Hydrogels for Cell Immobilization

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Abstract

Hydrogels are being investigated for mammalian cell immobilization. Their material properties can be engineered for biocompatibility, selective permeability, mechanical and chemical stability, and other requirements as specified by the application including uniform cell distribution and a given membrane thickness or mechanical strength. These aqueous gels are attractive for analytical and tissue engineering applications and can be used with immobilization in therapies for various diseases as well as to generate bioartificial organs. Recent advances have broadened the use of hydrogel cell immobilization in biomedical fields. This paper covers the methods and current development for immobilization of mammalian cells in hydrogels. It covers hydrogel requirements for use in adhesion, matrix entrapment and microencapsulation, the respective processing methods, as well as current applications.

Keywords: Hydrogel, Cell immobilization, Microencapsulation, Immunoisolation, Bioartificial organs

1. Introduction

Hydrogels are polymers cross linked via chemical bonds, ionic interactions, hydrogen bonds, hydrophobic interactions or physical bonds. These materials absorb water and swell readily without dissolving. Hydrogels are being investigated for cell immobilization in medicine and biotechnology. They chemically or physically hold the cells to provide stability, structural support or immunoisolation. Both synthetic and naturally derived hydrogels have been explored for immobilization of a variety of cell types. Hydrogels are promising for tissue reconstruction and regeneration as scaffolds and templates for entrapped cells. The hydrogels provide the structural support, which may be important in preserving the phenotype expression and function of the immobilized cells. Moreover, they maintain the high water content necessary for maintaining a chemical balance with polymer cell complex.

2. General Hydrogel Requirement

For successful immobilization, the hydrogel must be conducive to cell viability and function (biocompatible). It must also have proper permeability to allow sufficient diffusion and transport of oxygen and essential nutrients, metabolic waste and secretory products across the hydrogel network. Chemical and mechanical stability are two more demands of hydrogels as biomaterials. The ability to engineer the bulk and surface properties of hydrogels to satisfy these requirements makes hydrogels attractive for cell immobilization. Biocompatibility is necessary for successful cultivation of cells on or within the polymer. The hydrogel must be nontoxic; it should be relatively inert and not interfere with cell functions. The hydrophilicity of hydrogels renders most of them biocompatible.

Mass transport requirements also demand rigorous attention. Sufficient supply of oxygen and essential nutrients through the hydrogel network, plus adequate removal of metabolic waste and phenotype secretions are vital for sustaining the immobilized cells in the hydrogel complexes. The ability of hydrogel to facilitate diffusion and transport via media or body fluid limit the size and shape of hydrogel device feasible for immobilization. Proper design network, pore size, and chemical composition, which affect the interaction between the diffusing species and the molecular mesh is essential – otherwise cell morbidity results.

3. Methods

Three main methods for immobilizing cells in hydrogel are available: adhesion; matrix entrapment; and microencapsulation.

3.1 Adhesion

Adhesion, is based on the attachment of the cells to the polymer substrate. Immobilization by adhesion is generally used to stabilize cells for culture or analytical procedures, provide a structural template directing cell growth and differentiation or both.

3.1.1 Hydrogel requirements for adhesion

In addition to the general hydrogel requirements, successful immobilization by adhesion depends on cell attachment to the hydrogel substrate to preserve cell functions. Hydrogels can be engineered with bioadhesive properties. Several factors affect cell affinity and behavior of hydrogels: general chemistry of the monomers and the crosslinks; hydrophilic and hydrophobic properties; and surface properties involving charged functional

chemical groups. One way to enhance cell adhesion is by adding immobilized cell-adhesive proteins or oligopeptides, such as argine-glycine-aspartic acid (RGDs) sequence in the hydrogel. This approach preserves the bulk permeation properties of the hydrogel while improving its cell adhesion quality. Physical characteristics of the hydrogel also govern the adhesion affinity; therefore affecting the pore size and network structure can modify cell adhesion as well as morphology and function.

3.1.2 Methods

Macroporous hydrogel membranes are manufactured by several techniques. One method of constructing pores large enough by cell growth is by phase separation in polymer and solvent mixture. The "freeze-thaw" and the porosigen techniques are the other two approaches. Briefly, the hydrogel is polymerized around a crystalline matrix made from freezing the aqueous solvent around the porosigens of desired size (e.g. sucrose or dextrin). With the "freeze-thaw" method, the ice-based crystalline matrix is then thawed after UV polymerization, leaving macroporous foam. Another method recently reported for constructing hydrogel foams uses gas bubbles from sodium bicarbonate to create macroporous network. Bubbles are trapped during the gelation stage; thus, foam morphology is dependent on polymerization kinetics and varies for different hydrogel compositions.

3.1.3 Applications of adhesion

Hydrogels are used to assist in stabilizing cells and to provide cell growth template, especially for anchorage-dependent cell types. Naturally derived hydrogels, such as collagen and collagen mixtures, are popular as adhesion-based extracellular matrix analogs. The adhesive property of hydrogels can be enhanced by proteins or peptides. Collagen-based hydrogels were used to immobilize endothelial cells on hybrid vascular grafts. Neural tissue engineers have also attached neuroblast cells on collagen composites with polyglycerol methacrylate and other acrylic hydrogels. Results show that hydrogels successfully immobilized the cells and supported the cell functions.

Immobilized cell-specific proteins or peptides in hydrogels were also used to trap activated blood platelets and endothelial cells. Poly Ethylene Glycol, PEG microwells create regions of low shear stress for cell immobilization and subsequent spheroid formation while preventing random cell adhesions on substrate surface. Although these systems allow for 3D cell aggregations of a single cell type, they may not be suitable for co-cultures of additional cell types in a spatially controlled manner because of the non-adhesive property of the polymers. With the goal of generating spatially controlled 3D co-culture systems, photocrosslinkable chitosan is used. Chitosan is a hydrophilic and non-toxic polysaccharide. Because of its biocompatibility and similarities to naturally occurring glycosaminoglycans, chitosan is useful for various biomedical applications in tissue engineering, drug delivery, wound healing, and surgical adhesives. These polymers were used for studies of *in vitro* and *in vivo* growth of liver cells such as stellate, Hep G2, NIH 3T3 cells,etc. This study has made possible the making of an artificial liver.

Adhesion alone does not offer immunoisolation. For in vivo investigations, adhesion-based immobilization must be manufactured in conjunction with either polymeric membrane or matrix entrapment methods.

3.2 Matrix Entrapment

The primary distinction between adhesion and matrix entrapment is how the cells are held by the hydrogel. Unlike adhesion, matrix entrapment relies on physical constraint of the cells within the hydrogel network. Matrix entrapment is sometimes called microencapsulation when the hydrogel isolates the entrapped cells and provides immunoprotection. Hydrogels are ideal for matrix entrapment because the crosslinks of both synthetic and naturally derived hydrogels provide the essential three dimensional mesh and porous network to hold the cells in place while allowing the transport of nutrients, wastes, and other essential molecules via the bulk fluid. Matrix entrapment can be used with in vivo studies to protect transplanted cell-hydrogel complexes from mechanical and immunological damage.

3.2.1 Hydrogel Requirements for Matrix Entrapment

Hydrogels for matrix entrapment must be biocompatible to cells and host and have acceptable diffusive and transport properties. In addition, hydrogels for matrix entrapment must allow uniform cell distribution and must be formed by mild polymerization techniques. When cells are present during in situ polymerization of cell-hydrogel complexes, use of harsh solvents, toxic monomers, UV radiation, and high temperatures must be avoided to ensure adequate cell survival. Hydrogels used to protect transplanted cells for in vivo studies must also have selective permeability to allow diffusion of essential molecules but not host immune agents.

3.2.2 Methods

Hydrogels for matrix entrapment can be manufactured in various shapes. Gels are often polymerized in situ with the cells in molds or in air or oil (beads). Threads or tube-shaped gels can be manufactured using cylindrical molds.

3.2.3 Applications of Matrix Entrapment

One of the applications of matrix entrapment is stabilizing cells for cell culture and analysis.Matrix entrapment of hepatocytes incollagen was shown to preserve cell functions for much longer than standard culture methods, allowing extended in vitro metabolic studies of liver cells. A hydrogel sandwich configuration was also developed for long term metabolic studies with this cell types. Collagen entrapped hepatocytes succeeded the transplanted artificial livers. Other applications of matrix entrapment with collagen as an extracellular matrix analog include the development of hierarchically structured hybrid vascular grafts. Unlike the unimmobilized cultures,the organization of collagen-entrapped smooth muscle cells (SMCs) as well as the monolayer of endothelial cells (ECs) cultured above the cell hydrogel complex resembled that of normal muscular arteries after mechanical simulation. ECs and SMCs immobilized in collagen dermatan sulphate hydrogels also exhibited normal alignment and functions within a short period after transplantation.

Similarly, human esophageal epithelial cells were immobilized in collagen gel to crete a hybrid artificial esophagus. Furthermore, the success of collagen composites used to entrap neuroblasts promises potential reconstruction and regeneration of neural tissues. Matrix entrapment can be engineered for immunoprotection.

3.3 Microencapsulation

Microencapsulation surrounds single cells or small clusters of cells with a thin macroporous semipermeable membrane. The principal of mammalian cell encapsulation with hydrogels is that the permeability of the membrane is engineered to allow the passage of oxygen, important nutrients and cellular products but it stops the ingress of immunoglobulins or immune cells responsible for transplant rejection. The primary application of of encapsulation is immunoprotection of healthy xenogenic or allogenic cells for transplantation into a recipient in need of functional replacement of a metabolic tissue. The protected cells then function and secrete an effective supply of the needed hormone, protein or other bioactive secretory product for the host.

3.3.1 Hydrogels requirements for microencapsulation

In case of microencapsulation, the hydrogel must be semipermeable to allow the passage of low-molecular-weight molecules important for the cell survival and replacement of physiologic function; however, they must prevent the transport of immunoglobulins which are of higher molecular weights. The diffusive properties of hydrogels can be tailored by altering their hydrophilicity and the degree to which they are crosslinked. Most hydrogels engineered for encapsulation have a molecular weight cut-off in the range of 50,000 to 100,000 to exclude cytotoxic antibodies, whole cells, and other deleterious complex macromolecules. For optimum transport of molecules across the membrane and effective immunoprotection, the hydrogel microcapsules must have uniform wall thickness. Most microcapsules have wall thickness between 10 and 100 μm and have diameter less than 1 mm. Microcapsules can hold one or more cells and often larger microcapsules hold small clusters of cells. Hydrogel capsules must withstand prolonged implantation without breaking or dissolving to prevent loss of their immunoprotective ability. Postimplantation, the mechanical and structural stability of the membrane capsule must remain intact in the physiologic environment.

3.3.2 Methods

Microencapsulation involves the formation of polymer membranes around individual cells. The most widely used procedure involves the gelation of charged polyelectrolytes around the cell core.Coextrusion is another method for encapsulation of of mammalian cells. Cells can be co extruded with a polymer solution through a coaxial needle assembly. Shear force due to a coaxial air/liquid stream flowing past the tip of the needle assembly causes the hydrogel to envelop the cells and fall off. The encapsulated cells fall through a series of oil phases which cause precipitation of hydrogel around the cell. This process termed "simple coacervation". A potential disadvantage of this technique is that the organic solvents, which may be harmful to living cells, are used to precipitate the hydrogel. To eliminate the use of organic solvents, complex coacervation was developed using acidic and basic water-soluble polymers. Briefly, a droplet containing one of these polymers and cells is added to the other polymer. A thin membrane encapsulates the droplet due to ionic interactions of the two polymers. The major disadvantage of this method is that the capsules may be unstable due to high water uptake in the capsule wall. Modifications have been made to better control permeability and stability of the hydrogel capsules. Photopolymerization has been used to coat hydrogel capsules to improve their biocompatibility as well as to form the innermost hydrogel layer. Photopolymerization permits gelation of the polymer membrane in the presence of dissolved oxygen which is helpful for cell survival during the encapsulation process. Successful photopolymerization of polyethylene glycol based macromers in direct contact with cells and tissue has been achieved. The advantage of this technique is that the membrane is directly in contact with the encapsulated cells. Minimizing diffusion distance for oxygen,nutrients and cell products is important for eliminating necrosis at the center of the capsule and for improving therapeutic efficiency.

3.3.3 Applications of microencapsulation

Several different hydrogels have been investigated to determine the efficacy of encapsulation therapy as treatment for multiple diseases in a variety of animal models. For instance, alginate-polylysinealginate microcapsules have been reported to reverse diabetes in rats and mice. The mild encapsulation process preserved the integrity of the islet's secretory function and longterm viability was maintained. Acrylates have also been investigated for microencapsulation because they are well tolerated by the host's immune system and have exceptional hydrolytic stability. Acrylates investigated as hydrogel materials for mammalian cell encapsulation include the following: poly2-hydroxyethyl methacrylate, polymethyl methacrylate, polymethacrylic acid and polydimethylaminoethyl methacrylate. The most successful and widely used polyacrylate has been a poly-2-hydroxyethyl methacrylate-*co*-methyl methacrylate [P(HEMA-co-MMA)] coploymer with 75% HEMA coploymer fraction. The HEMA portion imparts the hydrogel with its biocompatible water-swollen character and the mma confers mechanical strength and durability. The [P(HEMA-co-MMA)] hydrogel has been used to encapsulate islets, heptocytes, human heptoma cells and variety of other cells.

4. Conclusions

Mammalian cell immobilization offers a useful method to preserve long-term viability and function of cells for in vitro models and for transplantation. The successful utilization of the immobilization methods adhesion, matrix entrapment and microencapsulation, can greatly enhance our understanding of cell development and phenotype expression as well as expand the tissue donor pool by providing whole-organ transplantation.

All three immobilization techniques have in common critical issues required for their success. In summary, the hydrogels must be biocompatible and nontoxic to the cells or host. They must allow sufficient transport of oxygen, nutrients, metabolic wastes and secretory products to and from the cells to the bulk media or the

Table 1: Hydrogels for cell immobilization

Methods	Type of hydrogel	Application
Adhesion	Poly(vinyl alcohol)	Cornea epithelial cells
	Poly(2-hydroxyethyl methacrylate)	Fibroblasts, chondrocytes
	Poly(N-vinyl pyrrolidone-methyl methacrylate-cellulose acetate butyrate)	Articular chondrocytes
	Poly(glycerol methacrylate)/collagen	Neuroblasts
	Collagen/dermatan sulphate	Vascular endothelial cells
Matrix entrapment	Collagen	Heptocytes, esophageal epithelial cells
	Agarose	Erythrocytes, pancreatic islets
	Alginate	Tumor cells
Microencapsulation	Agarose	Pancreatic islets
	Alginate-polylysine-alginate	Pancreatic islets
	alginate-polylysine-alginate-poly(ethylene glycol)	Pancreatic islets

vascular system. Hydrogels have shown very promising results for cell immobilization as culture substrate, structural support plus physical and immunological protection in the host. As technology progresses and immobilization method and properties are refined, cell immobilization methods and material properties are refined, cell immobilization with hydrogels will become a major therapeutic treatment for several diseases and valuable tool for tissue reconstruction and regeneration.

5. References

1. Junji Fukuda, Ali Khademhosseini, Yoon Yeo, Xiaoyu Yang, Judy Yeh, George Eng, James Blumling, Chi-Fong Wang, Daniel S. Kohane, Robert Langer. 2006. 'Micromolding of photocrosslinkable chitosan hydrogel for spheroid microarray and co-cultures.' *Biomaterials 27.* **5259–5267.**
2. Bell,C.L.,Peppas,N.A.1995. 'Biomedical membranes from ydrogels and interpolymer complexes'. *Adv. Polymer science.* 122 **125-176.**
3. Oxley, H.R., Corkhill, P.H., Fitton, J.H., Tighe, B.J.1993. 'Macroporous hydrogels for biomedical applications: methodology and morphology'. *Biomaterials 14.* **1064-1072.**
4. Sefton, M.V., Stevenson, W.T.K. 1993. 'Microencapsulation of live animal cell using polyacrylates.' *Adv. Polymer science 107.* **143-197.**
5. Smetana, K. Jr. 1993. 'Cell biology of hydrogels.' *Biomaterials 14,* **1046-1050.**
6. Wells, G.D.M, Fisher, M.M.,Sefton,M.V. 1993. 'Microencapsulation of viable hepatocytes in HEMA-MMA microcapsules: A preliminary study'. *Biomaterials 14,* **615-620.**
7. Woerly, S. 1993. 'Hydrogels for neural tissue reconstruction and transplantation.' *Biomaterials 14,* **1056-1058.**
8. Wiley and sons Encyclopedia of Polymer science.
9. Anna C. Jen, M. Conley Wake and Antonios G. Mikos 1996. 'Hydrogels for cell immobilization'. *Biotechnology and bioengineering,* vol 50 **357-364.**