

GELS FOR ENCAPSULATION OF BIOLOGICAL MATERIALS

This application is a continuation-in-part of U.S. Ser. No. 07/958,870 entitled "Gels for Encapsulation of Biological Materials" filed Oct. 7, 1992, now U.S. Pat. No. 5,529,914 which is a continuation-in-part of U.S. Ser. No. 07/870,540 entitled "Gels for Encapsulation of Biological Materials" filed Apr. 20, 1992 (now abandoned).

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to methods for coating and/or encapsulating surfaces and three-dimensional objects with cross-linked networks of water-soluble polymers.

Microencapsulation technology holds promise in many areas of medicine. For example, some important applications are encapsulation of cells for the treatment of diabetes (Lim, F., Sun, A.M. "Microencapsulated islets as bioartificial endocrine pancreas", (1980) *Science* 210, 908-910), encapsulation of hemoglobin for red blood cell substitutes, and controlled release of drugs. However, using the prior art methods, the materials to be encapsulated are often exposed to processing conditions, including heat, organic solvents and non-physiological pHs, which can kill or functionally impair cells or denature proteins, resulting in loss of biological activity. Further, even if cells survive the processing conditions, the stringent requirements of biocompatibility, chemical stability, immunoprotection and resistance to cellular overgrowth, of the encapsulating materials restrict the applicability of prior art methods.

For example, the encapsulating method based on ionic crosslinking of alginate (a polyanion) with polylysine or polyornithine (polycation) (Goosen, et al., (1985) *Biotechnology and Bioengineering*, 27:146) offers relatively mild encapsulating conditions, but the long-term mechanical and chemical stability of such ionically crosslinked polymers remains doubtful. Moreover, these polymers when implanted in vivo are susceptible to cellular overgrowth (McMahon, et al., (1990) *J. Nat. Cancer Inst.*, 82(22), 1761-1765) which over time restricts the permeability of the microcapsule to nutrients, metabolites and transport proteins from the surroundings. This has led to starvation and death of encapsulated islets of Langerhorns (O'Shea, G. M. et al. (1986) *Diabetes*, 35:943-946).

Thus, there remains a need for a relatively mild cell encapsulation method which offers control over properties of the encapsulating polymer and yields membranes in the presence of cells which are permeable, chemically stable, and very highly biocompatible. A similar need exists for the encapsulation of biological materials other than cells and tissues, as well as materials contacting biological materials.

Materials are considered biocompatible if the material elicits either a reduced specific humoral or cellular immune response or does not elicit a nonspecific foreign body response that prevents the material from performing the intended function, and if the material is not toxic upon ingestion or implantation. The material must also not elicit a specific reaction such as thrombosis if in contact with the blood.

Gels made of polymers which swell in water to form a hydrogel, such as poly(hydroxyethyl methacrylate) (poly-(HEMA)), water-insoluble polyacrylates, and agarose, have been shown to be useful for encapsulating islets and other

animal tissue (Iwata, et al., (1989) *Diabetes*, 38:224-225; Lamberti, et al., (1984) *Appl. Biochem. Biotech.*, 10, 101-105 (1984). However, these gels have undesirable mechanical properties. Agarose forms a weak gel, and the polyacrylates must be precipitated from organic solvents, which are potentially cytotoxic. Dupuy, et al. (1988) have reported the microencapsulation of islets by polymerization of acrylamide to form polyacrylamide gels. However, the polymerization process requires the presence of toxic monomers such as acrylamide and cross-linkers, and, if allowed to proceed rapidly to completion, generates local heat.

Microcapsules formed by the coacervation of alginate and poly(L-lysine) have been shown to be immunoprotective, for example, as described by O'Shea, et al., 1986. However, a severe fibrous overgrowth of these microcapsules was observed following implantation (McMahon, et al. 1990; O'Shea, et al., 1986). The use of poly(ethylene oxide) (PEO) to increase biocompatibility is well documented in literature. The biocompatibility of alginopoly(L-lysine) microcapsules has been reported to be significantly enhanced by incorporating a graft copolymer of PLL and PEO on the microcapsule surface (Sawhney, et al., "Poly(ethylene oxide)-Graft-Poly(L-Lysine) Copolymers to Enhance the Biocompatibility of Poly(L-Lysine)-Alginate Microcapsule Membranes," (1991) *Biomaterials*, 13, 863-870).

The PEO chain is highly water soluble and highly flexible. PEO chains have an extremely high motility in water and are essentially non-ionic in structure. Immobilization of PEO on a surface has been largely carried out by the synthesis of graft copolymers having PEO side chains (Sawhney, et al.; Miyama, et al., 1988; Nagoaka, et al.). This process involves the custom synthesis of monomers and polymers for each application. The use of graft copolymers, however, still does not guarantee that the surface "seen" by a macromolecule consists entirely of PEO.

Electron beam cross-linking has been used to synthesize PEO hydrogels, which have been reported to be non-thrombogenic by Sun, et al., (1987) *Polymer Prepr.*, 28:292-294; Dennison, K. A., (1986) Ph.D. Thesis. Massachusetts Institute of Technology. However, use of an electron beam precludes including with the polymer any living tissue since the radiation is cytotoxic. Also, the networks produced by this method are difficult to characterize due to the non-specific cross-linking induced by the electron beam.

Photopolymerization of PEG diacrylates in the presence of short wavelength ultraviolet light initiation has been used to entrap yeast cells for fermentation and chemical conversion (Kimura, et al. (1981), "Some properties of immobilized glycolysis system of yeast in fermentative phosphorylation of nucleotides," *Eur. J. Appl. Microbio. Biotechnol.*, 11:78-80; Omata et al., (1981), "Steroselective hydrolysis of dl-methyl succinate by gel-entrapped *Rhodotorula minuta* uzr. *texensis* cells in organic solvent," *Eur. J. Appl. Microbial Biotechnol.*, 11:199-204; Okada, T., et al., "Application of Entrapped Growing Yeast Cells to Peptide Secretion System," *Appl. Microbiol. Biotechnol.*, Vol. 26, pp. 112-116 (1987). Other methods for encapsulation of cells within materials photopolymerizable with short wavelength ultraviolet radiation have been used with microbial cells (Kimura, et al., 1981; Omata, et al., 1981; Okada, et al., 1987; Tanaka, et al., 1977; Omata, et al., 1979a; Omata, et al., 1979b; Chun, et al., 1981; Fukui, et al., 1976; Fukui, et al., 1984). However, yeast cells and some microbial cells are much harder and resistant to adverse environments, elevated temperatures, and short wavelength ultraviolet radiation than mammalian cells and human tissues.

There are several problems with these methods, including the use of methods and/or materials which are thrombogenic