometimes in the latter case crosslinking is not necessary. For example, in the hydrolysis of PAN to form amide and acid groups from the nitrile groups, if the nitrile groups remain in sufficient concentration and association, they can stabilize the hydrogel by hydrophobic interactions, thus forming a physical hydrogel. In the crosslinked state, crosslinked hydrogels reach an equilibrium swelling level in aqueous solutions which depends mainly on the crosslink density (estimated by the MW between crosslinks, M_c). Like physical hydrogels, chemical hydrogels are not homogeneous. They usually contain regions of low water swelling and high crosslink density, called 'clusters', that are dispersed within regions of high swelling, and low crosslink density. This may be due to hydrophobic aggregation of crosslinking agents, leading to high crosslink density clusters [46]. In some cases, depending on the solvent composition, temperature and solids concentration during gel formation, phase separation can occur, and water-filled 'voids' or 'macropores' can form. In chemical gels, free chain ends represent gel network 'defects' which do not contribute to the elasticity of the network. Other network defects are chain 'loops' and entanglements, which also do not contribute to the permanent network elasticity.

There are many different macromolecular structures that are possible for physical and chemical hydrogels. They include the following: crosslinked or entangled networks of linear homopolymers, linear copolymers, and block or graft copolymers; polyion–multivalent ion, polyion–polyion or H–bonded complexes; hydrophilic networks stabilized by hydrophobic domains; and IPNs or physical blends. Hydrogels may also have many different physical forms, including (a) solid molded forms (e.g., soft contact lenses), (b) pressed powder matrices (e.g., pills or capsules for oral ingestion), (c) microparticles (e.g., as bioadhesive carriers or wound treatments), (d) coatings (e.g., on implants or catheters; on pills or capsules; or coatings on the inside capillary wall in capillary electrophoresis), (e) membranes or sheets (e.g., as a reservoir in a transdermal drug delivery patch; or for 2D electrophoresis gels), (f) encapsulated solids (e.g., in osmotic pumps), and (g) liquids (e.g., that form gels on heating or cooling).

A wide and diverse range of polymer compositions have been used to fabricate hydrogels, and Table 1 summarizes the many varied compositions. The compositions can be divided into natural polymer hydrogels, synthetic polymer hydrogels and combinations of the two classes. Many different routes have been used to synthesize hydrogels, and they are summarized in Table 2 and shown schematically in Figs. 1–4.

Table 1
Hydrophilic polymers used to synthesize hydrogel matrices.[a]

Natural polymers and their derivatives (±*crosslinkers*)
Anionic polymers: HA, alginic acid, pectin, carrageenan, chondroitin sulfate, dextran sulfate
Cationic polymers: chitosan, polylysine
Amphipathic polymers: collagen (and gelatin), carboxymethyl chitin, fibrin
Neutral polymers: dextran, agarose, pullulan
Synthetic polymers (±*crosslinkers*)
Polyesters: PEG-PLA-PEG, PEG-PLGA-PEG, PEG-PCL-PEG, PLA-PEG-PLA, PHB, P(PF-co-EG)±acrylate end groups, P(PEG/PBO terephthalate)
Other polymers: PEG-bis-(PLA-acrylate), PEG±CDs, PEG-g-P(AAm-co-Vamine), PAAm, P(NIPAAm-co-AAc), P(NIPAAm-co-EMA), PVAc/PVA, PNVP, P(MMA-co-HEMA), P(AN-co-allyl sulfonate), P(biscarboxy-phenoxy-phosphazene), P(GEMA-sulfate)
Combinations of natural and synthetic polymers
P(PEG-co-peptides), alginate-g-(PEO-PPO-PEO), P(PLGA-co-serine), collagen-acrylate, alginate-acrylate, P(HPMA-g-peptide), P(HEMA/Matrigel®), HA-g-NIPAAm

[a] See Abbreviations for definitions of terms used.

2. Water in hydrogels

The character of the water in a hydrogel can determine the overall permeation of nutrients into and cellular products out of the gel. When a dry hydrogel begins to absorb water, the first water molecules entering the matrix will hydrate the most polar, hydrophilic groups, leading to '*primary bound water*'. As the polar groups are hydrated, the network swells, and exposes hydrophobic groups, which also interact with water molecules, leading to hydrophobically-bound water, or '*secondary bound water*'. Primary and secondary bound water are often combined and simply called the '*total bound water*'. After the polar and hydrophobic sites have interacted with and bound water molecules, the network will imbibe additional water, due to the osmotic driving force of the network chains towards infinite dilution. This additional swelling is opposed by the covalent or physical crosslinks, leading to an elastic network retraction force. Thus, the hydrogel will reach an equilibrium swelling level. The additional swelling water that is imbibed after the ionic, polar and hydrophobic groups become saturated with bound water is called '*free water*' or '*bulk water*', and is assumed to fill the space between the network chains, and/or the center of larger pores, macropores or voids. As the network swells, if the network chains or crosslinks are degradable, the gel will begin to disintegrate and dissolve, at a rate depending on its composition. It should be noted that a gel used as a tissue engineering matrix may never be dried, but the total water in the gel is still comprised of '*bound*' and '*free*' water.

There are a number of methods used by researchers to estimate the relative amounts of free and bound water, as fractions of the total water content. All of them are controversial, since there is proton NMR evidence that the interchange of water molecules between the so-called bound and free states is extremely rapid, perhaps as fast as one H_2O molecule every 10^{-9} s. The three major methods used to characterize water in hydrogels are based on the use of small molecular probes, DSC and NMR. When probe molecules are used, the labeled probe solution is equilibrated with the hydrogel, and the concentration of the probe molecule in the gel at equilibrium is measured. Assuming that only the free water in the gel can dissolve the probe solute, one can calculate the free water content from the amount of the imbibed probe molecule and the known (measured) probe molecule concentration in the external solution. Then the bound water is obtained by difference of the measured total water content of the hydrogel and the calculated free water content. Additional assumptions for use of this technique are that: (a) the solute does not affect the free and bound water distribution in the gel, (b) all of the free water in the gel

Table 2
Methods for synthesizing physical and chemical hydrogels.[a]

Physical gels
Warm a polymer solution to form a gel (e.g., PEO-PPO-PEO block copolymers in H_2O)
Cool a polymer solution to form a gel (e.g., agarose or gelatin in H_2O)
'Crosslink' a polymer in aqueous solution, using freeze–thaw cycles to form polymer microcrystals (e.g., freeze–thaw PVA in aqueous solution)
Lower pH to form an H-bonded gel between two different polymers in the same aqueous solution (e.g., PEO and PAAc)
Mix solutions of a polyanion and a polycation to form a complex coacervate gel (e.g., sodium alginate plus polylysine)
Gel a polyelectrolyte solution with a *multi*valent ion of opposite charge (e.g., $Na^+alginate^- + Ca^{2+} + 2Cl^-$)
Chemical gels
Crosslink polymers in the solid state or in solution with:
Radiation (e.g., irradiate PEO in H_2O)
Chemical crosslinkers (e.g., treat collagen with glutaraldehyde or a bis-epoxide)
Multi-functional reactive compounds (e.g., PEG + diisocyanate = PU hydrogel)
Copolymerize a monomer + crosslinker in solution (e.g., HEMA + EGDMA)
Copolymerize a monomer + a multifunctional macromer (e.g., bis-methacrylate terminated PLA-PEO-PLA + photosensitizer + visible light radiation)
Polymerize a monomer within a different solid polymer to form an IPN gel (e.g., AN + starch)
Chemically convert a hydrophobic polymer to a hydrogel (e.g., partially hydrolyse PVAc to PVA or PAN to PAN/PAAm/PAAc)

[a] See Abbreviations for definitions of terms used.

is accessible to the solute, (c) the solute concentration in the hydrogel's free water is equal to the solute concentration in the external solution, and (d) the solute does not interact with the gel matrix chains.

The use of DSC is based on the assumption that only the free water may be frozen, so it is assumed that the endotherm measured when warming the frozen gel represents the melting of the free water, and that value will yield the amount of free water in the HG sample being tested. Then the bound water is obtained by difference of the measured total water content of the HG test specimen, and the calculated free water content, similar to the above.

3. Pores and permeation in hydrogels

The amount of water in a hydrogel, i.e. the volume fraction of water, and its free vs. bound water 'character' will determine the absorption (or partitioning) and diffusion of solutes through the hydrogel. Pores may be formed in hydrogels by phase separation during synthesis, or they may exist as smaller pores within the network. The average pore size, the pore size distribution, and the pore interconnections are important factors of a hydrogel matrix that are often difficult to quantitate, and are usually included together in the parameter called 'tortuosity'. The effective diffusion path length across a HG film barrier is estimated by the film thickness times the ratio of the pore volume fraction divided by the tortuosity. These factors, in turn, are most influenced by the composition and crosslink density of the hydrogel polymer network.

Labeled molecular probes of a range of molecular weights (MWs) or molecular sizes are used to probe pore sizes in hydrogels [47]. Fluorescein-labeled dextrans are usually used. The same assumptions and restrictions apply to these probes as those for small molecular probes used to characterize free and bound water in a hydrogel.

Probe solute permeation is a useful method for characterizing pores and their interconnections in hydrogels. The probe solute size and shape, its relative hydrophilic and hydrophobic character, and the availability of 'free' water molecules to hydrate and dissolve the solute molecules are important factors governing solute permeation

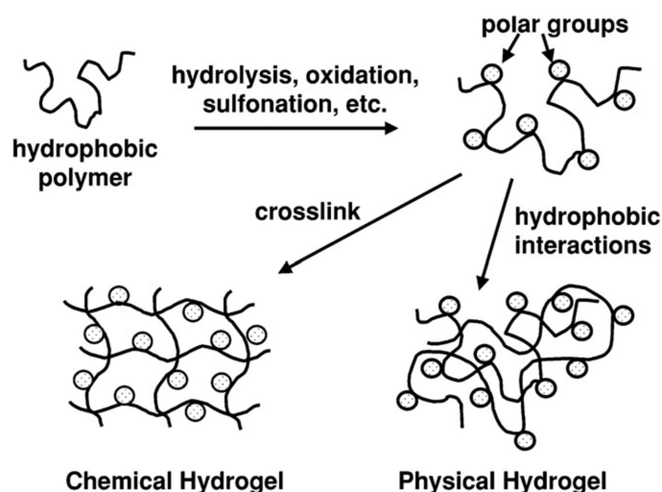

Fig. 2. Schematic of methods for formation of hydrogels by chemical modification of hydrophobic polymers. Examples of these types of hydrogels include (a) the partial hydrolysis of the acetate groups to –OH groups in conversion of PVAc to PVA, and (b) the partial hydrolysis of PAN to a polymer containing varying concentrations of acrylonitrile, amide and carboxyl pendant groups. In either case the resulting gel may be subsequently covalently crosslinked.

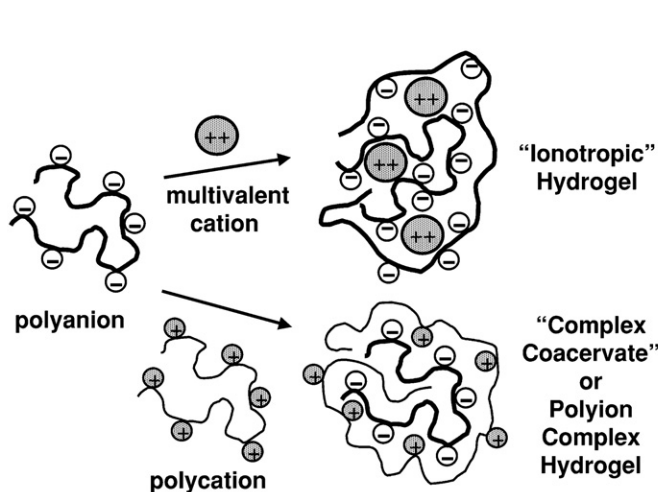

Fig. 1. Schematic of methods for formation of two types of ionic hydrogels. An example of an 'ionotropic' hydrogel is calcium alginate, and an example of a polyionic hydrogel is a complex of alginic acid and polylysine.

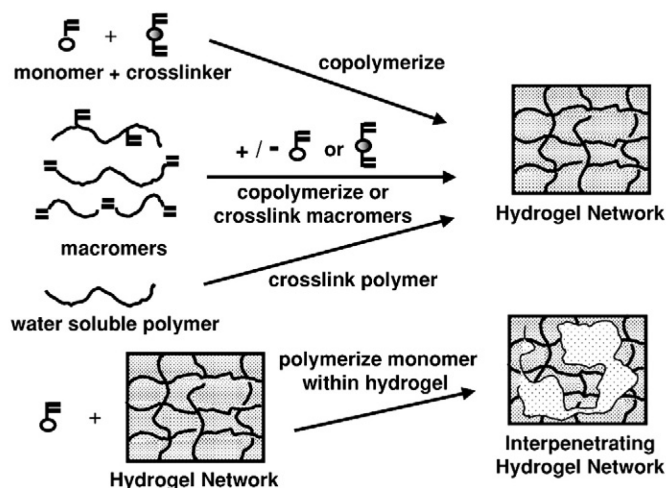

Fig. 3. Schematic of methods for formation of crosslinked hydrogels by free radical reactions, including a variety of polymerizations and crosslinking of water-soluble polymers. Examples include crosslinked PHEMA and PEG hydrogels.

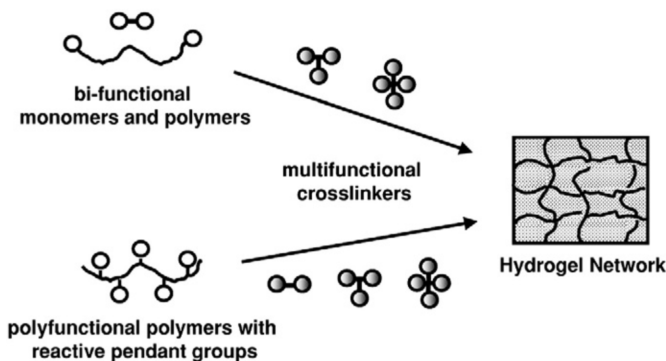

Fig. 4. Schematic of methods for formation of crosslinked hydrogels by condensation reactions of multifunctional reactants. Examples of the reactant groups include reactions of (a) isocyanates and amines or alcohols to form urea or urethane bonds, (b) amines or thiols and vinyl groups to form amines or sulfides by Michael additions, (c) amines and active esters such as *N*-hydroxy succinimide to form amides, (d) acids or acid chlorides and alcohols to form esters, (e) aldehydes and amines to form Schiff bases, etc. Typical examples of natural and synthetic polymers that are used to form hydrogels by such condensation reactions include many different types of polysaccharides, collagen, PAAc, PVA and PEG.

through any particular hydrogel. The permeation coefficient, *P*, is the product of the partition coefficient, *K*, and the apparent diffusion coefficient, $D_{app.}$

The partition coefficient, *K*, and uniformity of a protein/peptide drug loaded within a hydrogel will depend on the protein/peptide size, shape and net charge; the ionic, polar, apolar groups of the polymer, total available 'free' water within the hydrogel; the addition of partition enhancers to the solution; temperature, pH and ionic strength, and the drying method, if the hydrogel has been dried, since that often leaves a higher concentration of the drug at the outer regions of the hydrogel. If a protein drug is being loaded into a hydrogel, and if the protein has a net charge opposite to that of the hydrogel, then it may plug the pores at the surface during loading into the gel. On the other hand, if it has a net charge that is the same as that of the gel, then it may be excluded from the gel by Donnan exclusion. When loading a protein into a gel, the ionic strength, pH and buffer used in the protein solution may individually or together control the amount and distribution of the protein loaded into the gel.

The 'effective' or 'apparent' diffusion coefficient of the probe molecule, D_{app}, is equal to D_0 times the ratio of the pore volume fraction divided by the tortuosity, where D_0 is the diffusion coefficient in free water and the ratio of the pore volume fraction divided by the tortuosity is always <1. Diffusion in alginate hydrogels has been studied recently [48]. There will always be a portion of the imbibed water in a hydrogel that is not available for drug permeation due to pore 'dead ends', small pores that are less than the diameter of the drug molecule, H-bonded or hydrophobically 'bound' water, and drug–matrix polymer interactions.

Release of a macromolecular drug from a hydrogel will be controlled by the pore volume fraction, the pore sizes and their interconnections, the size of the drug molecule, and the type and strength of interactions of the drug with the polymer chains that make up the hydrogel network. In turn, the key factors that control the pore volume fraction, the pore sizes and their interconnections are the composition of the network polymer chains and the crosslink density. The interactions of the drug molecules with the network chains will be determined by their respective compositions. Thus, in designing a hydrogel network for controlled release of a drug, it will be necessary to 'match' the polymer composition and crosslink density with the particular size and composition of the drug molecule to be delivered.

4. Hydrogels as tissue engineering matrices

When parts or the whole of certain tissues or organs fail, there are several options for treatment, including repair, replacement with a synthetic or natural substitute, or regeneration. Fig. 5 shows how tissue or organ injury, disease or failure has evolved to reach the field of tissue engineering. Tissue repair or replacement with a synthetic substitute is limited to those situations where surgical methods and implants have achieved success. Although implants have been a reasonably successful option, tissue engineering holds out great promise for regeneration of the failed tissue. The first option for diseased or injured organs is extracorporeal treatment, in which blood is circulated through polymeric membrane exchange devices. These devices are usually passive exchange systems, but more recently experimental systems may contain entrapped or encapsulated cells from other human or animal sources. Those latter systems are called 'bioartificial' or 'biohybrid' organs. Total replacement of the diseased

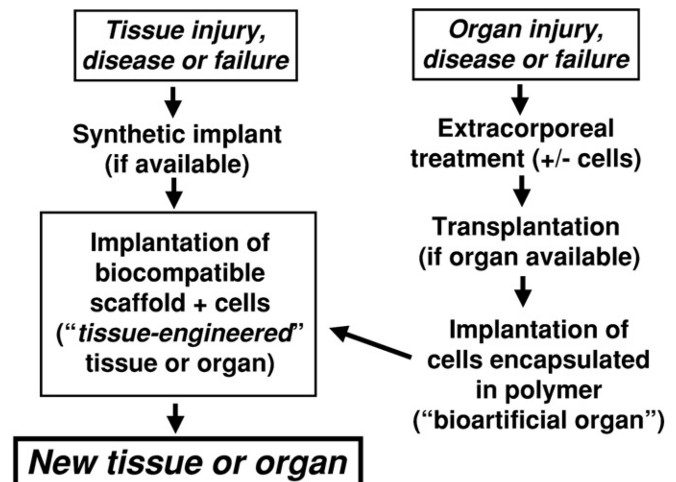

Fig. 5. Schematic showing the evolution of various therapeutic methods for treating injured or diseased tissues and organs, to tissue engineering for the repair, regeneration, or replacement of such tissues or organs.

Table 3
Important physico-chemical parameters and properties of hydrogels relevant to their use as matrices for tissue engineering.

Type of HG
Physical gel
Chemical gel
Molecular structures
Linear polymers
Block copolymers
Graft copolymers
Interpenetrating networks (IPNs)
Polyblends
Composition of HG
Natural polymers and their derivatives
Synthetic polymers
Combinations of natural and synthetic polymers
Important properties
Degradable or non-degradable
Injectable or pre-fabricated
Mechanical strength
Ease of handling
Shape and surface/volume ratio (sheets, cylinders, spheres)
Closed vs. open pores
Water content and character
Chemical modification (e.g., having attached cell adhesion ligands)
Added bioactive components (cells, drugs)
Sterilizability

Table 4
Advantages and disadvantages of hydrogels as tissue engineering matrices.

Advantages
Aqueous environment can protect cells and fragile drugs (peptides, proteins, oligonucleotides, DNA)
Good transport of nutrient to cells and products from cells
May be easily modified with cell adhesion ligands
Can be injected in vivo as a liquid that gels at body temperature
Usually biocompatible
Disadvantages
Can be hard to handle
Usually mechanically weak
May be difficult to load drugs and cells and then crosslink in vitro as a prefabricated matrix
May be difficult to sterilize

or malfunctioning organ or tissue with a natural substitute requires transplantation of an acceptable, healthy substitute, and there is a limited supply of such organs and tissues. Thus, tissue engineering holds out great promise for regeneration of organs.

Hydrogels have become increasingly studied as matrices for tissue engineering [49]. Hydrogels designed for use as tissue engineering scaffolds may contain pores large enough to accommodate living cells, or they may be designed to dissolve or degrade away, releasing growth factors and creating pores into which living cells may penetrate and proliferate. Table 3 lists the important parameters and properties of hydrogels for this application. Table 4 identifies the important advantages and disadvantages of hydrogels as matrices for tissue engineering. One significant advantage of hydrogels as tissue engineering matrices vs. more hydrophobic alternatives such as PLGA is the ease with which one may covalently incorporate cell membrane receptor peptide ligands, in order to stimulate adhesion, spreading and growth of cells within the hydrogel matrix. However, a significant disadvantage of hydrogels is their low mechanical strength, posing significant difficulties in handling [50]. Sterilization issues are also very challenging. It is clear that there are both significant advantages and disadvantages to the use of hydrogels in tissue engineering, and the latter will need to be overcome before hydrogels will become practical and useful in this exciting field.

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