

1 METHODS AND PHARMACEUTICAL COMPOSITIONS FOR THE CLOSURE
2 OF RETINAL BREAKS
3

4 ACKNOWLEDGEMENTS

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6 U.S. Government may have rights in this invention.
7

8 CROSS-REFERENCE TO RELATED APPLICATIONS

9 This application claims the benefit of the filing date of U.S. Patent Application
10 Serial No. 60/063,297, filed October 27, 1997, which is incorporated herein by
11 reference.
12

13 INTRODUCTION

14 Technical Field

15 The present invention relates to methods and pharmaceutical compositions
16 involving the use of polymers for the closure of retinal breaks.

17 Background

18 Successful management of rhegmatogenous retinal detachment is predicated
19 upon closure of all retinal breaks. A rhegmatogenous retinal detachment occurs when
20 vitreous fluid passes through a hole in the retina and the retina separates from the
21 retinal pigment epithelium. When retinal detachment is treated with vitrectomy,
22 closure of retinal breaks generally requires creation of a chorioretinal adhesion around
23 each break (Michels et al., Retinal Detachment, Klein EA, Ed. CV Mosby Co., St.
24 Louis, MO, 1990, pp 440, 847, 890-892). These adhesive lesions are generated with
25 either laser photocoagulation or cryotherapy. Maximal chorioretinal adhesion is
26 ordinarily achieved within 2 weeks following treatment (Bloch et al., *Am J Ophthalmol*
27 71:666-673 (1971); Yoon et al., *Ophthalmol* 95:1385-1388 (1988)). To keep the
28 retina in apposition with the retinal pigment epithelium during this time, prolonged
29 intraocular tamponade with gas or silicone oil is utilized (Norton et al., *Am J*
30 *Ophthalmol* 68:1011-1021 (1969); Norton et al., *Trans Am Acad Ophthalmol*
31 *Otolaryngol* 77:85-98 (1973); Lean et al., *Trans Ophthalmol Soc (UK)* 102:203-205



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1 (1982); Gonvers M, *Ophthalmologica* 184:210-218 (1982); Petersen J., *Graefe's Arch*
 2 *Clin Exp Ophthalmol* 225:452-456 (1987)).

3 When long acting gases such as SF₆ or C₃F₈ are used, patients often must keep
 4 their head in a face down position for 2 weeks after surgery (Michels et al., Retinal
 5 Detachment, Klein EA, Ed. CV Mosby Co., St. Louis, MO, 1990, pp 890-892). This
 6 causes considerable discomfort in most patients, and not uncommonly, is the most
 7 difficult hurdle in post-operative management. Additionally, intraocular gas may be
 8 associated with a number of ocular complications including, cataract, glaucoma,
 9 corneal edema, and creation of retinal folds (Fineberg et al., *Am J Ophthalmol* 79:67-
 10 76 (1975); Abrams et al., *Am J Ophthalmol* 94:165-171 (1982); Foulks et al., *Arch*
 11 *Ophthalmol* 105:256-259 (1987); Lewen et al., *Arch Ophthalmol* 105:1212-1214
 12 (1987)). Another potential disadvantage of gas as an intraocular tamponade is that by
 13 sequestering inflammatory factors between the bubble interface and the retina, it may
 14 promote scar tissue formation on the retinal surface (Charles, S., Vitreous
 15 Microsurgery, Williams & Wilkins, Baltimore, MD, 1987, p 135). As this scar tissue
 16 contracts, it can distort the retinal surface, and may cause re-detachment of the retina
 17 (proliferative vitreoretinopathy) (Machemer R., *Brit J Ophthalmol* 62:737-747 (1978);
 18 Laqua et al., *Am J Ophthalmol* 80:913-929 (1975)).

19 Using silicone oil as a post-operative intraocular tamponade has the advantage
 20 that the patient is not required to position face down for more than one day post-
 21 operatively. However, unlike gas, which is slowly reabsorbed into the blood stream,
 22 silicone must be surgically removed from the eye as a secondary procedure to prevent
 23 silicone induced ocular complications such as cataract, glaucoma, band keratopathy,
 24 corneal decompensation and promotion of proliferative vitreoretinopathy (PVR)
 25 (Federman et al., *Ophthalmol* 95:870-876 (1988); Sternberg et al., *Arch Ophthalmol*
 26 102:90-94 (1985)).

27 Other less effective methods of retinal fixation to the underlying retinal
 28 pigment epithelium include retinal tacks (de Juan et al., *Am J Ophthalmol* 99:272-274
 29 (1985); Burke et al., *Arch Ophthalmol* 105:404-408 (1987)), and cyanoacrylate glue
 30 (McCuen et al., *Am J Ophthalmol* 102:199-207 (1986)). Titanium or stainless steel
 31 metal retinal tacks have been used to attach the retina to the eye wall to treat giant
 32 retinal tears or after large relaxing retinotomies. The tacks do not create a confluent
 33 chorioretinal adhesion around the retinal tear and thus require supplemental laser or
 34 cryotherapy as well as intraocular tamponade with gas or silicone oil. Tacks are also

1 associated with complications such as choroidal hemorrhage and dislodgment (Lewis
2 et al., *Am J Ophthalmol* 103:672-680 (1987)).

3 Butyl-2-cyanocrylate glue has been used to close retinal breaks in animal
4 models of experimental retinal detachment (McCuen et al; Hida et al., *Am J*
5 *Ophthalmol* 103:782-789 (1987); Hida et al., 1988). The glue is applied directly to
6 retinal holes, polymerizing rapidly to form a seal over the retinal hole. While
7 successful at closing the break and creating a chorioretinal adhesion, some intraocular
8 glues can cause local retinal toxicity, possibly from release of formaldehyde and
9 cyanoacetate (Hida et al., 1987).

10 Patients suffering from retinal detachment are in need of a better method for
11 temporarily closing retinal breaks while chorioretinal adhesions form, thus allowing
12 recovery from surgery with a minimum of discomfort and/or ocular complications.

13 SUMMARY OF THE INVENTION

14 The present invention is directed to compositions, methods, and articles of
15 manufacture for the closure of retinal breaks by applying a polymer formulation to the
16 retinal surface in the vicinity of the retinal break. The invention provides methods for
17 closing a retinal break in a mammal, comprising applying to the retinal surface over
18 and around the retinal break a non-toxic polymer formulation comprising at least one
19 polymer precursor, and transforming the polymer formulation into a gel-like coat. In
20 a preferred embodiment, the polymer formulation comprises a photochemically
21 reactive polymer precursor species which can be transformed from a liquid to gel
22 form by exposure to light. Another preferred composition includes a mixture of two
23 mutually reactive polymer precursors.

24 The invention also provides methods for the management of retinal
25 detachment, comprising replacing the vitreous with gas, creating a chorioretinal
26 adhesion around a retinal break, applying to the retinal surface over and around the
27 retinal break a non-toxic polymer formulation comprising at least one polymer
28 precursor, and transforming the polymer into a gel-like coat.

29 Also provided are methods for preventing proliferative vitreoretinopathy,
30 comprising applying a non-toxic polymer formulation over and around the retinal
31 break and extending beyond the break by a substantial amount, preferably to cover
32 more than 75% of the retina.

1 In addition, the invention provides pharmaceutical compositions, methods for
2 preparing such pharmaceutical compositions, and articles for manufacture for use in
3 the methods described above.

4 DESCRIPTION OF SPECIFIC EMBODIMENTS

5 This invention pertains to the field of retinal surgery, particularly to the
6 closure of retinal breaks. The invention provides a superior alternative to silicone oil
7 or intraocular gas for post-operative tamponade. The methods of the invention
8 comprise the application of a polymer formulation to the retinal surface over and
9 around the retinal break. More extensive applications of the polymer formulation to
10 the retinal surface can prevent post operative scar tissue formation and recurrent
11 retinal detachment (proliferative vitreoretinopathy). The polymer formulation is
12 applied in liquid form, assuring conformity to irregular tissue surfaces. It is then
13 transformed to a thin, gel-like adhesive coat by photopolymerization with a light
14 source. Alternatively, a liquid polymer precursor that auto-polymerizes is applied
15 over the break and adjacent retina. The polymerized gel is bound to the retina and
16 retinal pigment epithelium, and resists displacement with overlying turbulent fluid
17 flow. It is water permeable and allows diffusion of small molecules such as oxygen,
18 glucose and other essential nutrients. While the polymer adheres to the retina, it
19 closes the retinal hole, preventing fluid from passing into the subretinal space.

20 Before or after application of the polymer formulation to the retinal surface,
21 laser photocoagulation or cryotherapy can be applied around the break to form a
22 chorioretinal adhesion, which reaches adequate strength to prevent retinal detachment
23 by about 10-14 days after surgery (Yoon et al.). Typically laser is applied around an
24 extra-macular hole, but not around macular holes. During this time, the polymer
25 slowly biodegrades, but remains in place long enough to maintain retinal attachment
26 and allow the retinopexy adhesion to reach maturity. Because the polymer closes the
27 hole, the vitreous cavity can be filled with balanced saline solution at the end of
28 surgery and no additional intraocular tamponade is required with gas or silicone.
29 Therefore, patients avoid the difficulty of post-operative positioning if gas is used and
30 avoid a second procedure to remove silicone oil if it is used instead of gas.
31 Furthermore, complications associated with gas or silicone oil are avoided.

1 In addition to obviating the need for gas or silicone tamponade, wider
2 application of the polymer formulation beyond the retinal break, to a portion of or the
3 entire retinal surface, has the added benefit of preventing post-operative scar tissue
4 formation on the retinal surface, which can distort the retinal surface and reopen
5 retinal breaks (proliferative vitreoretinopathy, PVR). This is due in part to the fact
6 that larger molecules, including proteins and cells which cause proliferative
7 vitreoretinopathy, cannot traverse or adhere to the polymer formulation, and thus will
8 not bind to the underlying retina during the post-operative period (West et al., *Proc*
9 *Natl Acad Sci (USA)* 93:13188-13193 (1986)).

10 One aspect of the invention is a method for closing a retinal break in an
11 animal, comprising applying a non-toxic polymer formulation to the retinal surface of
12 the animal over and around the retinal break, and transforming the polymer
13 formulation into a gel-like coat. Preferably, the resultant adhesive coat comprises a
14 biodegradable polymer. By "retinal break" is meant a hole, tear, or other abnormal
15 opening in the retina (also known as the neurosensory retina). Retinal breaks can
16 develop from several conditions, including, but not limited to, myopia, congenital
17 defects, trauma, and cataract surgery. Preferably the animal is a laboratory animal or
18 domesticated animal, is more preferably a mammal, and most preferably is a human.
19 Suitable laboratory animals include mice, rats, rabbits, monkeys, apes and other
20 research animals. Suitable domesticated animals include dogs, cats, cattle, horses,
21 goats, sheep, pigs, mules, donkeys, and other animals in the service or company of
22 man.

23 A key feature of the requirements for the materials to be used in closing retinal
24 breaks is that they adhere to the retina over and around the break. One way to provide
25 for this feature is to produce the material implant from a liquid polymer precursor
26 applied directly on and around the site of the retinal defect. By "polymer" is meant a
27 molecule formed by the union of two or more monomers. A "monomer" is a
28 repeating structure unit within a polymer. "Polymerization" is the bonding of two or
29 more monomers to produce a polymer. For example, polymerization of ethylene
30 forms a polyethylene chain, or polymerization of a monomer X and a monomer Y can
31 yield a polymer with the repeating subunit X-Y. It will be appreciated that polymers
32 can also be formed by the polymerization of more than two monomers, and that two
33 or more monomers can be present in unequal ratios in the resultant polymer. By
34 "polymer precursor" is meant a molecule that is subsequently linked by

1 polymerization to form a polymer, which is larger than the polymer precursor. As
2 discussed in greater detail below, polymerization can be achieved in various ways,
3 such as by photopolymerization, autopolymerization, or physicochemical
4 polymerization. The polymer precursor can itself be a polymer, such as, for example,
5 poly(ethylene glycol). Alternatively, the polymer precursor can be a molecule other
6 than a polymer, such as a protein, for example, albumin, collagen, gelatin, or other
7 non-polymeric molecules.

8 The polymer precursor is usually present in the polymer formulation at a
9 concentration in a range of about 0.01% to about 90%. The actual concentration
10 varies with the polymer precursor used and its toxicology. Most polymer precursors
11 are preferably used at a minimal concentration of about 5% because at lower
12 concentrations it may be difficult to form a gel. However, by increasing the
13 hydrophobicity of the ends of the polymer precursor, concentrations as low as about
14 1%, preferably about 3%, can be used to form a gel. High molecular weight
15 precursors (i.e., greater than about 70,000 g/mol, preferably greater than about
16 100,000 g/mol), such as, for example, acrylated hyaluronic acid are preferably present
17 at a concentration not greater than about 1%. See, for example, U.S. Patent Nos.
18 5,801,033; 5,820,882; 5,626,863; and 5,614,587, incorporated herein by reference.

19 Transformation of the polymer precursor to a thin, gel-like coat can be
20 accomplished in a number of ways, for example, by photochemical reactivity, by
21 chemical reactivity, and by physicochemical response. When such a liquid-to-solid
22 transition occurs directly upon the tissue surface, via any of the approaches described
23 above, the resulting biomaterial implant adheres to the tissue surface. Liquid polymer
24 precursor is applied over and around the retinal break, covering the breached area of
25 the retina and overlapping the unbreached area of the retina by an amount sufficient to
26 maintain adhesion of the polymerized implant to the retinal surface. Typically, the
27 polymerized implant extends over the unbreached area of the retina by about 0.1 mm
28 to about 5 mm, and can extend over a substantial portion of the retinal surface if
29 desired, up to the entire retinal surface. Preferably the polymerized implant extends
30 over the unbreached area of the retina by about 0.5 mm to about 2 mm. .

31 The transformation of polymer precursor into a gel-like coat can be achieved
32 by photopolymerization of the polymer formulation. Photochemically activatable
33 polymer precursors suitable for the methods of the invention include precursors
34 comprising a water-soluble polymer as the central domain, such as, for example,

1 poly(ethylene glycol) (PEG)-based polymers. PEG is a polymer of the formula
2 $\text{HOCH}_2(\text{CH}_2\text{OCH}_2)_n\text{CH}_2\text{OH}$, wherein n is an integer giving rise to molecules ranging
3 in molecular weight typically from about 200 g/mol to greater than about 75,000
4 g/mol, preferably between about 6,000 g/mol to about 35,000 g/mol. Some specific
5 PEG molecules have a molecular weight of about 400, 1350, 3350, 4000, 6000, 8000,
6 18500, 20000, or 35000. PEG molecules having a molecular weight not specifically
7 listed, but nonetheless within a range of about 200 g/mol to greater than about 75,000
8 g/mol are also contemplated. Lower molecular weight PEG formulations are referred
9 to as short chain PEG formulations and typically have a molecular weight of about
10 4,000 g/mole or less. Higher molecular weight PEG formulations are referred to as
11 long chain PEG formulations and have a molecular weight of greater than about 4,000
12 g/mol, preferably greater than about 8,000 g/mol, and can be greater than about
13 10,000 g/mol, and greater than about 20,000 g/mol. Preferably the long chain PEG
14 formulations have a molecular weight in the range of about 7,000 g/mol to about
15 20,000 g/mol, with about 8,000 g/mol to about 10,000 g/mol being most preferred.
16 One of ordinary skill in the art expects PEG molecules to be present in a distribution
17 centered around the stated molecular weight, commonly as much as plus or minus
18 about 20% of the stated molecular weight. Vendors often list the molecular weight of
19 a PEG product as an average molecular weight (See, for example, the Sigma catalog).

20 Preferably the polymer precursors of the invention comprise reactive termini
21 to allow for photopolymerization, such as, for example, free radical polymerizable
22 termini. Examples of such reactive termini include acrylates and methacrylates, with
23 acrylates being more preferred. Preferably the polymer precursor is a PEG diacrylate
24 or tetracrylate.

25 Preferably the polymer precursor also comprises degradable regions of a
26 molecular weight, relative to that of the water-soluble central domain, to be
27 sufficiently small that the properties of the polymer precursor in solution, and the gel
28 properties, are determined primarily by the central water-soluble chain. Typically the
29 polymer precursor comprises about 0% to about 20%, preferably about 1% to about
30 10%, degradable regions. Examples of such degradable regions include, but are not
31 limited to, hydrolytically labile oligomeric extensions, such as, for example, poly(α -
32 hydroxy esters). Examples of poly(α -hydroxy esters) include poly(*dl*-lactic acid)
33 (PLA), poly(glycolic acid) (PGA), poly (3-hydroxybutyric acid) (HBA), and polymers

1 of ϵ -caprolactone. The hydrolytic susceptibility of some of the ester linkages is in the
 2 following order: glycolidyl > lactoyl > ϵ -caprolactyl.

3 In a preferred embodiment, the polymer precursor has the formula:



5 wherein W is a water-soluble polymer; D is a degradable moiety; P is a
 6 photopolymerizable moiety; m and q are integers from 1 to about 10; o is an integer
 7 from 1 to about 100; and n and p are integers from 0 to about 120. W can be a linear
 8 polymer or a branched polymer. One of ordinary skill in the art would understand the
 9 formula provided above to include branched polymers having more than two termini
 10 and having degradable and/or photopolymerizable moieties on some or all of the
 11 termini of the branched polymer. A "degradable moiety" is an oligomeric compound
 12 that when integrated into a polymer precursor, creates within the polymer precursor a
 13 degradable region as described above. A "photopolymerizable moiety" is a moiety
 14 that allows the polymer precursor to polymerize upon exposure to light. Some
 15 wavelengths suitable for catalyzing polymerization are discussed in more detail
 16 below.

17 Typically, the values of m and q are varied so as to achieve the desired degree
 18 of cross-linking and rate of transition from liquid-to-gel upon polymerization. The
 19 values of n and p are varied so as to achieve a desirable percentage of the degradable
 20 moiety, preferably between about 0.1% to about 25% degradable moiety, with about
 21 1% to about 10% being most preferred. One of ordinary skill in the art would know
 22 to vary the values for n and p according to the value of o and the molecular weight of
 23 W in order to achieve this goal. Preferably m and q are integers from 1 to about 5, n
 24 and p are integers from 0 to about 10, and o is an integer from 1 to about 40.
 25 Alternatively, the polymer formulation can comprise in varying molar ratios polymer
 26 precursors having differing values for m, n, o, p and q so as to achieve a desirable
 27 percentage of the degradable moiety upon polymerization. For example, if W is a
 28 water soluble polymer having a molecular weight of at least 4,000 g/mol and o=1, n
 29 and p are integers from 0 to about 60, more preferably from 0 to about 25, even more
 30 preferably 1 to about 15, with 1 to about 5 being most preferred. Preferably W is a
 31 PEG molecule having a molecular weight from about 200 g/mol to about 75,000

1 g/mol. Preferably, if W is a PEG molecule having a molecular weight greater than
2 4,000, o is an integer from 1 to about 5, with 1 being most preferred.

3 Preferably the polymer precursor comprises a PEG central chain with
4 degradable regions and photopolymerizable end groups that terminate the degradable
5 regions. The polymer precursors of the invention can be synthesized by methods
6 known in the art (Sawhney et al., *Macromolecules* (1993) 26:581-587; Hill-West et
7 al., *Proc. Natl. Acad. Sci. USA* (1994) 91:5967-5971) and described herein in
8 Examples 1-3.

9 A preferred polymer chain comprises lactic acid, glycolic acid or epsilon-
10 caproic acid in the degradable region D. Incorporation of oligolactic acid into the
11 polymer will increase its hydrophobic content. The polymer's hydrophobic content,
12 and hence its strength of adhesion, varies directly with its % oligolactic, oligoglycolic,
13 or oligoepsilon-caproic acid content. PEG is used to initiate the ring-opening
14 polymerization of *dl* lactide, *ll* lactide, glycolide, or epsilon caprolactone to an extent
15 such that from about 0.1% to about 25%, preferably about 1% or 10%, of the mass of
16 the polymer chain is comprised of oligolactic acid, oligoglycolic acid, or oligoepsilon-
17 caproic acid. This ratio is controlled via the reaction stoichiometry: the
18 polymerization, if performed on dry polymer precursor, will produce very little lactic
19 acid, glycolic acid, or epsilon-caproic acid homopolymer.

20 Biocompatibility of various biodegradable polymers can easily be assessed as
21 described in Example 6 by injecting rabbits intravitreally with a polymer formulation,
22 photopolymerizing the polymer precursor, and observing the animal clinically or
23 histologically for signs of intraocular inflammation or toxicity.

24 The polymer precursors can be photopolymerized to form cross-linked
25 networks directly upon the retinal surface. In addition to the polymer precursors, the
26 biodegradable polymer formulation can also comprise reagents to facilitate the
27 photopolymerization process, such as at least one photoinitiator, and one or more co-
28 catalysts, such as, for example, N-vinylpyrrolidone and triethanolamine. Preferably a
29 nontoxic photoinitiator such as eosin Y photoinitiator is used. Other initiators include
30 2,2-dimethoxy-2-phenylacetophenone and ethyl eosin. The polymerization process
31 can be catalyzed by light in a variety of ways, including UV polymerization with a
32 low intensity lamp emitting at about 365 nm, visible laser polymerization with an
33 argon ion laser emitting at about 514 nm, visible illumination from a conventional
34 endoilluminator used in vitreous surgery, and most preferably by illuminating with a

1 lamp that emits light at a wavelength between 400 - 600 nm, such as, for example, a
2 1-kW Xe arc lamp. Illumination occurs over about 1-120 seconds, preferably less
3 than 30 seconds.. Since the heat generated is low, photopolymerization can be carried
4 out in direct contact with cells and tissues. Indeed, similar materials have been
5 successfully utilized for the encapsulation of pancreatic islet cells and for the
6 prevention of post-operative adhesion formation (Hill-West et al. *Obstet Gynecol* 83:
7 59-64 (1994).

8 Alternatively, the transformation of the polymer formulation into a gel-like
9 coat can be achieved by autopolymerization of the polymer formulation. Auto-
10 chemically reactive polymer gels may be formed by mixing two or more mutually
11 reactive polymer precursors to result in a cross-linked polymer network. Usually, the
12 polymer formulation comprises a first polymer precursor and a second polymer
13 precursor, the first and second polymer precursors being mutually reactive. Preferably
14 the first and second polymer precursors are present in about equimolar amounts.
15 Typically, at least one of the reactive polymer precursors is a PEG based polymer
16 precursor. Preferably, both polymer precursors are PEG based polymer precursors.

17 Suitable first polymer precursors include proteins, such as, for example,
18 albumin, proteins derived from skin, connective tissue, or bone, such as collagen or
19 gelatin, other fibrous proteins and other large proteins, tetra-amino PEG, copolymers
20 of poly(N-vinyl pyrrolidone) containing an amino-containing co-monomer, aminated
21 hyaluronic acid, other polysaccharides, and other amines. Preferably the tetra-amino
22 PEG has a molecular weight of at least about 3,000 g/mol, preferably more than about
23 6,000 g/mole, even more preferably more than about 10,000 g/mol, and more
24 preferably at least about 20,000 g/mol.

25 Suitable second polymer precursors include, but are not limited to, terminally-
26 functionalized PEG, such as difunctionally activated forms of PEG. Some activating
27 groups include epoxy groups, aldehydes, isocyanates, isothiocyanates, succinates,
28 carbonates, propionates, etc. Examples of such forms of PEG include, but are not
29 limited to, PEG di-succinimidyl glutarate (SG-PEG), PEG di-succinimidyl (S-PEG),
30 PEG di-succinimidyl succinamide (SSA-PEG), PEG di-succinimidyl carbonate (SC-
31 PEG), PEG di-propionaldehyde (A-PEG), PEG succinimidyl propionate, and PEG di-
32 glycidyl ether (E-PEG) (U.S. Patent No. 5,614,587) and other epoxy-derivatized PEG
33 molecules, PEG nitrophenyl carbonate, PEG dialdehydes, PEG di-isocyanates, PEG
34 di-isothiocyanates, and the like. Particularly preferred is a di-N-

1 hydroxysuccinimidyl-activated dicarboxyl (PEG), such as a di-N-
2 hydroxysuccinimidyl PEG. Other suitable difunctionally activated forms of PEG can
3 be obtained from the Shearwater Polymers Catalog (see, for example, the
4 "Electrophilically Activated" section of their website at
5 <http://www.swpolymers.com>).

6 Preferred autochemically reactive polymer precursor pairs include (1) a tetra-
7 amino PEG and a di-N-hydroxysuccinimidyl PEG; (2) a tetra-amino PEG and a di-
8 succinimidyl carbonate PEG; (3) collagen, gelatin, or albumin and a di-N-
9 hydroxysuccinimidyl PEG; (4) collagen, gelatin, or albumin and a di-succinimidyl
10 carbonate PEG; and (5) other suitable autochemically reactive polymer pairs. Most
11 preferred for the methods of the invention is the combination of a tetra-amino PEG
12 and a di-N-hydroxysuccinimidyl PEG. If a di-N-hydroxysuccinimidyl active PEG is
13 mixed with a di-amino PEG, a high molecular weight polymer results, but not a cross-
14 linked hydrogel. However, if a di-N-hydroxysuccinimidyl activated PEG is mixed
15 with a tetra-amino PEG, a cross-linked hydrogel network is formed, liberating only N-
16 hydroxysuccinate as a reaction product. N-hydroxysuccinate is water-soluble and of
17 very low toxicity. Preferably the di-N-hydroxysuccinimidyl PEG used in
18 combination with a tetra-amino PEG is a di-N-hydroxy-succinimidyl activated
19 succinate-terminated PEG. Di-N-hydroxy-succinimidyl activated glutarate-
20 terminated PEG is less preferred because, when used in combination with a tetra-
21 amino PEG, can produce ocular inflammation. These hydrogels can degrade by
22 spontaneous hydrolysis at the linking group at the end of the polymer chain and can
23 degrade within the protein backbone of a protein-containing gel. With gels formed
24 from a PEG-containing first component and a PEG-containing second component,
25 one can include a hydrolytically degradable oligolactic acid, oligoglycolic acid, or
26 oligoepsilon-caproic acid domain, for example. Gels formed from protein-based,
27 peptide-based, or polysaccharide-based precursors can also degrade under the
28 enzymatic influences of the body.

29 Biocompatibility of various reactive polymer precursor pairs can easily be
30 assessed as described in Example 7 by injecting a rabbit intravitreally with a mixture
31 of the members of the polymer precursor pair, and observing the animal visually or
32 histologically for signs of intraocular inflammation or toxicity.

33 The extent of incorporation into the gel phase can be optimized by
34 manipulating various parameters, such as the pH of the reaction solution and the ratio

1 of the first polymer precursor to the second polymer precursor. Typically, when PEG
2 tetra-amine and di-N-hydroxy succinimidyl PEG are to be used, polymer precursors
3 are separately reconstituted immediately before use in physiological saline at pH 8.
4 They are mixed to yield a total final concentration of about 10% using an optimal
5 ratio of molar amounts of each precursor, preferably equimolar. Given that reaction
6 begins immediately after mixing, injection onto the retina is preferably performed
7 immediately. The mixing is performed with two syringes and a connector.
8 Alternatively, a syringe with two barrels can be used. Static mixture occurs on the tip
9 of the syringe immediately before the polymer precursor solutions pass through a
10 needle or cannula. The time between the initiation of mixing and injection is usually
11 less than about 30 seconds. This can be achieved by positioning a 30 gauge cannula
12 (or other suitable sized cannula, or a needle) attached to a syringe(s) containing
13 polymer over the break prior to mixing the components.

14 Toward physicochemical transition, block copolymers of poly(ethylene
15 glycol)--poly(propylene glycol)--poly(ethylene glycol), commonly referred to as
16 Pluronics™, can be used to form polymer solutions that are liquid at 4°C but gels at
17 37°C, permitting injection of the cold fluid with solidification to form a
18 physicochemically cross-linked polymer network on the surface of the tissue. Other
19 thermoreversible biocompatible biodegradable polymers are known. For example,
20 Jeong et al., *Nature* (1997) 388:860-862, recently described copolymers of PEG and
21 lactic acid that display favorable liquid-to-solid gelation transitions. Such materials
22 can either be applied warm and fluid and allowed to cool *in vivo* into a gel form, or
23 can be applied cool and fluid and allowed to warm *in vivo* into a gel form, depending
24 upon the physicochemical characteristics of the gel and its precursor.

25 Polymers that display a physicochemical response to stimuli have been
26 explored as potential drug-delivery systems. Stimuli studied to date include chemical
27 substances and changes in temperature, pH and electric field. Homopolymers or
28 copolymers of N-isopropylacrylamide and poly(ethylene oxide)-poly(propylene
29 oxide)-poly(ethylene oxide) (known as poloxomers) are typical examples of
30 thermosensitive polymers, but their use in drug delivery is problematic because they
31 are toxic and non-biodegradable. Biodegradable polymers used for drug delivery to
32 date have mostly been in the form of injectable microspheres or implant systems,
33 which require complicated fabrication processes using organic solvents. Such
34 systems have the disadvantage that the use of organic solvents can cause denaturation

1 when protein drugs are to be encapsulated. Furthermore, the solid form requires
2 surgical insertion, which often results in tissue irritation and damage. The methods of
3 the invention involve the synthesis of a thermosensitive, biodegradable hydrogel
4 consisting of polymer precursor blocks of poly(ethylene oxide) and poly(L-lactic
5 acid). Aqueous solutions of these polymer precursors exhibit temperature-dependent
6 reversible gel-sol transitions. By "sol" is meant a polymer precursor solution which is
7 more liquid than solid. By "gel" is meant a polymer solution which is more solid than
8 liquid. The hydrogel can be loaded in an aqueous phase at an elevated temperature
9 (around 45 degrees C), where they form a sol. In this form, the polymer is injectable.
10 On subcutaneous injection and subsequent rapid cooling to body temperature, the
11 loaded copolymer forms a gel.

12 The polymer formulations described above are applied in a manner consistent
13 with the surgical procedure as a whole. Typically, the subretinal fluid is drained with
14 fluid/gas exchange in order to flatten the retina. Laser photocoagulation or
15 cryotherapy can then be performed around the break. The polymer formulation is
16 then applied to the retinal surface as described above. Polymerization is effected as
17 discussed above. Usually at least about 1 second to five minutes or longer is allowed
18 to pass to ensure complete polymerization has occurred, and preferably the delay is
19 less than 30 seconds. The gas is then removed and replaced with a balanced saline
20 solution.

21 Another aspect of the invention is a method for management of retinal
22 detachment in an animal, comprising applying a non-toxic, biodegradable polymer
23 formulation to the retinal surface of the animal over and around the retinal break, and
24 transforming the polymer formulation into a gel-like coat. As discussed above,
25 closure of the retinal break prevents fluid from leaking into the potential space
26 between the retina and the retina pigment epithelium. If desired, chorioretinal
27 adhesions can be created, preferably by laser photocoagulation before or after
28 application of the biodegradable polymer. A "chorioretinal adhesion" is an adhesion
29 between the retina and underlying retinal pigment epithelium and choroid.

30 Yet another aspect of the invention is a method for the prevention of
31 proliferative vitreoretinopathy (PVR), comprising applying a non-toxic,
32 biodegradable polymer formulation more extensively to the retinal surface of an
33 animal in need thereof than otherwise applied to close a retinal break. Preferably the
34 polymer formulation is applied to at least about 25% of the retinal surface

1 surrounding the retinal break, preferably to more than about 50% and applications to
2 more than about 75% of the retinal surface to the entire retinal surface are most
3 preferred. In a preferred embodiment, autopolymerizable polymer precursors are
4 applied to the retinal surface as described above. In another preferred embodiment, a
5 polymer precursor solution containing at least one photoinitiator is applied to the
6 retinal surface around the retinal hole. Polymerization is then effected by any of the
7 methods described above to close the retinal break. The eye is then filled with a
8 solution containing at least one photoinitiator but no polymer precursor to coat the
9 surface of the retina. Excess photoinitiator is drained from the eye. Next, polymer
10 precursor solution that does not contain photoinitiator is applied to the remainder of
11 the retinal surface and polymerization is again effected. The polymerization reaction
12 results in a thin, transparent gel where the polymer precursor contacts the
13 photoinitiator, but not in areas free of photoinitiator. This results the formation of a gel
14 only on the surface of the retina. The eye is once again filled with fluid.
15 Unpolymerized precursors are then irrigated from the eye. The adherent polymer
16 biodegrades over a 2-10 week period. The polymerized gel overlying the retina both
17 closes the retinal break and prevents adherence of scar tissue that could cause
18 proliferative vitreoretinopathy and recurrent retinal detachment. Another embodiment
19 omits the initial step of applying a polymer precursor solution containing
20 photoinitiator directly to the hole.

21 A further aspect of the invention is the use of at least one non-toxic,
22 biodegradable polymer precursor for the preparation of a pharmaceutical composition
23 for closing a retinal break in a mammal. Suitable polymer precursors and other
24 components of the pharmaceutical composition are discussed in detail above in the
25 sections describing the components of suitable polymer formulations. Additional
26 components can include any other reagents that catalyze polymerization of the
27 polymer precursor, pharmaceutically suitable delivery vehicles for ocular
28 administration, such as for delivery to the interior of the eye, and any other
29 pharmaceutically acceptable additives.

30 The invention also provides articles of manufacture for use in closing a retinal
31 break in a mammal with a non-toxic biodegradable polymer. In one embodiment, the

1 article of manufacture comprises a first container comprising a polymer precursor of
2 the formula:



4 wherein W is a water-soluble polymer; D is a degradable moiety; P is a
5 photopolymerizable moiety; m and q are integers from 1 to about 10; o is an integer
6 from 1 to about 100; and n and p are integers from 0 to about 120. The first container
7 can optionally contain at least one photoinitiator and can also optionally contain at
8 least one co-catalyst. Where the first container does contain a photoinitiator in
9 addition to the polymer precursor, the article of manufacture can optionally contain a
10 second container comprising polymer precursor but no photoinitiator. The article of
11 manufacture can optionally contain a third container comprising a photoinitiator
12 solution but no polymer precursor. An article of manufacture comprising all three
13 containers or just the second and third containers are useful for preventing PVR as
14 described above. An article of manufacture comprising the first container only is
15 sufficient for closing retinal breaks. The article of manufacture preferably further
16 comprises instructions for use according to the methods described above involving
17 photopolymerization.

18 In another embodiment, the article of manufacture comprises a first container
19 comprising a first polymer precursor and a second container comprising a second
20 polymer precursor, the first and second polymer precursors being mutually reactive.
21 The first and second polymer precursors can be present in the container in admixture
22 with a pharmaceutically suitable vehicle for delivery to the interior of the eye.
23 Alternatively, any such vehicle can be added separately, if necessary, for example, to
24 reconstitute the polymers. Suitable first and second polymer precursors are any of
25 those polymer precursor pairs discussed above that can autopolymerize. Preferably
26 the first polymer precursor is albumin, collagen or gelatin, and the second polymer
27 precursor is a terminally-functionalized poly(ethylene glycol) (PEG). Typically, the
28 first and second containers are separate syringes or are separate barrels of a single
29 syringe having static mixture device at the tip of the syringe, and can also be vials or
30 other cylindrical containers, such as, for example a segment of tubing. The article of
31 manufacture can further comprise printed instructions for a method for closing a
32 retinal break by combining the first and second polymer precursors immediately

1 before applying to the retinal surface of the mammal over and around the retinal
2 break. Usually, the first and second polymer precursors are combined by extruding
3 from each container simultaneously into and through a connector onto the retinal
4 surface. Suitable connectors are any structures that permit mixing of the first and
5 second polymer precursors immediately before application to the retinal surface, such
6 as, for example, a structure that is Y-shaped and comprises two tubular segments,
7 each of which fits over an aperture in each container, and which are united into a
8 single tubular segment.

9 The following examples illustrate, but in no way are intended to limit the
10 present invention.

11 EXAMPLES

12 Example 1: Synthesis of 6 kD PEG Polymer Precursor

13 A PEG-co-poly(α -hydroxy acid) copolymer is synthesized. A total of 30 g of
14 dry PEG 6K, 3.60 g of *dl*-lactide (5 mol *dl*-lactide/mol of PEG), and 15 mg of
15 stannous octanoate are charged into a 100-mL round-bottomed flask under a nitrogen
16 atmosphere. The reaction mixture is stirred under vacuum at 200°C for 4 h and at
17 160°C for 2 h and is subsequently cooled to room temperature. The resulting
18 copolymer is dissolved in dichloromethane, precipitated in anhydrous ether, filtered,
19 and dried. The α - and ω -hydroxyl end groups of PEGs with various molecular
20 weights are used as ring-opening reagents to initiate the polymerization of either *dl*-
21 lactide or glycolide to similarly form several other copolymers.

22 The copolymers are end-capped with acrylate groups to form a polymerizable
23 polymer precursor. A total of 30 g of the intermediate copolymer is dissolved in 300
24 mL of dichloromethane in a 500-mL round-bottomed flask and is cooled to 0°C in an
25 ice bath. A total of 1.31 mL of triethylamine and 1.58 mL of acryloyl chloride are
26 added to the flask, and the reaction mixture is stirred for 12 h at 0°C and 12 h at room
27 temperature. The reaction mixture is filtered to remove triethanolamine
28 hydrochloride, and the polymer precursor is obtained by pouring the filtrate in a large
29 excess of dry diethyl ether. It is further purified by dissolution and reprecipitation
30 once using dichloromethane and hexane, respectively. Finally, it is dried at 70°C
31 under vacuum for 1 day.

1 **Example 2: Synthesis of 10,000-Da PEG Polymer Precursor**

2 A macromolecular precursor is synthesized that consists of a central chain of
3 poly(ethylene glycol) (PEG) with flanking regions of lactic acid oligomer and
4 tetraacrylate termini. The precursor is synthesized by dissolving 50 g of 10,000-Da
5 PEG (Sigma) in toluene (Mallinckrodt, ACS grade) and refluxing under argon for 1
6 hour. 4.5 g of DL-lactide (Aldrich) and 50 μ l of 50% (vol/vol) stannous octanoate
7 (ICN) in toluene are added. The solution is refluxed under argon for 16 hours to
8 achieve an average of five lactic acid groups per end; as estimated by proton NMR.
9 The solution is cooled to about 20°C, and the polymer is precipitated with hexane
10 (Mallinckrodt, ACS grade), filtered, washed, and dried. This polymer is dissolved in
11 tetrahydrofuran (Mallinckrodt, ACS grade) under argon and cooled to about 15°C.
12 5.23 ml of triethylamine (Aldrich) and 3 ml of acryloyl chloride (Aldrich) are added
13 to the mixture while bubbling argon through the solution. The mixture is then
14 refluxed under argon for 24 hours. Triethylamine hydrochloride precipitate is
15 removed by filtration. The macromolecular precursor is precipitated with hexane,
16 filtered, washed, and dried. The precursor is stored at 0°C under argon until use.

17 **Example 3: Synthesis of PEG Diacrylates of Various Molecular Weights.**

18 PEG diacrylates of various molecular weights are synthesized as described in
19 Cruise et al., *Biomaterials* 19:1287-1294 (1998). All solvents used in the synthesis
20 are reagent grade or better and the reactants are used as received.

21 Fifty grams of PEG diol (Union Carbide) with a molecular weight of either
22 1350 (2K), 3350 (4K), 8000 (8K) or 20,000 (20K) were dissolved in 750 ml of
23 benzene (Fisher) and water was removed by azeotropically distilling 250 ml of
24 benzene. Triethylamine (Aldrich), in four fold molar excess based on PEG diol end
25 groups, is added to the PEG solution at room temperature. Acryloyl chloride
26 (Aldrich), in four fold molar excess based on PEG diol end groups, is added dropwise
27 to the PEG solution to form acrylate diesters of PEG. The mixture is stirred overnight
28 at 35°C under argon. The insoluble triethylamine salts formed during the reaction are
29 removed by filtration and the PEG diacrylate product is precipitated by the addition of
30 1.4 liters of diethyl ether (Fisher) chilled to 4°C. The PEG diacrylate precipitate is
31 collected on a fritted funnel, redissolved in 100 ml of benzene, and reprecipitated with
32 1.4 liters of chilled diethyl ether twice more. The polymer is dried 24 h in a vacuum
33 oven at 35° C.

1 PEG diacrylates are analyzed using nuclear magnetic resonance (NMR)
 2 spectroscopy and gel permeation chromatography (GPC). The degree of substitution
 3 of the PEG terminal alcohol for acrylate is determined using the NMR spectrum of
 4 PEG diacrylates and the method of Dust et al., *Macromolecules* 23:3743-3746 (1990),
 5 which compares the ratio of the integration from the PEG backbone (~3.5 ppm) and
 6 the acrylate peaks (~ 5.8–6.4 ppm) to the known PEG weight average molecular
 7 weight. The extent of acrylation substitution is calculated using the following
 8 formula: % acrylation = {PEG molecular weight}/{(integral of PEG
 9 backbone)/[(integral of acrylates)/6]/4X44}.

10 **Example 4: Visible Laser Polymerization of 10,000-Da PEG Polymer Precursor**

11 The tissue is incubated in 1 mM eosin Y (Sigma), a nontoxic photoinitiator, in
 12 Hepes-buffered saline (10 mM, pH 7.4) for 1 minute to adsorb the photoinitiator onto
 13 the surface of the tissue. The tissue is then rinsed twice in Hepes-buffered saline and
 14 infused with a 23% solution of the macromolecular precursor that also contains 100
 15 mM triethanolamine (Aldrich) and 0.15% *N*-vinylpyrrolidone (Aldrich). The tissue is
 16 illuminated using an argon ion laser (514 nm, 70 mW/cm², 2-s exposure; American
 17 Laser, Salt Lake City) to convert the liquid precursor to a hydrogel on the surface of
 18 the tissue.

19 **Example 5: Polymerization of 10,000-Da PEG Polymer Precursor with Emitted** 20 **Light Between 400 and 600 nm**

21 The tissue is contacted with 1 mM eosin Y in Hepes-buffered saline, which is
 22 allowed to adsorb to the tissue for 1 minute. The eosin Y is withdrawn, and the tissue
 23 is rinsed twice with saline. The tissue is then contacted with a 23% solution of the
 24 precursor that also contains 100 mM triethanolamine and 0.15% *N*-vinylpyrrolidone.
 25 The tissue is then externally illuminated with a 1-kW Xe arc lamp that emits light
 26 between 400 and 600 nm (Optomed, Austin, TX) at an irradiance of 35 mW/cm².
 27 Illumination times are between 2 and 15 s.

28 **Example 6: Assessing Biocompatibility of Photochemically Reactive Polymer** 29 **Formulations**

30 Dutch Banded Rabbits are given general anesthesia with an intramuscular
 31 injection of xylazine and ketamine. Two Dutch Rabbits eyes are injected
 32 intravitreally with 100µl of a mixture of a photochemically reactive polymer

1 precursor, N-vinylpyrrolidone (1500 ppm), triethanolamine (20 mM), and eosin Y
2 photoinitiator (10 μ M) in a balanced saline solution. An external, hand-held Xenon
3 arc light source (400 - 600 nm) is used to irradiate the globe of the eye for 1 minute.

4 The eyes are examined clinically with slit lamp and indirect ophthalmoscopy
5 at days 1 and 5 post-injection for media opacity or other signs of ocular toxicity.
6 Rabbits are then sacrificed on day 5 and the eyes are examined for histologic evidence
7 of intraocular inflammation or toxicity.

8 Long chain PEG (20,000 g/mol)

9 At days 1 and 5 post-injection of a polymer formulation containing 23% long
10 chain PEG (20,000) g/mol), no media opacity or other signs of ocular toxicity were
11 evident and the fundus was clearly visible in both eyes. The rabbits were sacrificed
12 on day 5 and the eyes were processed for light microscopy. The animals showed no
13 histologic evidence of intraocular inflammation or toxicity. The iridocilliary
14 processes showed none of the inflammatory processes evident in rabbit eyes injected
15 with di-N-hydroxy succinimidyl activated glutarate-terminated PEG (Example 7).
16 There was no fibrinoid reaction in the vitreous cavity. There was no inflammatory
17 process evident in the retina or in the vitreous cavity.

18 Short chain PEG (4,000 g/mol)

19 Rabbits were treated as described above, except that a retinal break was
20 created as described below in Example 8. The animals were examined at 1 and 7 days
21 after injection of a polymer formulation containing 23% short chain PEG (4,000
22 g/mol) by penlight and indirect ophthalmoscopy. Severe intraocular inflammation
23 was evident in both treated eyes. A fibrinous pupillary membrane obscured the pupil
24 of one eye and no view of the fundus was possible in either treated eye.

25 Example 7: Assessing Biocompatibility of Autochemically Reactive Polymer 26 Formulations

27 Dutch Banded Rabbits are given general anesthesia with an intramuscular
28 injection xylazine and ketamine. Auto-chemically reactive polymer precursors are
29 mixed in a balanced saline solution and 100 μ l is injected intravitreally using a 27
30 gauge needle on a tuberculin syringe. A gel is allowed to form.

The eyes are examined at days 1 and 5 for signs of intraocular inflammation and opacification of the ocular media. The rabbits are sacrificed on day 5 and the eyes are examined for histological evidence of intraocular inflammation or toxicity.

Di-N-hydroxysuccinimidyl activated glutarate-terminated PEG

PEG tetra-amine (molecular weight 20,000 g/mol) and di-N-hydroxysuccinimidyl activated glutarate-terminated PEG (molecular weight 3,500 g/mol) were mixed to yield a polymer formulation containing 11.5% of each polymer precursor and injected intravitreally. At days 1 and 5 post-injection, severe intraocular inflammation and opacification of the ocular media were evident. The pupil was obscured and no view of the fundus was possible. The rabbits were sacrificed on day 5 and the eyes were processed for light microscopy. Both eyes showed marked inflammatory cell infiltration of the uveal tract and vitreous cavity. The iridocilliary processes were haemorrhagic and edematous. A marked suppurative reaction with multiple eosinophilic polymorphonucleocytes was observed. A marked fibrinoid reaction was visible in the vitreous cavity. A subretinal inflammatory process was evident, with multiple eosinophilic polymorphonucleocytes that extended into the vitreous cavity. The inflammatory processes also extended into the anterior chamber.

Di-N-hydroxysuccinimidyl activated succinate-terminated PEG

PEG tetra-amine (molecular weight 20,000 g/mol) and di-N-hydroxysuccinimidyl activated succinate-terminated PEG (molecular weight 3,500 g/mol) were mixed to yield a polymer formulation containing 11.5% of each polymer precursor and injected intravitreally. At days 1 and 5 post-injection, no media opacity or other signs of ocular toxicity were evident. The rabbits were sacrificed on day 5 and the eyes were processed for light microscopy. The rabbit eyes showed no histologic evidence of intraocular inflammation or toxicity. The iridocilliary processes showed none of the inflammatory processes evident in rabbit eyes injected with glutarate-terminated PEG as described above. There was no fibrinoid reaction in the vitreous cavity. There was no inflammatory process evident in the retina or in the vitreous cavity.

Example 8: Assessing Adherent Properties of Polymer Implant

1 Two New Zealand White Rabbits are given general anesthesia with an
 2 intramuscular injection of xylazine and ketamine. They are then pre-treated with
 3 cryotherapy behind the nasal and temporal limbus in the ora serrata region under
 4 direct visualization. Two weeks later, using sterile technique, the animals undergo
 5 vitrectomy and lensectomy. Endodiathermy is then used to create an approximate 1
 6 disc diameter retinal break just superior to the medullary wing. Balanced saline
 7 solution is injected into the subretinal space using a 30 gauge cannula to create a
 8 localized retinal detachment. Fluid-gas exchange is then performed, and the retina is
 9 flattened. The polymer formulation is applied over the retinal break using a 30 gauge
 10 cannula. The fiberoptic endo-illuminator of the Premier Vitrector (Storz Instruments)
 11 is then used to irradiate the mixture for 1 minute, causing a thin, transparent
 12 polymerized gel to form over the retinal break. The eyes are then refilled with
 13 balanced saline solution. Attempts are made to displace the gel with the fiberoptic
 14 illuminator tip and the 30 gauge cannula.

15 Short chain PEG

16 Short chain PEG diacrylate (molecular weight 4000 g/mol, ca. 10%
 17 concentration), N-vinylpyrrolidone (1500 ppm), and triethanolamine (20 mM)
 18 precursors were mixed with an eosin Y photoinitiator (10 μ M) and applied over the
 19 retinal break. The polymer remained adherent to the hole and surrounding retina.
 20 Thus, it is possible to precisely apply the polymer precursor solution under gas,
 21 polymerize it with visible light, and form an adherent gel over the hole that resists
 22 mechanical displacement.

23 Example 9: Assessing Rate of Degradation of Polymer Implant

24 The duration of presence of non-toxic hydrogels on the retina is determined by
 25 incorporating commercially available 1 μ M diameter fluorescence polymer beads
 26 (Polysciences) in the hydrogel precursor and thus in the hydrogel. This fluorescence
 27 can readily be observed in the eye non-invasively by the same type of fluorescence
 28 biomicroscopy commonly used to visualize the eyes of human patients given
 29 fluorescein. Eighteen Dutch Banded rabbits are given general anesthesia with an
 30 intramuscular injection of xylazine and ketamine. The right eyes are treated with
 31 cryotherapy behind the nasal and temporal limbus in the ora serrata region under
 32 direct visualization. Two weeks later, the animals are again given general anesthesia

1 with an intramuscular injection of xylazine and ketamine. Lensectomy and
 2 vitrectomy are performed on the right eyes. A bent 30 gauge needle or vitrector is
 3 then used to create an approximately 1 disc diameter retinal break just superior to the
 4 medullary wing. Balanced saline solution is injected into the subretinal space using a
 5 30 gauge cannula to create a localized retinal detachment. Fluid-gas exchange is then
 6 performed, and the retina is flattened. For example, the rabbits are divided into 3
 7 groups of 6 rabbits each and given the treatments outlined below:

8 GROUP 1: 1% oligolactic acid photochemically reactive polymer

9 GROUP 2: 10% oligolactic acid photochemically reactive polymer

10 GROUP 3: Auto-chemically reactive polymer

11 All rabbits undergo vitrectomy, lensectomy, creation of a retinal break and
 12 detachment as described above. Fluid-gas exchange is then performed and laser
 13 photocoagulation applied around the retinal break in customary fashion. In each
 14 group of rabbits one of the hydrogel formulations and incorporated fluorescence
 15 polymer beads (Polysciences) is injected over and around the retinal break using a 30
 16 gauge cannula. In the case of photochemical hydrogels, the fiberoptic endo-
 17 illuminator is used to irradiate the mixture for 1 minute to form an adherent gel
 18 overlying the retinal hole. The eyes are filled with balanced saline solution,
 19 sclerotomies and conjunctiva are closed, and a subconjunctival injection of
 20 gentamycin is given. On post-operative days 1, 3, 7, 14, 21, and 28 the rabbits are
 21 examined by fluorescence biomicroscopy to determine whether polymer remains
 22 adherent to the retina. Because a chorioretinal adhesion may take up to 2 weeks to
 23 reach maximal strength, polymer formulations should ideally remain adherent to the
 24 retina for at least this amount of time but not more than 4 weeks. The animals are
 25 sacrificed after 28 days and the eyes are examined histologically.

26 **Example 10: Short-term Retinal Break Closure with Polymer Formulation**

27 Two New Zealand White Rabbits are given general anesthesia with an
 28 intramuscular injection of xylazine and ketamine. To prevent intraoperative bleeding,
 29 the animals are then euthanized with an intracardiac injection of Pentobarbital. The
 30 animals then immediately undergo vitrectomy and lensectomy. Endodiathermy is
 31 then used to create an approximate 1 disc diameter retinal break just superior to the
 32 medullary wing. Balanced saline solution is injected into the subretinal space using a

20 gauge cannula to create a localized retinal detachment. Fluid-gas exchange is then performed, and the retina is flattened. In one rabbit, balanced saline solution is then injected over the retinal break, which causes a localized retinal detachment. In the other rabbit, a photoreactive polymer formulation is applied over the retinal break using a 20 gauge cannula. The fiberoptic endo-illuminator of the Premier Vitrector (Storz Instruments) is then used to irradiate the mixture for 1 minute, causing a thin, transparent polymerized gel to form over the retinal break. Balanced saline solution is then injected over the polymer-covered retinal break.

9 PEG diacrylate

10 PEG diacrylate (molecular weight 8,000 g/mol) containing 5% lactide was
11 applied to a retinal break created as described above. Despite forceful injection of 3
12 cc of saline solution through a 20 gauge cannula, the retina remained attached.

13 Example 11: Assessing Ability of Polymer Implant to Seal Retinal Break

14 Twenty-four Dutch Banded rabbits are given general anesthesia with an
15 intramuscular injection of xylazine and ketamine. The right eyes are treated with
16 cryotherapy behind the nasal and temporal limbus in the ora serrata region under
17 direct visualization. Two weeks later, the animals are again given general anesthesia
18 with an intramuscular injection of xylazine and ketamine. Lensectomy and
19 vitrectomy are performed on the right eyes. A bent 30 gauge needle or vitrector is
20 then used to create an approximately 1 disc diameter retinal break just superior to the
21 medullary wing. Balanced saline solution is injected into the subretinal space using a
22 30 gauge cannula to create a localized retinal detachment. Fluid-gas exchange is then
23 performed, and the retina is flattened. For example, the rabbits are divided into 4
24 groups of 6 rabbits each and given the treatments outlined below:

25 GROUP 1: No polymer treatment

26 GROUP 2: 1% oligolactic acid photochemically reactive polymer

27 GROUP 3: 10% oligolactic acid photochemically reactive polymer

28 GROUP 4: Autochemically reactive polymer

29 Rabbits in group 1 receive no polymer treatment. Rabbits in groups 2-4
30 receive one of the hydrogel formulations injected over and around the retinal break
31 using a 30 gauge cannula. In the case of photochemical hydrogels, the fiberoptic

1 endo-illuminator is used to irradiate the mixture for 1 minute to form an adherent gel
 2 overlying the retinal hole. Next, the eyes are filled with saline through the infusion
 3 port until the retinas of all control rabbits re-detach, which typically occurs after a few
 4 minutes of continuous infusion. Rabbits treated with polymer application to the
 5 retinal break are observed for re-detachment of the retina as they are infused with
 6 saline for 5 minutes. Since the goal is to assess the ability of the hydrogels to adhere
 7 to and close a retinal break, laser photocoagulation is not performed. The eyes are
 8 filled with balanced saline solution, sclerotomies and conjunctiva are closed, and a
 9 subconjunctival injection of gentamycin is given. Clinical examination with slit lamp
 10 and indirect ophthalmoscopy is performed on post-operative days 1, 3, 7, 14, 21, and
 11 28. Evidence of retinal detachment, spontaneous retinal re-attachment, or signs of
 12 ocular inflammation is noted. At 28 days rabbits are sacrificed and the eyes are
 13 examined histologically for any evidence of toxicity of the procedure.

14 **Example 12: Closure of a Retinal Break in a Human Patient with a**
 15 **Photoreactive Polymer Formulation**

16 A patient with rhegmatogenous retinal detachment requiring vitrectomy, such
 17 as retinal detachment with proliferative vitreoretinopathy or a detachment with a
 18 posterior retinal break, undergoes vitrectomy. Fluid-gas exchange with sterile air,
 19 CO₂, or xenon gas is performed to flatten the retina. Laser is applied around the hole
 20 if it is extra-macular. Laser is not applied around macular holes. 10-25% long chain
 21 PEG diacrylate (molecular weight 8,000 g/mol) containing about 5% lactide, N-vinyl
 22 pyrrolidone (1500 ppm), and triethanolamine (5 nM) are mixed with an eosin Y
 23 photoinitiator (1 mM) and applied to the retinal surface over and around the retinal
 24 hole under gas. Polymerization is then accomplished with 15-60 seconds of
 25 irradiation with either the fiberoptic endoilluminator or a xenon arc light source via a
 26 transpupillary route. The polymerization reaction results in a thin, transparent gel
 27 which closes the retinal break and remains adherent to the retinal surface. The eye is
 28 filled with a balanced saline solution. Over a 2-12 week period, preferably 2-3 weeks,
 29 the adherent polymer biodegrades. Thereafter, the retinal break is closed by a
 30 chorioretinal adhesion. No post-operative positioning is required.

31
 32 **Example 13: Closure of a Retinal Break in a Human Patient with an**
 33 **Autoreactive Polymer Formulation**

1 A patient with rhegmatogenous retinal detachment requiring vitrectomy, such
2 as retinal detachment with proliferative vitreoretinopathy or a detachment with a
3 posterior retinal break, undergoes vitrectomy. Fluid-gas exchange is performed to
4 flatten the retina. Laser is applied around the hole if it is extra-macular. Laser is not
5 applied around macular holes. Albumin and di-N-hydroxysuccinimidyl activated
6 succinate-terminated PEG are applied simultaneously by mixing the contents of
7 separate syringes through a connector to the retinal surface over and around the retinal
8 hole under gas. The polymerization reaction begins immediately upon mixing and
9 results in a thin, transparent gel which closes the retinal break and remains adherent to
10 the retinal surface. The eye is filled with a balanced saline solution. Over a 2-12 week
11 period, preferably 2-3 weeks, the adherent polymer biodegrades. Thereafter, the
12 retinal break is closed by a chorioretinal adhesion. No post-operative positioning is
13 required.

14

15 **Example 14: Prevention of Proliferative Vitreoretinopathy (PVR)**

16 Dutch Belted rabbits pre-treated with peripheral retinal cryotherapy undergo
17 pars plana lensectomy and vitrectomy. A posterior retinal tear is then created with a
18 bent 30 gauge needle or a vitrector. Balanced saline is then injected in the subretinal
19 space to create a retinal detachment. Fluid-gas exchange is then performed and the
20 retina flattened. Laser is applied around the hole(s) if it is extra-macular. Laser is not
21 applied around macular holes. Then, a polymer precursor solution containing
22 photoinitiator is applied to the retinal surface around the retinal hole. Polymerization
23 is then accomplished with 15-60 seconds of irradiation with either the fiberoptic
24 endoilluminator or a xenon arc light source via a transpupillary route, which closes
25 the retinal break and remains adherent to the retinal surface.

26 The eye is then filled with photoinitiator to coat the surface of the retina.
27 Excess photoinitiator is then drained from the eye. Next, polymer precursor solution
28 that does not contain photoinitiator is applied to the remainder of the retinal surface
29 and polymerization is again accomplished with 15-60 second of irradiation with either
30 the fiberoptic endoilluminator or a xenon arc light source via a transpupillary route.
31 The polymerization reaction results in a thin, transparent gel where the polymer
32 precursor contacts the photoinitiator, but not in areas free of photoinitiator. This results
33 the formation of a gel only on the surface of the retina. The eye is once again filled

1 with fluid. Unpolymerized precursors are then irrigated from the eye. Over a 2-10
2 week period the adherent polymer biodegrades. The polymerized gel overlying the
3 retina both closes the retinal break and prevents adherence of scar tissue that could
4 cause proliferative vitreoretinopathy and recurrent retinal detachment. No post-
5 operative positioning is required.

6 **Example 15: Prevention of Proliferative Vitreoretinopathy (PVR) in a Human**
7 **Patient by Using a Photoreactive Polymer Formulation After Vitrectomy**

8 A patient with rhegmatogenous retinal detachment requiring vitrectomy, such as
9 retinal detachment with proliferative vitreoretinopathy or a detachment with a
10 posterior retinal break, undergoes vitrectomy. Fluid-gas exchange is performed to
11 flatten the retina. Laser is applied around all retinal breaks. A solution containing 10
12 μ M eosin Y photoinitiator is then applied to the entire retinal surface covering all
13 retinal breaks as well as intact retina. The photoinitiator solution is then washed from
14 the vitreous cavity, but it remains adherent to the retinal surface. The vitreous cavity
15 is then filled with a mixture of long chain PEG diacrylate (molecular weight 20,000
16 g/mol) containing about 10% lactide, N-vinyl pyrrolidone (1500 ppm), and
17 triethanolamine (20 nM), which contacts the photoinitiator coated retina. The
18 intraocular cavity is then irradiated for 15-120 seconds with a fiberoptic
19 endoilluminator or a xenon arc light source via a transpupillary route to effect
20 polymerization. The polymerization reaction results in a thin, transparent gel which
21 closes the retinal breaks and remains adherent to the retinal surface. Coverage of the
22 retinal tissue prevents cells and proteins that cause PVR from adhering to the retinal
23 surface. The eye is irrigated with balanced saline solution to remove all unreacted
24 polymer precursor. Balanced saline is left in the vitreous cavity and the patient has no
25 positional requirement. Over a 2-16 week period, preferably 6-12 weeks, the adherent
26 polymer biodegrades. Thereafter, the retinal break is closed by a chorioretinal
27 adhesion. No post-operative positioning is required. The likelihood of PVR is
28 reduced.

29

30 All publications and patent applications mentioned in this specification are
31 herein incorporated by reference to the same extent as if each individual publication
32 or patent application was specifically and individually indicated to be incorporated by
33 reference.

1 The invention now being fully described, it will be apparent to one of ordinary
2 skill in the art that many changes and modifications can be made thereto without
3 departing from the spirit or scope of the appended claims.

4

5

Claims

1. A method for closing a retinal break in a mammal, which method comprises:

5 applying over and around the retinal break a non-toxic polymer formulation comprising at least one polymer precursor that is a poly (ethylene glycol) (PEG) based polymer precursor and transforming the polymer formulation into a gel-like coat.

2. A method for closing a retinal break in a mammal, which method comprises:

10 applying over and around the retinal break a non-toxic polymer formulation comprising at least one polymer precursor, and transforming the polymer formulation into a gel-like coat by photopolymerization, autopolymerization, or physicochemical polymerization of the polymer precursor.

15 3. The method of Claim 1 or Claim 2, wherein the polymer formulation comprises a polymer precursor of the formula:



wherein W is a water-soluble polymer; D is a degradable moiety; P is a photopolymerizable moiety; m and q are integers from 1 to about 10; o is an
20 integer from 1 to about 100; and n and p are integers from 0 to about 120.

4. The method of Claim 1 or Claim 2, wherein the PEG comprises reactive termini.

5. The method of Claim 4, wherein the reactive termini are free radical polymerizable termini.

6. The method of Claim 4, wherein the reactive termini are acrylate termini.
7. The method of Claim 4, wherein the PEG comprises a long chain PEG having a molecular weight of at least about 8,000 g/mol.
8. The method of Claim 4, wherein the PEG comprises a long chain PEG
5 having a molecular weight of at least about 20,000 g/mol.
9. The method of Claim 4, wherein the PEG based polymer precursor further comprises degradable regions.
10. The method of Claim 9, wherein the degradable regions comprise from about 0.1% to about 25% oligolactic acid, and the formulation optionally
10 comprises photoinitiator and at least one co-catalyst, which can include N-vinylpyrrolidone and triethanolamine.
11. The method of Claim 8, wherein the PEG based polymer precursor further comprises about 1% oligolactic acid.
12. The method of Claim 8, wherein the PEG based polymer precursor
15 further comprises about 10% oligolactic acid.
13. A method for closing a retinal break in mammal, which method comprises:

applying over and around the retinal break a non-toxic polymer formulation comprising at least one polymer precursor, transforming the polymer
20 formulation into a gel-like coat, and applying at least one photoinitiator to the retinal surface.
14. The method of Claim 13, wherein the photoinitiator is an eosin Y photoinitiator.

15. The method of Claim 13, wherein the formulation further comprises at least one co-catalyst.

16. The method of Claim 1, Claim 2 or Claim 13, wherein the transformation is by autopolymerization of the polymer precursor.

5 17. A method for closing a retinal break in a mammal, which method comprises:

applying over and around the retinal break a non-toxic polymer formulation comprising at least one polymer precursor and transforming the polymer formulation into a gel-like coat, wherein the polymer formulation

10 comprises a first polymer precursor and a second polymer precursor, the first and second polymer precursors being mutually reactive.

18. The method of Claim 17, wherein the first polymer precursor is an amine.

19. The method of Claim 18, wherein the amine is a tetra-amino poly(ethylene glycol) (PEG).

15 20. The method of Claim 17, wherein the first polymer precursor is a protein and the second polymer precursor is a terminally-functionalized poly(ethylene glycol)(PEG).

21. The method of Claim 20, wherein the protein is albumin, collagen, or gelatin.

20 22. The method of Claim 21, wherein the protein is albumin.

23. The method of Claim 17, wherein the second PEG molecule is a di-N-hydroxysuccinimidyl PEG.

24. The method of Claim 17, wherein the second polymer precursor is a difunctionally activated form of PEG wherein the activating group is a succinate.

25. The method of Claim 24, wherein the second polymer precursor is a PEG di-succinimidyl carbonate.
26. The method of any preceding claim, wherein the mammal is a human.
27. The method of any preceding claim, wherein the gel-like coat comprises
5 a biodegradable polymer.
28. The method of any preceding claim, wherein the polymer formulation is applied to more than about 50% of the retinal surface.
29. The method of Claim 28, wherein the polymer formulation is applied to the entire retinal surface.
- 10 30. A method for management of retinal detachment in a mammal, comprising:
applying over and around a retinal break a non-toxic polymer formulation comprising at least one polymer precursor, and
transforming the polymer formulation into a gel-like coat.
- 15 31. The method of Claim 30, further comprising, prior to the overlapping, draining the subretinal fluid with fluid/gas exchange.
32. The method of Claim 30, further comprising creating at least one chorioretinal adhesion around the retinal break.
33. The method of Claim 32, wherein the chorioretinal adhesion is created
20 before the polymer formulation is applied to the retinal surface.
34. The method of Claim 32, wherein the chorioretinal adhesion is created by laser photocoagulation.
35. A method for preventing proliferative vitreoretinopathy in a mammalian eye having a retinal break, comprising:

(a) applying over and around the retinal break and to more than about 50% of the retinal surface of the mammal a first solution comprising a non-toxic polymer formulation comprising at least one polymer precursor, and

(b) transforming the polymer formulation into a gel-like coat.

5 36. The method of Claim 35, further comprising, prior to step (a), draining the subretinal fluid with fluid/gas exchange.

37. The method of Claim 35, wherein the transforming is by photopolymerization of the polymer precursor.

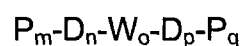
38. The method of Claim 35, wherein step (a) further comprises, prior to
10 applying the first solution, the steps of (i) filling the eye with a second solution comprising at least one photoinitiator but no polymer precursor, and (ii) draining the eye, wherein the applying is by filling the eye with the first solution, wherein the first solution lacks a photoinitiator.

39. The method of Claim 35, further comprising creating at least one
15 chorioretinal adhesion around the retinal break.

40. The method of Claim 38, further comprising, prior to the filling of the eye with the second solution, applying over and around the retinal break a third solution comprising polymer precursor and at least one photoinitiator.

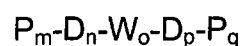
41. A pharmaceutical composition for the closure of retinal breaks with a
20 non-toxic, biodegradable polymer, which composition comprises at least one photopolymerizable polymer precursor and at least one reagent that catalyzes photopolymerization of the precursor in admixture with a suitable vehicle for delivery to the interior of the eye.

42. The pharmaceutical composition of Claim 41, wherein the polymer precursor is a poly(ethylene glycol) (PEG) based polymer precursor of the formula:



5 Wherein W is a long chain PEG having a molecular weight of at least 8,000 g/mol; D is a degradable moiety; P is a photopolymerizable moiety; m and q are integers from 1 to about 10; o is an integer from 1 to about 100; and n and p are integers from 0 to about 120, and the reagents include at least one photoinitiator and at least one co-catalyst.

10 43. An article of manufacture for the closure of retinal breaks with a non-toxic polymer comprising a first container comprising poly(ethylene glycol) (PEG) based polymer precursor of the formula:



Wherein W is a long chain PEG having a molecular weight of at least 8,000
15 g/mol; D is a degradable moiety; P is a photopolymerizable moiety: m and q are integers from 1 to about 10; o is an integer from 1 to about 100; and n and p are integers from 0 to about 120.

44. The article of manufacture of Claim 43, further comprising a second container comprising a photoinitiator solution but no polymer precursor.

20 45. The article of manufacture of Claim 43, further comprising printed instructions for closing a retinal break in a mammal, comprising applying the polymer precursor over and around the retinal break and transforming the polymer precursor into a gel-like coat.

46. The article of manufacture of Claim 44, further comprising printed instructions for preventing proliferative vitreoretinopathy in a mammalian eye, comprising filling the eye with the contents of the second container, draining the eye, and filling the eye with the contents of the first container, wherein the first
5 container does not contain a photoinitiator.
47. An article of manufacture for the closure of retinal breaks with a non-toxic polymer, comprising a first container comprising a first polymer precursor, a second container comprising a second polymer precursor, said first and second polymer precursors being mutually reactive.
- 10 48. The article of manufacture of Claim 47, wherein the first polymer precursor is albumin, collagen or gelatin, and the second polymer is a terminally-functionalized poly(ethylene glycol) (PEG).
49. The article of manufacture of Claim 47, wherein the first and second containers are syringe barrels.
- 15 50. The article of manufacture of Claim 47, further comprising printed instructions for a method for closing a retinal break in a mammal, comprising:
combining the first and second polymer precursors immediately before
applying to the retinal surface of the mammal over and around the retinal break.
51. The article of manufacture of Claim 50, wherein the combining occurs
20 during extrusion into and through a connector connecting the first and second containers and onto the retinal surface.