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Polymer Substrates for Controlled Biological Interactions

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Abstract The identification of a myriad of small peptide and carbohydrate ligands recognized by cell surface receptors has generated enthusiasm for the use of these ligands of components of biomaterials for controlling cellular interactions. Achieving control of cell interactions via ligand modification of materials also requires that nonspecific interactions of cells with these materials due to surface adsorption of biological macromolecules is minimized. Polyethylene oxide (PEO) exhibits extraordinary inertness toward most biological macromolecules and is thus receiving increasing attention as a component of new materials for controlling cell behavior. Both surface and bulk modifications with PEO are being applied to develop a range of bland substrate materials as vehicles for ligand immobilization.

Key words: polyetheylene oxide, receptor-mediated interactions, biomaterials, tissue regeneration, ligands

Tailoring surface properties to control cellular interactions with biomaterials is a well-established strategy for enhancing biocompatibility. The early clinical use of nylon fabrics in vascular grafts in the mid 1950s, for example, was followed almost immediately by attempts to improve nylon's hemocompatibility by altering its water contact angle through various chemical treatments [Edwards and Tapp, 1955]. This traditional goal, controlling cellular interactions by preventing them altogether, remains important in a diverse number of applications such as catheters, contact lenses, and artificial kidneys. Creating "inert" surfaces which meet the needs of these applications is an ongoing challenge. It is an alternative approach to controlling biological interactions, though, creating surfaces which are highly cell-interactive, which has fueled the expansion in the biomaterials community which has occurred over the past decade. Modification of surfaces to foster cell interactions via specific receptor-mediated phenomena provides a basis for expanding the use of biomaterials to a broad variety of applications in which cells are an integral part of the final device. The majority of implantable devices currently in clinical use serve to replace original tissue with a synthetic materials, and clinical successes are most notable in the connective tissues, e.g., diseased blood vessels are replaced with dacron tubes, damaged cartilage is replaced with silicone rubber, and cataracts are replaced with polymethylmethacrylate lenses. The potential for controlling cell migration and tissue growth and organization by means of selective interactions between cells and biomaterials means that new clinical products based on transplantation of metabolically active cells, such as liver, will likely be developed, and that the long-term performance of prosthetic tissue replacements can be improved by incorporating cells as an active component of the replacement devices. The success of this new strategy, however, often relies on building an inert surface first and then incorporating the desired cell-interactive ligands to the bland surface. Thus, the two strategies for controlling cell interactions with biomaterial surfaces are interrelated.

There are many similarities between these design strategies for biomedical materials and design strategies for immobilizing proteins and cells for biotechnology applications, and it is not surprising that some materials are used in both areas. One material which illustrates the crossover between biotechnology and biomaterials particularly well is polyethylene oxide (PEO),

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which has been found by both the biotechnology and biomedical materials communities to be a versatile "inert" material.

Perhaps more than with any other polymer, attention has been focused on using PEO as a means of preventing non-specific interactions of biological macromolecules with materials and thus enhancing biocompatibility. Polyethylene oxide (the dihydroxy-terminated polymer of low molecular weight is also referred to as polyethylene glycol, or "PEG") is a linear polyether, $-(CH_2CH_2O)_n$. It exhibits both hydrophilic (due to hydrogen bonding with the ether oxygen) and hydrophobic character and is infinitely soluble in water and many organic solvents. Although it interacts with cell membranes and can form complexes with certain polyanions, its remarkable quality is that it tends to exclude other polymers in aqueous solutions. It is thus virtually non-interactive with proteins and most biological macromolecules, and it has been used extensively in pharmaceutical applications as a non-toxic excipient. An excellent compendium of the biologically related applications of PEO is given in a recently published monograph [Harris, 1992].

In clinical biomaterials applications, PEO is most often used as a component of block, segmented, or graft copolymers; few clinically approved devices are made solely from PEO. The first widespread biomaterials application of PEO came about in the late 1960s when Spandex-type polyether urethanes were adapted for use in the artificial heart and other devices requiring the strength and elasticity offered by polyurethanes, and the greater hydrolytic stability of polyether over polyester soft segments [Boretos, 1984]. Their relatively good blood and tissue compatibility was attributed in part to the hydrophilicity of the PEO soft segment. Many efforts to enhance compatibility of polyurethanes further, particularly for blood-contacting applications, have thus centered on increasing the content of PEO at the surface to cause a decrease in protein and platelet deposition [Sa Da Costa, Brier-Russell et al., 1981; Okkema, Grasel et al., 1989]. This approach has produced mixed results. The complex relationships between bulk chemistry, processing conditions, and surface chemistry have proved difficult to delineate in these materials [Tyler et al., 1992]. Other elastomers which incorporate PEO into the bulk have been developed but have likewise proved insufficiently inert for demanding applications such as small diameter vascular grafts [Chaikof et al., 1989]. This has given researchers strong motivation to pursue approaches based on surface modification with PEO rather than relying just on bulk phase modifications to impart changes in surface properties.

Many strategies have been used to coat or modify surfaces with PEO layers to enhance biocompatibility. Adsorption of PEO-containing block copolymers has been shown to reduce protein adsorption significantly, but such adsorbed polymers desorb over time in the biological environment [Lee et al., 1989]. Stable PEG surface layers have been achieved using a variety of covalent grafting techniques. However, obtaining complete surface coverage and thus complete protein resistance is difficult because PEG chains naturally repel each other in aqueous solution [Gölander et al., 1992]. Direct measurement of the amount of PEO linked to the surface is difficult, and the efficacy of surface modification with PEO is typically assessed by reduction in nonspecific adsorption of plasma proteins (fibrinogen and albumin). Variability is thus inherent in results reported by different groups. It is often reported, though, that the efficacy of surface grafting in imparting protein resistance depends on PEO molecular weight in a nonlinear fashion; intermediate chain lengths are more effective than short or long chains. Theoretical analysis of the dependence of surface repulsive forces developed by PEO chains grafted to hydrophobic surfaces predicts protein repulsion in aqueous solution increases with increasing chain length and increasing surface density (i.e., decreasing chain-to-chain spacing) [Jeon et al., 1991]. In practice, high surface densities of long chains are difficult to achieve using standard grafting techniques. Novel approaches to increasing surface density, and thus surface repulsive forces, include grafting under solvent conditions where the chains are highly compressed (near the cloud point) and grafting of branched and star PEOs [Bergström et al., 1992; Merrill, 1993]. High surface chain densities may also be achieved by attaching to one end of the PEO chain a molecule which will self assemble, thus overcoming repulsive forces between chains [Needham et al., 1992].

The role of PEO in providing cell repellence is evolving into a role in promoting specific, controlled interactions between cells and materials. As more and more is understood about the molecular basis of cellular interactions with extra-

cellular matrix, rational design of surfaces to foster cell interactions is increasingly being viewed as a means of creating biocompatibility. In the past, fostering cell interactions by surface modification typically meant adsorption of proteins which modified cell behavior, e.g., adsorption of serum or fibronectin to the surface of vascular grafts to promote endothelialization. Protein adsorption has a number of drawbacks, though, for modification of implant materials. The conformation of adsorbed proteins is difficult to control, and surfaces which allow adventitious protein adsorption may allow displacement of the desired proteins in vivo by other biological macromolecules. Specificity of interaction with desired cell type is also difficult to achieve because ECM molecules often have multiple cell and protein-binding sites and can interact promiscuously with many cell types. And for products intended for human implantation, protein purity and immunogenecity may raise concerns about safety. The identification of minimal cell-binding epitopes in ECM proteins and receptors which recognize them led to efforts to develop completely synthetic materials which interact with cells via well-defined peptide or carbohydrate ligands. Since it is often important that the synthetic base materials which incorporate these cell-interactive ligands exhibit minimal nonspecific interactions with biological fluids, PEO retains a key role in the development of new materials for controlled cellular interactions. A wide range of PEO derivatives are now available commercially to meet these needs (Shearwater Polymers, Huntsville, AL).

Selectivity of cell interactions with ligands linked to PEO-modified surfaces can potentially be controlled either by using highly specific ligands or by controlling the surface concentration of a nonspecific ligand. Vascular graft materials modified with PEO-linked cell adhesion peptides, for example, demonstrate selective interactions of human endothelial cells, in comparison to smooth muscle cells and fibroblasts, if a selective peptide (REDV) is immobilized or if a nonselective peptide (YIGSR) is immobilized in low concentration [Hubbell et al., 1991].

CURRENT RESEARCH

The development of techniques for surface modification which render polymer surfaces noninteractive in the biological milieu remains a critical problem in biomaterials and it is being approached from many perspectives. A high level of interest in PEG-modified surfaces is sustained by the fact that the end(s) of the PEO chain can be used as sites for ligand attachment. The problems encountered with modification of surfaces by grafting with linear PEG have not diminished the interest in PEO-modified surfaces but have instead stimulated novel approaches to building PEO-rich surface layers.

An approach which addresses the problem of surface coverage is synthesis of materials composed entirely, or almost entirely, of PEO. In contrast to materials such as polyurethanes which incorporate PEO but which have a significant fraction of another component, materials comprised primarily of PEO have limited mechanical strength in their hydrated state. Nevertheless, there are a many applications where the hydrogel nature of such PEO-based materials is entirely appropriate and even desirable, for example, engineering of soft tissues such as liver, nerve, skin, and breast; encapsulation of islets and other secretory cells in a permselective membrane for transplant; and formation of barriers between tissues to prevent postsurgical adhesions.

Formation of PEO hydrogels can be achieved by chemical or radiation crosslinking in aqueous solution. PEO can be chemically crosslinked only by the end groups because there are no chemically reactive groups along the backbone. Various approaches have been taken to such crosslinking. A versatile approach has been developed by Hubbell and co-workers [1991], who formed PEO gels by photo-crosslinking end-activated PEO macromers with a photo-sensitive initiator. These gels are formed in situ by a nontoxic process and have been applied to clinical problems where prevention of cell adhesion is desired for example, to form semi-permeable membranes around islets, where the permeability of the membrane is controlled by the macromer size and the crosslinking conditions. Modification of the macromers to incorporate hydrolytically labile ester groups imparts controlled degradation of the resulting gels, and these degradable materials are in clinical trials for prevention of post-surgical adhesions.

Increasing Ligand Capacity on PEO: Radiation Crosslinking

Chemical crosslinking via the end groups inherently limits the availability of end groups for further reaction, specifically, linkage of ligands for promotion of cell interactions. Crosslinking

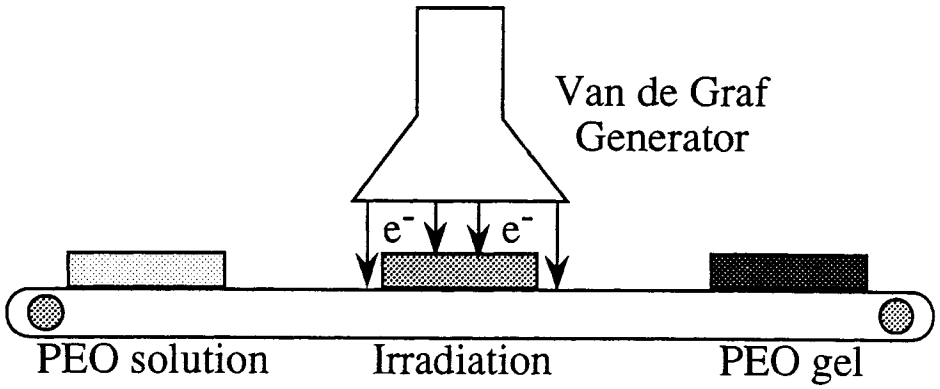
via the backbone carbons of PEO can be achieved by high-energy radiation (gamma rays or electron beam), and this is an approach we are taking as a route to forming PEO gels which contain free terminal hydroxyl residues for ligand attachment. Radiation crosslinking of PEO is free-radical mediated and is typically carried out in aqueous solution containing 5–20% polymer by weight (Fig. 1). In solution, crosslinking reactions dominate over chain scission reactions, while chain scission dominates under most conditions in the dry powder. The reaction chemistry leading to backbone crosslinking of PEO is shown in Figure 1. Side reactions leading to chain scission and to formation of reactive carbonyls and unsaturated bonds can be minimized in most cases by using high radiation dose rates and purging oxygen from solutions before irradiation.

Radiation crosslinked gels with suitable mechanical properties can be formed from linear PEO if the molecular weight is greater than 100,000 and such gels from crosslinked high molecular weight PEO have been commercialized by Union Carbide as a wound-healing product (Vigilon™). However, gels formed from linear PEO contain a relatively low concentration (<1 mM) of reactive end groups for ligand derivatization. The free terminal hydroxyl concentrations which can be obtained in linear gels may be great enough to obtain desirable cell interactions with certain ligands (notably RGD cell-adhesion peptides) but may be too low for many receptor-mediated interactions. Considering RGD peptides as model ligands, it has been shown that cells will adhere and spread on polyacrylamide gels which contain <1 μM covalently linked peptide [Brandley and Schnaar, 1989]. These results compare well with observations that cells form stress fibers on RGD linked to rigid surfaces when the spacing is no greater than 140 nm [Massia and Hubbell, 1991]; if one assumes the top 1 nm of gel is accessible to the cell surface, 140 nm spacing corresponds to 0.9 μM concentration of ligand in the gel. If adhesion and stress fiber formation are the desired interactions, gels formed from linear PEO may suffice. However, other biological responses, such as migration, replication, or retention of differentiated function, which are known to be sensitive to ligand concentration [DiMilla et al., 1992; Mooney et al., 1992], may require higher surface densities of adhesion peptide, as may other receptor-ligand pairs. Liver cells, for example, adhere to galactose-modified substrates via the hepatic asialoglycoprotein receptor. A minimum ligand concentration of 3 mM is required for hepatocyte adhesion to galactose-modified polyacrylamide gels, and cell spreading is not observed unless the concentration is at least an order of magnitude higher [Oka and Weigel, 1986]. Obtaining such high ligand concentrations (>10 mM) in a PEO gel material while maintaining the other desirable features of PEO which contribute to its biocompatibility requires that the concentration of terminal hydroxyls be increased over that in linear PEO gels.

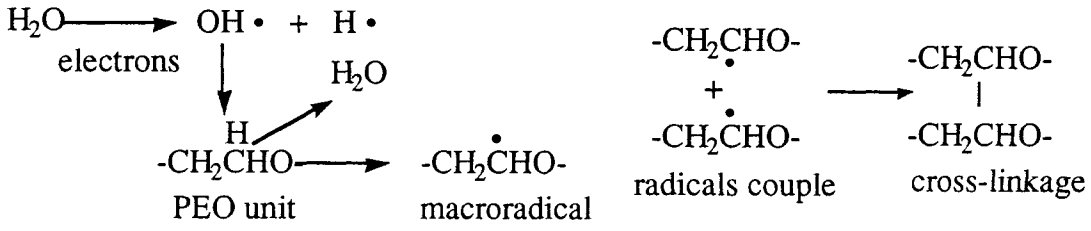
Further Enhanced Ligand Capacity on PEO: Star Polymers

One way to increase the ligand concentration in a PEO-based gel is to use a multifunctional or branched form of PEG. Relatively few such types of molecules have been synthesized. We have been developing materials for receptor-mediated cell interactions based on polyethylene oxide star polymers synthesized by Rempp and co-workers [Merrill et al., 1990; Rempp et al., 1990] in order to obtain PEO gels with terminal hydroxyl concentrations (and ultimately, ligand concentrations) as high as 20–30 mM. These PEO star polymers comprise 10–50 PEO arms emanating from a divinyl benzene (DVB) core and are synthesized by living anionic polymerization. Each arm has a molecular weight of 3,000–10,000, and the core represents $<3\%$ of the molecular weight. Thus, a molecule of M_W = 100,000 will have 10–30 terminal hydroxyl residues, compared to a linear molecule, which bears only 2. The solution properties of star PEO molecules are significantly different than comparable molecular weight linear PEO. Star molecules are denser due to crowding of chains near the core; for a 500,000 M_W molecule, the effective Einstein radius of a star molecule (10,000 molecular weight arms) is about half that of a linear molecule [Merrill, 1993]. This crowding may be advantageous from a biocompatibility perspective because it makes the DVB core relatively inaccessible. The gel-forming behavior of star PEO is different from that of linear PEO as would be expected based on the different solution properties. Experimentally, stars exhibit a significantly lower swelling ratio compared to linear PEO when crosslinked in aqueous solutions of identical mass concentration of polymer using the same radiation dosage [Merrill et al., 1990]. Swelling ratios of star gels are generally

(a) gel formation



(b) crosslinking chemistry



(c) linear vs. star PEO gels

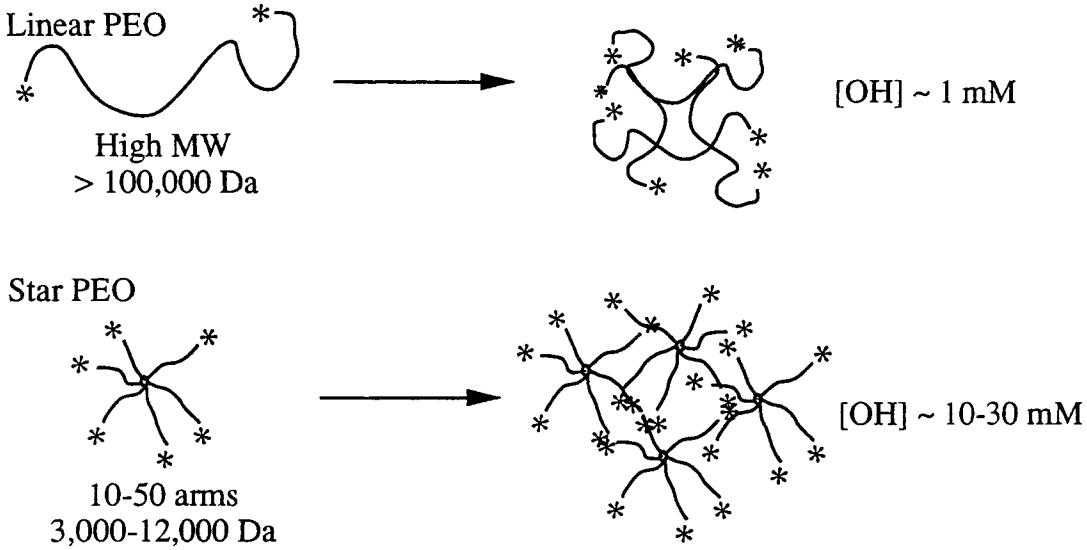


Fig. 1. Formation of radiation-crosslinked hydrogels. **a:** Aqueous solutions of PEO (5–20% w/v) are degassed and passed beneath the beam of 3 MeV Van de Graf generator source which delivers a dose up to 3 Mrad/s. Total doses in the range 2–10 Mrad are used to form gels. **b:** Major reactions which occur during crosslinking of PEO by e-beam irradiation. Radiolysis of water generates free radicals which extract hydrogen from the backbone. Combination of backbone radicals leads to crosslinking. **c:** Comparison of linear and star PEO gels.

close to 1. This is consistent with the observation that the chains in PEO stars are extended.

We are currently developing materials for liver cell transplant and culture using radiation crosslinked star PEO gels modified with ligands recognized by the unique hepatic asialoglycoprotein (ASGP) receptor. The physiological function of this receptor is to remove damaged proteins from the blood via receptor-mediated endocytosis. Although it is not a classical adhesion receptor, cell immobilization via this receptor may offer some advantages from a biomaterials perspective in terms of selectivity, provided appropriate biological function can be maintained. The mammalian ASGP receptor recognizes oligosaccharides terminated in galactose linked via a β 1–4 linkage, and shows an increasing affinity for ligands with increasing valency, i.e., relatively low affinity for oligosaccharides terminated in a single galactose (K_d = 283,000 nM), intermediate affinity for branched oligosaccharides with two terminal galactose units (K_d = 13,000–41,000 nM), and maximum affinity for ligands with three terminal units (K_d = 2–200 nM) [Lee, 1989]. (For comparison, the dissociation constant for integrin receptors is 100–1,000 nM [Duband et al., 1991]). The feasibility of immobilizing hepatocytes via this receptor-ligand interaction has previously been demonstrated using galactose-modified polyacrylamide gels [Oka and Weigel, 1986] and galactose-modified polystyrene [Kobayashi et al., 1988]. The former studies focused on the nature of receptor-ligand interactions in the initial stages of adhesion in the first 24 h, and the latter addressed effects of ligand concentration on behavior of hepatocytes in extended culture and demonstrated that many programs of gene expression could be maintained by cells on galactose-modified surfaces. From an implantation perspective, polyacrylamide and polystyrene are undesirable, and this motivates the use of alternative materials. Also, from the perspective of obtaining natural biological behavior of the receptor following binding of the ligand, the materials used in the previous studies have limitations because the ligand is attached to an immobile substrate via relatively short spacers (up to 20 atoms in the case of polyacrylamide) and thus restrict mobility of the receptor following binding, and likely allow only monovalent binding of galactose. In contrast, PEO chains are highly flexible in aqueous solution and can thus potentially allow for receptor mobility following binding.

PROSPECTS

The widespread clinical use of polymeric biomaterials originated in the 1940s in devices which served as prosthetic replacements for structural components in the body: blood vessels, joints, trachea, etc. The ability to manipulate cellular interactions with materials via specific receptor-mediated phenomena promises to influence the development of new clinical devices in at least two important ways. First, in existing applications, such as replacement of blood vessels, cartilage, and bone, there is increasing emphasis on the development of technologies which incorporate cells as an integral part of the final device, and on tissue regeneration rather than tissue replacement. This may be in the form of an endothelial lining on a permanent vascular graft, or a device which gradually dissolves as cells transplanted with the device grow into tissue, an approach proposed for regeneration of cartilage. Second, the cell-based approach to device design expands the possibilities for using biomaterials from connective tissue repair to regeneration of metabolically active tissue by cell transplantation.

Clinical realization of these goals in each case depends both on finding appropriate moeities to interact with cells and presenting these moeities in the context of a material which suppresses undesired nonspecific interactions. Significant challenges remain in the first arena, particularly because prediction of in vivo behavior from in vitro cell culture is almost an art. New or modified materials based on PEO are good prospects for the inert base materials needed to present ligands to cells. Many versions of PEO are already approved by the FDA for human implantation, and more are in clinical trials. The empirical observations about the repulsive nature of PEO-based materials are currently being balanced by rational analysis of the underlying physicochemical phenomena responsible, allowing better design of materials and better prediction of stability.

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