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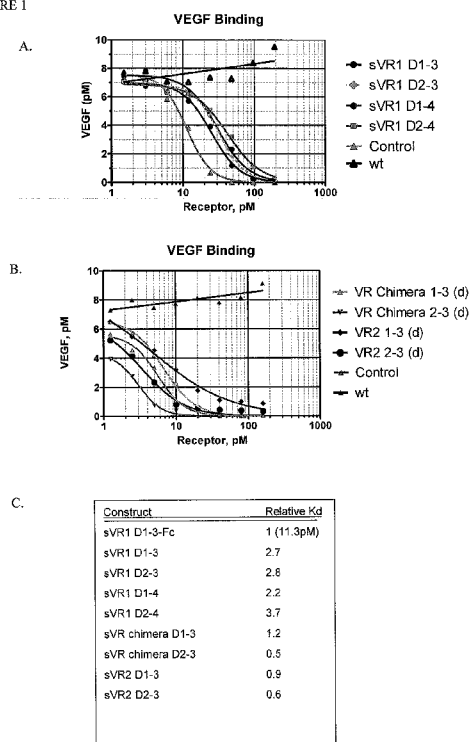
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(54) Title: CELL LINES THAT SECRETE SOLUBLE VEGF RECEPTORS AND USES THEREOF

FIGURE 1



(57) Abstract: The invention provides isolated nucleic acid and polypeptide sequences encoding Vascular Endothelial Growth Factor (VEGF) receptors, such as soluble VEGF receptors. Also encompassed are cell lines encoding such VEGF receptors. The invention also provides encapsulated cell therapy devices that are capable of delivering such VEGF receptors as well as methods of using these devices to deliver the VEGF receptors to the eye and to treat ophthalmic and cell proliferation disorders in patients suffering therefrom.



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CELL LINES THAT SECRETE SOLUBLE VEGF RECEPTORS AND USES THEREOF

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FIELD OF THE INVENTION

The present invention relates generally to the field of encapsulated cell therapy.

BACKGROUND OF THE INVENTION

Many clinical conditions, deficiencies, and disease states can be remedied or
10 alleviated by supplying to the patient one or more biologically active molecules produced by
living cells or by removing from the patient deleterious factors which are metabolized by
living cells. In many cases, these molecules can restore or compensate for the impairment or
loss of organ or tissue function. Accordingly, many investigators have attempted to
reconstitute organ or tissue function by transplanting whole organs, organ tissue, and/or cells,
15 which provide secreted products or affect metabolic functions. However, while such
transplantation can provide dramatic benefits, it is limited in its application by the relatively
small number of organs that are suitable and available for grafting. Moreover, in general,
transplantation patients must be immunosuppressed in order to avert immunological rejection
of the transplant, which results in loss of transplant function and eventual necrosis of the
20 transplanted tissue or cells. Likewise, in many cases, the transplant must remain functional
for a long period of time, even for the remainder of the patient's lifetime. It is both
undesirable and expensive to maintain a patient in an immunosuppressed state for a
substantial period of time.

A number of vision-threatening disorders of the eye exist for which additional
25 effective therapies are still needed. One major problem in treatment of such diseases is the
inability to deliver therapeutic agents into the eye, due to the presence of the blood-retinal
barrier, and to maintain them there at therapeutically effective concentrations.

Many growth factors have shown promise in the treatment of ocular disease. For
example, BDNF and CNTF have been shown to slow degeneration of retinal ganglion cells

and decrease degeneration of photoreceptors in various animal models. *See, e.g.*, Genetic Technology News, vol. 13, no. 1 (Jan. 1993). Additionally, nerve growth factor has been shown to enhance retinal ganglion cell survival after optic nerve section and has also been shown to promote recovery of retinal neurons after ischemia. *See, e.g.*, Siliprandi, et al., Invest. Ophthalmol. & Vis. Sci., 34, pp. 3232-3245 (1993).

A desirable alternative to transplantation procedures is the implantation of cells or tissues within a physical barrier which will allow diffusion of nutrients, metabolites, and secreted products, but will block the cellular and molecular effectors of immunological rejection. A variety of devices which protect tissues or cells producing a selected product from the immune system have been explored. *See, e.g.*, US Patent No. 5,158,881; WO92/03327; WO91/00119; and WO93/00128, each of which is incorporated herein by reference in its entirety. These devices include, for example, extravascular diffusion chambers, intravascular diffusion chambers, intravascular ultrafiltration chambers, and implantation of microencapsulated cells. *See* Scharp, D. W., et al., World J. Surg., 8, pp. 221-9 (1984). *See, e.g.*, Lim et al., Science 210: 908-910 (1980); Sun, A. M., Methods in Enzymology 137: 575-579 (1988); WO 93/03901; and U.S. Pat. No. 5,002,661. The use of such devices would alleviate the need to maintain the patient in an immunosuppressed state. However, none of these approaches have been satisfactory for providing long-term transplant function.

Thus, methods of delivering appropriate quantities of needed substances, such as, for example, neurotrophic factors, anti-angiogenic factors, anti-inflammatory factors, enzymes, hormones, or other factors, or of providing other needed metabolic functions, to the eye for an extended period of time are needed.

SUMMARY OF THE INVENTION

Provided herein are isolated nucleic acids encoding a Vascular Endothelial Growth Factor (VEGF) receptor wherein the nucleic acids comprise or consist of a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, and SEQ ID NO:13. The invention also provides nucleic acid molecules encoding a polypeptide comprising or consisting of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14.

Also provided are nucleic acid molecules encoding a polypeptide having a sequence that is at least 95% identical to any SEQ ID NOs: 2, 4, 6, 8, 10, 12, or 14. Alternatively, the nucleic acid molecules may be the complement of such a nucleic acid molecule. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

An "isolated" nucleic acid molecule is one that is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the nucleic acid molecules of the invention can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention (*e.g.*, a nucleic acid molecule having a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, and SEQ ID NO: 13, encoding a polypeptide having a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14, or a complement of any of these nucleotide sequences) can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of these nucleic acid sequences a hybridization probe, soluble VEGF receptor molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook *et al.*, (eds.), MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

Any of the nucleic acids of the invention can be amplified using cDNA, mRNA or, alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according

to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to soluble VEGFR nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term “oligonucleotide” refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue.

Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at least 6 contiguous nucleotides of SEQ ID NO: 1, 3, 5, 7, 9, 11, or 13 or a complement thereof. Oligonucleotides may be chemically synthesized and may be used as probes.

In other embodiments, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, or 13. A nucleic acid molecule that is complementary to these nucleotide sequences is one that is sufficiently complementary to the nucleotide sequence that it can hydrogen bond with little or no mismatches, thereby forming a stable duplex.

As used herein, the term “complementary” refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term “binding” means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, Van der Waals, hydrophobic interactions, etc. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, or 13, *e.g.*, a fragment that can be used as a probe or primer or a fragment encoding a biologically active portion of any of the soluble VEGF receptors of the invention. Fragments provided herein are defined as

sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type. Homologs are nucleic acid sequences or amino acid sequences of a particular gene that are derived from different species.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid. Derivatives or analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 30%, 50%, 70%, 80%, or 95% identity (with a preferred identity of 80-95%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, or 13 due to degeneracy of the genetic code and thus encode the same soluble VEGFR proteins as that encoded by the nucleotide sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, or 13.

In another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11 or 13. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500, 1000, 1500, 2000, or more

nucleotides in length. In another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region, for example SEQ ID NO: 1, 3, 5, 7, 9, 11, or 13.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other. Moreover, as used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at T_m, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in Ausubel *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions are hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C, followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well-known in the art. See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and

Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (e.g., as employed for cross-species hybridizations). See, e.g., Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981, *Proc Natl Acad Sci USA* 78: 6789-6792.

Also provided are polypeptide encoded by any of the nucleic acid molecules described herein. For example, these polypeptide can be soluble VEGF receptors. In some embodiments, the soluble VEGF receptors described herein bind (e.g., preferentially) to VEGF. The binding of these soluble VEGF receptors to VEGF inhibits endothelial cell proliferation and vascular permeability.

The invention also involves an isolated polypeptide that is at least 80% identical to a polypeptide having an amino acid sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14. Alternatively, the isolated polypeptide is at least 80% homologous to a fragment (*i.e.*, at least 6 contiguous amino acids) of a polypeptide having an amino acid sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14. Moreover, the invention also includes isolated polypeptides that are at least 80% homologous to a derivative, analog, or homolog of a polypeptide having an amino acid sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14. Similarly, the invention also provides an isolated polypeptide that is at least 80% identical to a naturally occurring allelic variant of a polypeptide having an amino acid sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14. Those skilled in the art will recognize that such polypeptides should be encoded by a nucleic acid molecule capable of hybridizing to a nucleic acid molecule of SEQ ID NOs: 1, 3, 5, 7, 9, 11, or 13 under stringent conditions.

As used herein, the terms "protein" and "polypeptide" are intended to be interchangeable. The novel polypeptides of the invention include the soluble VEGF receptor polypeptides whose sequence is provided in SEQ ID NOs: 2, 4, 6, 8, 10, 12, or 14. The invention also includes mutant or variant polypeptides any of whose residues may be changed from the corresponding residue shown in SEQ ID NOs: 2, 4, 6, 8, 10, 12, or 14, while still encoding a polypeptide that maintains its soluble VEGF receptor activities and physiological

functions, or a functional fragment thereof. In the mutant or variant protein, up to 20% or more of the residues may be so changed.

In general, a soluble VEGF receptor variant that preserves soluble VEGFR-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution.

Those skilled in the art will recognize that the invention also pertains to isolated soluble VEGFR polypeptides, and biologically active portions thereof, or derivatives, fragments, analogs or homologs thereof. Soluble VEGFR constructs described herein can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, the soluble VEGFR polypeptides of the invention are produced by recombinant DNA techniques. As an alternative to recombinant expression, a soluble VEGFR protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" polypeptide or biologically active portion thereof is substantially free of cellular material or other contaminating proteins or polypeptides from the cell or tissue source from which the soluble VEGFR polypeptide is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of soluble VEGFR polypeptides in which the polypeptide is separated from cellular components of the cells from which it is isolated or recombinantly produced. For example, the language "substantially free of cellular material" includes preparations of VEGFR polypeptide having less than about 30% (by dry weight) of non-VEGFR protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-VEGFR protein, still more preferably less than about 10% of non-VEGFR protein, and most preferably less than about 5% non-VEGFR protein. When the VEGFR polypeptide or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

Similarly, the language "substantially free of chemical precursors or other chemicals" includes preparations of soluble VEGFR polypeptide in which the polypeptide is separated from chemical precursors or other chemicals that are involved in the synthesis of the polypeptide. For example, the language "substantially free of chemical precursors or other chemicals" includes preparations of soluble VEGFR polypeptide having less than about 30% (by dry weight) of chemical precursors or non-VEGFR chemical, more preferably less than about 20% chemical precursors or non-VEGFR chemicals, still more preferably less than about 10% chemical precursors or non-VEGFR chemicals, and most preferably less than about 5% chemical precursors or non-VEGFR chemicals.

Biologically active portions of a soluble VEGFR polypeptide construct of the invention include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the soluble VEGFR polypeptides, *e.g.*, the amino acid sequence shown in SEQ ID NOs: 2, 4, 6, 8, 10, 12, or 14 that include fewer amino acids than the full length soluble VEGFR constructs described herein, and exhibit at least one activity of a soluble VEGFR polypeptide of the invention. Typically, biologically active portions comprise a domain or motif with at least one activity of the soluble VEGFR polypeptide.

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. *See*, Needleman and Wunsch 1970 *J Mol Biol* 48: 443-453. The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison,

determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region.

The invention also provides for soluble VEGF receptor chimeric or fusion proteins. A soluble VEGFR chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Ausubel *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide).

The invention further provides vectors containing any of the nucleic acid molecules of the invention. Specifically, the invention also pertains to vectors, preferably expression vectors, containing a nucleic acid encoding the soluble VEGFR polypeptides of the invention, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced

(e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise any of the nucleic acids of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., soluble VEGFR polypeptides, mutant forms of soluble VEGFR polypeptides, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of soluble VEGFR constructs of the invention in prokaryotic or eukaryotic cells. Other suitable expression systems for both prokaryotic and eukaryotic cells are known in the art. (See, e.g., Chapters 16 and 17 of Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY
5 MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989).

In addition, the invention also provides host cells or cell lines containing such vectors (or any of the nucleic acid molecules described herein). As used herein, the terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms
10 refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. By way of non-limiting
15 example, the host cell may be an ARPE-19 cell. However, other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing
20 foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989),
25 and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into
30 the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding the soluble VEGF receptor construct or can be introduced on a separate vector.

Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

5 A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) any of the soluble VEGFR polypeptide constructs described herein. Accordingly, the invention further provides methods for producing these soluble VEGFR polypeptides using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding soluble VEGFR has been introduced) in a suitable medium such that soluble
10 VEGFR polypeptide is produced. In another embodiment, the method further comprises isolating soluble VEGFR from the medium or the host cell.

Likewise, the invention also provides cell lines of ARPE-19 cells genetically engineered to produce a soluble VEGF receptor, wherein the soluble VEGF receptor is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO:1,
15 SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, and SEQ ID NO:13. Similarly, the invention also provides cell lines of ARPE-19 cells genetically engineered to produce a soluble VEGF receptor comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14.

20 Also described herein are implantable cell culture devices containing a containing one or more ARPE-19 cells that are genetically engineered to produce any of the polypeptides of the invention and a semipermeable membrane surrounding the core, wherein the membrane permits the diffusion of the soluble VEGF receptor there through. In some embodiments, the core additionally contains a matrix disposed within the semipermeable membrane. By way of
25 non-limiting example, the matrix may include a hydrogel or extracellular matrix components. For example, the hydrogel may contain alginate cross-linked with a multivalent ion. In other embodiments, the matrix includes a plurality of monofilaments, wherein the monofilaments are twisted into a yarn or woven into a mesh or are twisted into a yarn that is in non-woven strands, and wherein the cells or tissue are distributed thereon. Those skilled in the art will
30 recognize that the filamentous cell-supporting matrix can be made from a biocompatible material selected from the group consisting of acrylic, polyester, polyethylene, polypropylene, polyacetonitrile, polyethylene terephthalate, nylon, polyamides, polyurethanes, polybutester, silk, cotton, chitin, carbon, and/or biocompatible metals.

In some embodiments, the cell encapsulation devices described herein also have a tether anchor. For example, the tether anchor may be an anchor loop that is adapted for anchoring the capsule to an ocular structure.

Any of the devices described herein are suitable for implantation into the eye. By way of non-limiting example, the capsules can be implanted into the vitreous, the aqueous humor, the Subtenon's space, the periocular space, the posterior chamber, and/or the anterior chamber of the eye.

The jackets of the devices described herein preferably are made from a permselective, immunoisulatory membrane. For example, the jackets are made from an ultrafiltration membrane or a microfiltration membrane. Those skilled in the art will recognize that an ultrafiltration membrane typically has a pore size of 1-100 nm, whereas a microfiltration membrane typically has a pore size of 0.1-10 μ m. In other embodiments, the jacket may be made from a non-porous membrane material (*e.g.*, a hydrogel or a polyurethane).

In any of the devices described herein, the capsule can be configured as a hollow fiber or a flat sheet. Moreover, in various embodiments, at least one additional biologically active molecule can be co-delivered from these devices. For example, the at least one additional biologically active molecule can be from a non-cellular or a cellular source (*i.e.*, the at least one additional biologically active molecule is produced by one or more genetically engineered ARPE-19 cell in the core).

Also provided herein are methods for treating ophthalmic disorders by implanting the implantable cell culture devices of the invention into the eye of a patient and allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye, thereby treating the ophthalmic disorder. For example, the ophthalmic disorder to be treated can be selected from the group consisting of retinopathy of prematurity, diabetic macular edema, diabetic retinopathy, age-related macular degeneration, glaucoma, retinitis pigmentosa, cataract formation, retinoblastoma and retinal ischemia. In one preferred embodiment, age-related macular degeneration is wet form age-related macular degeneration. In another preferred embodiment, the ophthalmic disorder is diabetic retinopathy.

The invention further provides methods for inhibiting endothelial cell proliferation by implanting the implantable cell culture devices of the invention into a patient suffering from a cell proliferation disorder and allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF, wherein the binding inhibits endothelial cell proliferation in the patient.

For example, the disorder is selected from the group consisting of hematologic disorders, atherosclerosis, inflammation, increased vascular permeability and malignancy.

Also provided are methods of delivering a soluble VEGF receptor to a recipient host by implanting the implantable cell culture device described herein into a target region of the recipient host, wherein the encapsulated one or more ARPE-19 cells secrete the soluble VEGF receptor at the target region. Preferred target regions include the central nervous system, including the brain, ventricle, spinal cord, or the aqueous and vitreous humors of the eye.

Those skilled in the art will recognize that in any of the methods described herein, between 0.1 pg and 1000 µg per patient per day of the soluble VEGF receptors of the invention can diffuse from the implantable cell culture devices.

In addition, the invention also provides methods for making the implantable cell culture devices of the invention. In one method, at least one ARPE-19 cell is genetically engineered to secrete a soluble VEGF receptor encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, and SEQ ID NO:13 and the genetically modified ARPE-19 cells are encapsulated within a semipermeable membrane, wherein said membrane allows the diffusion of the soluble VEGF receptor therethrough. In another method, at least one ARPE-19 cell is genetically engineering to secrete a soluble VEGF receptor comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14, and the genetically modified ARPE-19 cells are encapsulated within a semipermeable membrane, wherein said membrane allows the diffusion of the soluble VEGF receptor therethrough.

The invention also describes the use of one or more ARPE-19 cells that are genetically engineered to produce any of the polypeptides of the invention (*e.g.*, SEQ ID NOs: 2, 4, 6, 8, 10, 12, or 14) in the manufacture of any of the implantable cell culture devices according to the invention for treating ophthalmic disorders by implantation of the device into the eye of a patient and by allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye. Moreover, any of the implantable cell culture devices described herein can be used for treating ophthalmic disorders by implantation of the device into the eye of a patient and by allowing the soluble VEGF receptors of the invention to diffuse from the device and bind to VEGF in the eye.

Also provided are one or more ARPE-19 cells that are genetically engineered to produce any of the polypeptides of the invention for treating ophthalmic disorders by implantation of any of the implantable cell culture devices of the invention into the eye of a patient and by allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye.

Any of the isolated nucleic acid molecules described herein can also be used in the manufacture of one or more ARPE-19 cells that are genetically engineered to produce one of the polypeptides of the invention for treating ophthalmic disorders by implantation into the eye of a patient an implantable cell culture device of the invention and by allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye. Moreover, any of the isolated nucleic acid molecules of the invention can also be used for treating ophthalmic disorders where treating comprises implanting into the eye of a patient an implantable cell culture device according to the instant invention and allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye, wherein said one or more ARPE-19 cells in said device have been genetically engineered with said isolated nucleic acid molecule to thereby produce any of the isolated polypeptides described herein.

In any of the embodiments described herein, the ophthalmic disorder is selected from the group consisting of retinopathy of prematurity, diabetic macular edema, diabetic retinopathy, age-related macular degeneration (*e.g.* wet form age-related macular degeneration), glaucoma, retinitis pigmentosa, cataract formation, retinoblastoma and retinal ischemia.

The invention also provides for the use of one or more ARPE-19 cells that are genetically engineered to produce a polypeptide of the invention in the manufacture of an implantable cell culture device according to the invention for inhibiting endothelial cell proliferation by implantation of the device into the eye of a patient suffering from a cell proliferation disorder and by allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye and to thereby inhibit endothelial cell proliferation in said patient. Likewise, the invention also provides implantable cell culture devices of the invention for inhibiting endothelial cell proliferation by implantation of the device into the eye of a patient suffering from a cell proliferation disorder and by allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye and to thereby inhibit endothelial cell proliferation in said patient.

In other embodiments, the invention provides one or more ARPE-19 cells that are genetically engineered to produce any of the polypeptides of the invention for inhibiting endothelial cell proliferation by implantation of any of the implantable cell culture devices described herein into the eye of a patient suffering from a cell proliferation disorder and by
5 allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye and to thereby inhibit endothelial cell proliferation in said patient.

The use of any of the isolated nucleic acid molecules described herein in the manufacture of one or more ARPE-19 cells that are genetically engineered to produce the polypeptide(s) of the invention for inhibiting endothelial cell proliferation by implantation
10 into the eye of a patient suffering from a cell proliferation disorder an implantable cell culture device according to the instant invention and by allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye and to thereby inhibit endothelial cell proliferation in said patient is also contemplated.

Moreover, the invention also provides any of the isolated nucleic acid molecules
15 according to the invention for inhibiting endothelial cell proliferation, the treating comprising implanting into the eye of a patient suffering from a cell proliferation disorder an implantable cell culture device according to the invention and allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye and to thereby inhibit endothelial cell proliferation in said patient, and wherein said one or more ARPE-19 cells in said device have
20 been genetically engineered with said isolated nucleic acid molecule to thereby produce a polypeptide of the invention.

Those skilled in the art will recognize that the cell proliferation disorder may be selected from the group consisting of hematologic disorders, atherosclerosis, inflammation, increased vascular permeability and malignancy.

Also provided is the use of one or more ARPE-19 cells that are genetically engineered to produce any of the polypeptides described herein in the manufacture of an implantable cell culture device according of the invention for delivering a soluble VEGF receptor to a recipient host by implantation of the device into a target region of the recipient host and wherein the encapsulated one or more ARPE-19 cells secrete the soluble VEGF receptor at
30 the target region. Similarly, any of the implantable cell culture devices of the invention can be used for delivering a soluble VEGF receptor to a recipient host by implantation of the device into a target region of the recipient host and wherein the encapsulated one or more ARPE-19 cells secrete the soluble VEGF receptor at the target region.

Moreover, one or more ARPE-19 cells that are genetically engineered to produce any of the polypeptides described herein can be used for delivering a soluble VEGF receptor to a recipient host by implantation of any implantable cell culture devices of the invention into a target region of the recipient host and wherein the encapsulated one or more ARPE-19 cells
5 secrete the soluble VEGF receptor at the target region.

Likewise, any of the isolated nucleic acid molecules described herein can be used in the manufacture of one or more ARPE-19 cells that are genetically engineered to produce any of the polypeptides described herein for delivering a soluble VEGF receptor to a recipient host by implantation into a target region of the recipient host an implantable cell culture
10 device of the invention, wherein the encapsulated one or more ARPE-19 cells secrete the soluble VEGF receptor at the target region.

Any of the isolated nucleic acid molecules described herein can also be used for delivering a soluble VEGF receptor to a recipient host, said delivering comprising implanting into a target region of the recipient host an implantable cell culture device of the invention,
15 wherein the encapsulated one or more ARPE-19 cells secrete the soluble VEGF receptor at the target region, and wherein said one or more ARPE-19 cells in said device have been genetically engineered with said isolated nucleic acid molecule to thereby produce any of the polypeptides of the invention.

Those skilled in the art will recognize that the target region is selected from the group
20 consisting of the central nervous system, including the brain, ventricle, spinal cord, and the aqueous and vitreous humors of the eye.

In addition, the invention also provides methods of producing an isolated polypeptide, the method comprising expressing any of the isolated nucleic acid molecules described herein and harvesting the expressed polypeptide.
25

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can
30 be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the

present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B are graphs showing VEGF saturation binding of truncated VR isoforms. Using VR1 D1-3-Fc as a positive control, the relative dissociation constant K_d , is shown in **Figure 1C**. Briefly, increasing amounts of VEGF receptor from condition media was incubated overnight with VEGF, and free VEGF was measured using a commercially available ELISA.

10

Figures 2A -2C are graphs showing the results of the HUVEC Inhibition Bioassay. Increasing amounts of VEGF receptor from condition media were analyzed for their ability to inhibit human endothelial cell proliferation using a HUVEC bioassay.

15

Figure 3 is a schematic showing the pKAN3 Plasmid Map.

DETAILED DESCRIPTION OF THE INVENTION

Vascular endothelial growth factor (VEGF) is a signaling protein involved in both vasculogenesis, the formation of the embryonic circulatory system, and angiogenesis, the growth of blood vessels from pre-existing vasculature. While VEGF is mostly known for its effects on cells of the vascular endothelium, it also effects a broad range of other cells types, *e.g.*, stimulation monocyte/macrophage migration, neurons, cancer cells, kidney epithelial cells, *etc.*

20

There are a number of proteins within the VEGF family, which arise as a result of alternate splicing of mRNA. The various splice variants impact the function of VEGF, as they determine whether the resulting proteins are pro- or anti-angiogenic. Additionally, the splice variants also effect the interaction of VEGF with heparin sulfate proteoglycans (HSPGs) and neuropilin co-receptors on the cell surface, which, in turn, enhances the ability of VEGF to bind to and activate VEGF signaling receptors (VEGFRs).

25

30

The VEGF splice variants are released from cells as glycosylated disulfide-bonded dimers. Structurally, VEGF belongs to the PDGF family of cysteine-knot growth factors, and, thus, several closely-related proteins exist, *i.e.*, placenta growth factor (PlGF), VEGF-B,

VEGF-C and VEGF-D, which together comprise the VEGF sub-family of growth factors. VEGF, itself, is commonly referred to as VEGF-A in order to differentiate it from these other, related growth factors.

The VEGF family of proteins stimulates cellular response by binding to the VEGFRs

5 or to the tyrosine kinase receptors present on a cell surface. VEGF receptors have an extracellular portion consisting of seven immunoglobulin-like domains, a single transmembrane spanning region, and an intracellular portion containing a split tyrosine-kinase domain. VEGF-A binds to both VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1). VEGFR1 is expressed as a full-length receptor tyrosine kinase (RTK) as well as in a soluble
10 form, which carries only the extracellular domain. VEGFR-2 appears to mediate almost all of the known cellular responses to VEGF and is expressed in mesodermal progenitor cells that are destined to differentiate into hemangioblasts and angioblasts. The function of VEGFR-1 is less well-defined, although it is thought to modulate VEGFR-2 signaling. VEGF-C and VEGF-D, but not VEGF-A, are also ligands for a third receptor (VEGFR-3), which mediates
15 lymphangiogenesis.

A gene of interest (*i.e.*, a gene that encodes a given VEGF receptor construct) can be inserted into a cloning site of a suitable expression vector by using standard techniques. The nucleic acid and amino acid sequences of the human (and other mammalian) genes encoding VEGF receptor molecules are known. *See, e.g.*, U.S. Pat. Nos. 4,997,929; 5,141,856;
20 5,364,769; 5,453,361; WO 93/06116; WO 95/30686, incorporated herein by reference.

Various specific truncated VEGF receptor constructs are contemplated by the instant invention. The proteins described herein comprise fragments of secretory VEGF receptor 1 (sVR1), secretory VEGF receptor 2 (sVR2), and/or a chimera of VEGF binding domains of VEGF receptor 1 and secretory VEGF receptor 2. These secretory proteins bind VEGF and
25 contain multiple Ig-like domains. Several immunoglobulin-like domains from both VR1 and VR2 are used in the constructs disclosed herein. Domain 2 (D2) is the VEGF-binding domain ("VBD") of sVR1, but it requires domain 3 (D3) for high affinity binding of VEGF. Truncations of sVR1 containing domains 1 through 3 (D1-3) or domains 2-3 (D-3) bind VEGF with higher affinity than domain 2 alone. Moreover, these truncations also neutralize
30 the angiogenic effects of VEGF. VR2 has similar requirements for high affinity VEGF-binding but additionally must be dimeric, since the monomeric version binds with very low affinity. For example, dimeric SVR2 D2-3 may be used in the methods and compositions

described herein. In addition, the chimera described herein includes sVR1D1-2 or sVR1D2 along with sVR2D3. Versions may be monomeric or dimeric in form.

The specific VEGF receptor constructs of the invention include:

- 1) sVR1 D1-3
- 2) sVR1 D2-3
- 3) sVR1 D1-4
- 4) sVR1 D2-4
- 5) sVR D1-3 R1/R2 chimera dimer
- 6) sVR D2-3 R1/R2 chimera dimer
- 7) sVR2 D2-3 dimer

Construct 5 is composed of VR1 D1-2 fused to VR2 D3. The dimeric form includes a carboxy human IgG hinge region that is fused to VR2 D3. Construct 6 is composed of VR1 D2 fused to VR2 D3. The dimeric form includes a carboxy human IgG hinge region fused to VR2 D3.

The specific nucleotide and amino acid sequences of each of these VEGF antagonist constructs are shown below.

As used herein, capitalized nucleotide sequence represents CDS, while lower case letters indicate intron (IgSP). Underlined 5' terminus indicates leader sequence. Underlined 3' terminus indicates IgG hinge region. Underlined N-terminus indicates signal sequence.

Underlined C-terminus indicates IgG hinge region. Moreover, the predicted mature protein is shaded.

sVR1D1-3

ATGGTCAGCTACTGGGACACCGGGTCCTGCTGTGCGCGCTGCTCAGCTGTCTGCTTCTCACAGGA
TCTAGTTCAGGTTCAAAATTAAAAGATCCTGAACTGAGTTTAAAAGGCACCCAGCACATCATGCAA
 GCAGGCCAGACACTGCATCTCCAATGCAGGGGGGAAGCAGCCCATAAATGGTCTTTGCCTGAAAT
 GGTGAGTAAGGAAAGCGAAAGGCTGAGCATAACTAAATCTGCCTGTGGAAGAAATGGCAAACAA
 TTCTGCAGTACTTTAACCTTGAACACAGCTCAAGCAAACCACACTGGCTTCTACAGCTGCAAATAT
 CTAGCTGTACCTACTTCAAAGAAGAAGGAAACAGAATCTGCAATCTATATATTTATTAGTGATACA
 GGTAGACCTTTCGTAGAGATGTACAGTGAAATCCCCGAAATTATACACATGACTGAAGGAAGGGA
 GCTCGTCATTCCCTGCCGGGTACGTCACCTAACATCACTGTTACTTTAAAAAAGTTTCCACTTGAC
 ACTTTGATCCCTGATGGAACACGCATAATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGC
 AACGTACAAAGAAATAGGGCTTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAA
 ACTATCTCACACATCGACAAACCAATACAATCATAGATGTCCAAATAAGCACACCACGCCAGTC
 AAATTACTTAGAGGCCATACTCTTGTCCTCAATTGTACTGCTACCACTCCCTTGAACACGAGAGTTC
 AAATGACCTGGAGTTACCCTGATGAAAAAATAAGAGAGCTTCCGTAAGGCGACGAATTGACCAA
 AGCAATTCCCATGCCAACATATTCTACAGTGTTCTTACTATTGACAAAATGCAGAACAAAGACAAA
 GGACTTTATACTTGTCGTGTAAGGAGTGACCATCATTCAAATCTGTTAACACCTCAGTGCATATA
 TGA (SEQ ID NO:1)

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPESLKGTHIMQAGOTLHLQCRGEAAHKWSLPEMV
 SKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGFYSCKYLAVPTSCKKETESAIYIFISDTGRPFVE
 MYSEIPEIIHMTGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSDRGKFHISNATYKEIGLLTCEAT
 VNGHLYKTNLYLTHROTNTHIDVQISTPRPVKLLRGHTLVNLCTATTPLNTRVQMTWSYPDEKNKRASV
 RRRIDQSNHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFKSVNTSVHI* (SEQ ID NO:2)

IgSP-VR1D2-3

ATGAAATGCAGCTGGGTTATCTTCTCCTGATGGCAGTGGTTACAGGtaaggggctcccaagtcccaacttgagggt
 ccataaactctgtgacagtggcaatcactttgctttctttctacaggGGTGAATTCGAGTGATACAGGTAGACCTTTCGTAGAG
 ATGTACAGTGAAATCCCCGAAATTATACACATGACTGAAGGAAGGGAGCTCGTCATTCCCTGCCG
 GGTTACGTCACCTAACATCACTGTTACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGA
 AAACGCATAATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAGG
 GCTTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAACTATCTCACACATCGACA
 AACCAATACAATAGATGTCAAATAAGCACACACCAGCCAGTCAAATTACTTAGAGGCCATA
 CTCTTGCTCTCAATTGTACTGCTACCACTCCCTTGAACACGAGAGTTCAAATGACCTGGAGTTACCC
 TGATGAAAAAATAAGAGAGCTTCCGTAAGGCGACGAATTGACCAAAGCAATTCCCATGCCAACA
 TATTCTACAGTGTCTTACTATTGACAAAATGCAGAACAAAGACAAAGGACTTTATACTTGTCTGTG
 TAAGGAGTGGACCATCATTCAAATCTGTAAACACCTCAGTGCATATATGA (SEQ ID NO:3)

MKCSWVIFFLMAVVTGVNSSDTGRPFVEMYSEIPEIIHMTGRELVIPCRVTSPNITVTLKKFPLDTLIPD
 GKRIIWDSDRGKFHISNATYKEIGLLTCEATVNGHLYKTNLYLTHROTNTHIDVQISTPRPVKLLRGHTLVNL
 CTATTPLNTRVQMTWSYPDEKNKRASVRRRIDQSNHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSF
 KSVNTSVHI* (SEQ ID NO:4)

sVR1D1-4

ATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTGTCTGCTTCTCACAGGA
 TCTAGTTCAGGTTCAAAATTAAGATCCTGAACTGAGTTTAAAAGGCACCCAGCACATCATGCAA
 GCAGGCCAGACACTGCATCTCCAATGCAGGGGGGAAGCAGCCCATAAATGGTCTTTGCCTGAAAT
 GGTGAGTAAGGAAAGCGAAAGGCTGAGCATAACTAAATCTGCCTGTGGAAGAAATGGCAAACAA
 TTCTGCAGTACTTTAACCTTGAACACAGCTCAAGCAAACCACACTGGCTTCTACAGCTGCAAATAT
 CTAGCTGTACCTACTTCAAAGAAGAAGGAAACAGAATCTGCAATCTATATATTTATTAGTGATACA
 GGTAGACCTTTCGTAGAGATGTACAGTGAAATCCCCGAAATTATACACATGACTGAAGGAAGGGA
 GCTCGTCATTCCCTGCCGGGTACGTCACCTAACATCACTGTTACTTTAAAAAAGTTTCCACTTGAC
 ACTTTGATCCCTGATGGAAAACGCATAATCTGGGACAGTAGAAAGGGCTTCATCATATACTCAAAT
 AACGTACAAAGAAATAGGGCTTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAA
 ACTATCTCACACATCGACAAACCAATACAATCATAGATGTCAAATAAGCACACCACGCCAGTC
 AAATTACTTAGAGGCCATACTCTTGCTCTCAATTGTACTGCTACCACTCCCTTGAACACGAGAGTTC
 AAATGACCTGGAGTTACCCTGATGAAAAAATAAGAGAGCTTCCGTAAGGCGACGAATTGACCAA
 AGCAATTCCCATGCCAACATATTCTACAGTGTCTTACTATTGACAAAATGCAGAACAAAGACAAA
 GGACTTTATACTTGTCTGTGTAAGGAGTGGACCATCATTCAAATCTGTAAACACCTCAGTGCATATA
 TATGATAAAGCATTATCACTGTGAAACATCGAAAACAGCAGGTGCTTGAAACCGTAGCTGGCAA
 GCGGTCTTACCGGCTCTCTATGAAAGTGAAGGCATTTCCCTCGCCGGAAGTTGTATGGTTAAAAGA
 TGGGTTACCTGCGACTGAGAAATCTGCTCGCTATTTGACTCGTGGCTACTCGTTAATTATCAAGGA
 CGTAACTGAAGAGGATGCAGGGAATTATACAATCTTGCTGAGCATAAAACAGTCAAATGTGTTTA
 AAAACCTCACTGCCACTTAG (SEQ ID NO:5)

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPESLKGTHIMQAGOTLHLQCRGEAAHKWSLPEMV
 SKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGFYSCKYLAVPTSCKKETESAIYIFISDTGRPFVE
 MYSEIPEIIHMTGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSDRGKFHISNATYKEIGLLTCEAT
 VNGHLYKTNLYLTHROTNTHIDVQISTPRPVKLLRGHTLVNLCTATTPLNTRVQMTWSYPDEKNKRASV
 RRRIDQSNHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFKSVNTSVHIYDKAFITVKHRKQOVLET
 VAGKRSYRLSMKVKAFFSPSEVWVKDGLPATEKSARYLTRGYSIIKDVTEEDAGNYTILLSIKQSNVFK
 NLAT* (SEQ ID NO:6)

sVR1D2-4

ATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTGTCTGCTTCTCACAGGA
 TCTAGTTCAGGTTTCGCGAAGTGATACAGGTAGACCTTTCGTAGAGATGTACAGTGAAATCCCCGAA
 5 ATTATACACATGACTGAAGGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTCACCTAACATCACT
 GTTACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGCATAATCTGGGACAGT
 AGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAGGGCTTCTGACCTGTGAAGCAAC
 AGTCAATGGGCATTTGTATAAGACAAACTATCTCACACATCGACAAACCAATACAATCATAGATGT
 10 CCAAATAAGCACACCACGCCAGTCAAATTACTTAGAGGCCATACTCTTGCTCAATTGTACTGC
 TACCACTCCCTTGAACACGAGAGTTCAAATGACCTGGAGTTACCCTGATGAAAAAATAAGAGAG
 CTTCCGTAAGGCGACGAATTGACCAAAGCAATTCCCATGCCAACATATTCTACAGTGTTCTTACTA
 TTGACAAAATGCAGAACAAAGACAAAGGACTTTATACTTGTCTGTAAGGAGTGGACCATCATTC
 AAATCTGTTAACACCTCAGTGCATATATATGATAAAGCATTTCATCACTGTGAAACATCGAAAACAG
 CAGGTGCTTGAACCGTAGCTGGCAAGCGGTCTTACCGGCTCTCTATGAAAGTGAAGGCATTTCCTC
 15 TCGCCGGAAGTTGTATGGTTAAAAGATGGGTTACCTGCGACTGAGAAATCTGCTCGCTATTGACT
 CGTGGCTACTCGTTAATTATCAAGGACGTAAGAGGATGCAGGGAATTATACAATCTTGCTG
 AGCATAAAACAGTCAAATGTGTTAAAAACCTCACTGCCACTTAG (SEQ ID NO:7)

MVSYWDTGVLLCALLSCLLLTGSSSGSRSDTGRPFVEMYSEIPEIIHMTGRELVIPCRVTSPNITVTLKK
 20 FPLDTLIPDGKRIHWSRKGFIHSNATYKEIGLLTCEATVNGHLYKTNLYLTHROTNTHIDVQISTPRPVKLL
 RGHTLVLNCTATTPLNTRVQMTWSYPDEKNKRASVRRRIDQSNHANIFYSVLTIDKMONKDKGLYT
 CRVRSGPSFKSVNTSVHIYDKAFITVKHRKQOVLETVAGKRSYRLSMKVKAFFSPEVVWLKDGLPATE
 KSARYLTRGYSLHKDVTEEDAGNYTILLSIKQSNVEKNLTAT* (SEQ ID NO:8)

sVR D1-3 R1/R2 Chimera Dimer

ATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTGTCTGCTTCTCACAGGA
 TCTAGTTCAGGTTCAAAATTAAGATCCTGAACTGAGTTTAAAAGGCACCCAGCACATCATGCAA
 30 GCAGGCCAGACACTGCATCTCCAATGCAGGGGGGAAGCAGCCCATAAATGGTCTTTGCCTGAAAT
 GGTGAGTAAGGAAAGCGAAAGGCTGAGCATAACTAAATCTGCCTGTGGAAGAAATGGCAAACAA
 TTCTGCAGTACTTTAACCTTGAACACAGCTCAAGCAAACCACACTGGCTTCTACAGCTGCAAATAT
 CTAGCTGTACCTACTTCAAAGAAGAAGGAAACAGAATCTGCAATCTATATATTTATTAGTGATACA
 GGTAGACCTTTCGTAGAGATGTACAGTGAAATCCCCGAAATTATACACATGACTGAAGGAAGGGA
 35 GCTCGTCATTCCCTGCCGGGTACGTCACCTAACATCACTGTTACTTTAAAAAAGTTTCCACTTGAC
 ACTTTGATCCCTGATGGAAAACGCATAATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGC
 AACGTACAAAGAAATAGGGCTTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAA
 ACTATCTCACACATCGACAAACCAATACAATCATCGATGTGGTTCTGAGTCCGTCTCATGGAATTG
 AACTATCTGTTGGAGAAAAGCTTGTCTTAAATTGTACAGCAAGAACTGAACTAAATGTGGGGATTG
 40 ACTTCAACTGGGAATACCCTTCTTGAAGCATCAGCATAAGAACTTGTAACCCGAGACCTAAAA
 ACCCAGTCTGGGAGTGAGATGAAGAAATTTTGAAGCACCTTAAGTATAGATGGTGTAAACCCGGAG
 TGACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCACATTG
 TCAGGGTCCATGAAAAAGAAATTCGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCGTGC
 45 CCAGCACCTGAACTCCTGTAG (SEQ ID NO:9)

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPELSLKGTOHIMQAGOTLHLQCRGEAAHKWSLPEMV
 SKESERLSITKSACGRNGKQFCSTLTNTAQANHTGFYSCKYLAVPTSKKKETESAIYIFISDTGRPFVE
 MYSEIPEIIHMTGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIHWSRKGFIHSNATYKEIGLLTCEAT
 50 VNGHLYKTNLYLTHROTNTHIDVLSPSHGIELSYGEKLVNCTARTELNVGIDFNWYFSSKHQHKLVNRD
 LKTQSGSEMKKFLSTLTIDGVTTRSDQGLYTCAASSGLMTKKNSTFVRVHEKEFEFPKSCDKTHTCPPCPAPEL
 L* (SEQ ID NO:10)

sVR D2-3 R1/R2 Chimera Dimer

5 ATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTGTCTGCTTCTCACAGGA
TCTAGTTCAGGTTTCGCGAAGTGATACAGGTAGACCTTTCGTAGAGATGTACAGTGAAATCCCCGAA
ATTATACACATGACTGAAGGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTACCTAACATCACT
GTTACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAAACGCATAATCTGGGACAGT
AGAAAGGGCTTCATCATATCAAAATGCAACGTACAAAGAAATAGGGCTTCTGACCTGTGAAGCAAC
10 AGTCAATGGGCATTTGTATAAGACAACTATCTCACACATCGACAAACCAATACAATCATCGATGT
GGTCTGAGTCCGTCTCATGGAATTGAACTATCTGTTGGAGAAAAGCTTGTCTTAAATTGTACAGC
AAGAACTGAACTAAATGTGGGGATTGACTTCAACTGGGAATACCCTTCTTCGAAGCATCAGCATA
AGAACTTGTAACCGAGACCTAAAAACCCAGTCTGGGAGTGAGATGAAGAAATTTTGTAGCACC
TTAACTATAGATGGTGTAAACCCGGAGTGACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTG
ATGACCAAGAAGAACAGCACATTTGTGAGGGTCCATGAAAAA**GAATTC**GAGCCCAAATCTTGTGA
15 CAAACTCACACATGCCACCGTGCCAGCACCTGAACTCCTGTAG (SEQ ID NO:11)

MVSYWDTGVLLCALLSCLLLTGSSSGSRSDTGRPFVEMYSEIPEIHHMTEGRELVIPCRVTSFNITVTLKK
FPLDTLIPDGKRIIWDNRKGFHISNATYKEIGLLTCEATVNGHLYKTNYLTHRONTIHDVVLSPSHGIELS
VGEKLVNLNCTARTELVNGIDFNWEYPSSKHQHKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLY
20 TCAASSGLMTKKNSTFVRVHEK**EFEPKSCDKTHTCPPCPAPELL*** (SEQ ID NO :12)

sVR2 D2-3 dimer

25 ATGGAGAGCAAGGTGCTGCTGGCCGTCGCCCTGTGGCTCTGCGTGGAGACCCGGGCCGCCTCTGTG
GGTTATGTTCAAGATTACAGATCTCCATTTATTGCTTCTGTTAGTGACCAACATGGAGTCGTGTACA
TTACTGAGAACAAAAACAAACTGTGGTGATTCCATGTCTCGGGTCCATTTCAAATCTCAACGTGT
CACTTTGTGCAAGATACCCAGAAAAGAGATTTGTTCTGATGGTAACAGAATTTCTGGGACAGCA
AGAAGGGCTTTACTATTCCCAGCTACATGATCAGCTATGCTGGCATGGTCTTCTGTGAAGCAAAAA
30 TTAATGATGAAAGTTACCAGTCTATTATGTACATAGTTGTCGTTGTAGGGTATAGGATTTATGATGT
GGTCTGAGTCCGTCTCATGGAATTGAACTATCTGTTGGAGAAAAGCTTGTCTTAAATTGTACAGC
AAGAACTGAACTAAATGTGGGGATTGACTTCAACTGGGAATACCCTTCTTCGAAGCATCAGCATA
AGAACTTGTAACCGAGACCTAAAAACCCAGTCTGGGAGTGAGATGAAGAAATTTTGTAGCACC
TTAACTATAGATGGTGTAAACCCGGAGTGACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTG
ATGACCAAGAAGAACAGCACATTTGTGAGGGTCCATGAAAAACCTTTTGTGCTTTTGGAAAGTGGC
35 **GAATTC**GAGCCCAAATCTTGTGACAAACTCACACATGCCACCGTGCCAGCACCTGAACTCCT
GTAG (SEQ ID NO: 13)

MESKVLLAVALWLCVETRAASVGYVQDYRSPFIASVSDQHGVVYITENKNKTVVIPCLGSISNLNVSLC
ARYPEKRFVDPDGNRISWDSKKGFITPSYMSYAGMVFC EAKINDESYSIMYIVVVGYRIYDVVLS
40 HGIELSVGEKLVNLNCTARTELVNGIDFNWEYPSSKHQHKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLY
TCAASSGLMTKKNSTFVRVHEKPFVAFSG**EFEPKSCDKTHTCPPCPAPELL*** (SEQ ID NO:14)

In the VR chimera dimers and the sVR2 D2-3 dimer shown above, the 3' underlining depicts that human hinge sequence. In these sequences, the bolded nucleotides (GAATTC) and amino acids (EF) were introduced as part of the subcloning. These are a subcloning artifact (GAATTC is an EcoR1 restriction site that codes for the amino acids Glu and Phe) and are not part of the receptor or hinge sequences per se.

A wide variety of host/expression vector combinations may be used to express the gene encoding the growth factor, or other biologically active molecule(s) of interest. Long-term, stable *in vivo* expression is achieved using expression vectors (*i.e.*, recombinant DNA molecules) in which the gene encoding the VEGF receptor is operatively linked to a promoter that is not subject to down regulation upon implantation *in-vivo* in a mammalian host.

Suitable promoters include, for example, strong constitutive mammalian promoters, such as beta-actin, eIF4A1, GAPDH, etc. Stress-inducible promoters, such as the metallothionein 1 (MT-1) or VEGF promoter may also be suitable. Additionally, hybrid promoters containing a core promoter and custom 5' UTR or enhancer elements may be used. Other known non-retroviral promoters capable of controlling gene expression, such as CMV or the early and late promoters of SV40 or adenovirus are suitable.

The expression vector containing the gene of interest may then be used to transfect the desired cell line. Standard transfection techniques such as liposomal, calcium phosphate coprecipitation, DEAE-dextran transfection or electroporation may be utilized. Commercially available mammalian transfection kits, such as Fugene6 (Roche Applied Sciences), may be purchased. Additionally, viral vectors may be used to transduce the desired cell line. An example of a suitable viral vector is the commercially available pLenti family of viral vectors (Invitrogen). Human mammalian cells can be used. In all cases, it is important that the cells or tissue contained in the device are not contaminated or adulterated.

Preferred promoters used in the disclosed constructs include the SV40 promoter, the Amp promoter and the MT1 promoter, as shown in Figure 3.

Other useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences, such as various known derivatives of SV40 and known bacterial plasmids, e.g., pUC, pBlueScriptTM plasmids from *E. coli* including pBR322, pCR1, pMB9 and their derivatives. Expression vectors containing the geneticin (G418) or hygromycin drug selection genes (Southern, P. J., *In Vitro*, 18, p. 315 (1981), Southern, P. J. and Berg, P., *J. Mol. Appl. Genet.*, 1, p. 327 (1982)) are also useful. These vectors can employ a variety of different enhancer/promoter regions to drive the expression of both a biologic gene of interest and/or a gene conferring resistance to selection with toxin such as G418 or hygromycin B. A variety of different mammalian promoters can be employed to direct the expression of the genes for G418 and hygromycin B and/or the biologic gene of interest. The G418 resistance gene codes for aminoglycoside phosphotransferase (APH) which enzymatically inactivates G418 (100-1000 µg/µl) added to the culture medium. Only those cells expressing the APH gene will survive drug selection usually resulting in the expression of the second biologic gene as well. The hygromycin B phosphotransferase (HPH) gene codes for an enzyme which specifically modifies hygromycin toxin and inactivates it. Genes co-transfected with or contained on the same plasmid as the hygromycin B phosphotransferase gene will be preferentially expressed in the

presence of hygromycin B at 50-200 µg/ml concentrations.

Examples of expression vectors that can be employed include, but are not limited to, the commercially available pRC/CMV, pRC/RSV, and pCDNA1NEO (Invitrogen). Other suitable commercially available vectors include pBlast, pMono, or pVito.

5 In one embodiment, the pNUT expression vector, which contains the cDNA of the mutant DHFR and the entire pUC18 sequence including the polylinker, can be used. *See, e.g.,* Aebischer, P., et al., Transplantation, 58, pp. 1275-1277 (1994); Baetge et al., PNAS, 83, pp. 5454-58 (1986). The pNUT expression vector can be modified such that the DHFR coding sequence is replaced by the coding sequence for G418 or hygromycin drug resistance.

10 The SV40 promoter within the pNUT expression vector can also be replaced with any suitable constitutively expressed mammalian promoter, such as those discussed above.

Those skilled in the art will recognize that any other suitable, commercially available expression vectors (*e.g.*, pCDNA family (Invitrogen), pBlast, pMono, pVito, or pCpG-vito (Invivogen)) can also be used. Principal elements regulating expression are typically found

15 in the expression cassette. These elements include the promoter, 5' untranslated region (5' UTR) and 3' untranslated region (3' UTR). Other elements of a suitable expression vector may be critical to plasmid integration or expression but may not be readily apparent. The skilled artisan will be able to design and construct suitable expression vectors for use in the claimed invention. The choice, design, and/or construction of a suitable vector is well within

20 the routine level of skill in the art.

The genes encoding the VEGF1 and VEGF2 receptors have been cloned and their nucleotide sequences published. (GenBank Accession U01134 and AF063658). Other genes encoding the biologically active molecules useful in this invention that are not publicly available may be obtained using standard recombinant DNA methods such as PCR

25 amplification, genomic and cDNA library screening with oligonucleotide probes. Any of the known genes coding for biologically active molecules may be employed in the methods of this invention.

The cell of choice is the ARPE-19 cell line, a spontaneously arising continuous human retinal pigmented epithelial cell line. However, those skilled in the art will recognize

30 that other suitable cells, including by not limited to CHO cells, BHK cells, RPE (primary cells or immortalized cells), can also be used. The choice of cell depends upon the intended application. The encapsulated cells may be chosen for secretion of a particular VEGF receptor construct. Cells can also be employed which synthesize and secrete agonists,

analog, derivatives or fragments of the construct, which are active. Those skilled in the art will recognize that other suitable cell types may also be genetically engineered to secrete any of the VEGF receptor constructs described herein.

To be a platform cell line for an encapsulated cell based delivery system, the cell line should have as many of the following characteristics as possible: (1) the cells should be hardy under stringent conditions (the encapsulated cells should be functional in the avascular tissue cavities such as in the central nervous system or the eye, especially in the intra-ocular environment); (2) the cells should be able to be genetically modified (the desired therapeutic factors needed to be engineered into the cells); (3) the cells should have a relatively long life span (the cells should produce sufficient progenies to be banked, characterized, engineered, safety tested and clinical lot manufactured); (4) the cells should preferably be of human origin (which increases compatibility between the encapsulated cells and the host); (5) the cells should exhibit greater than 80% viability for a period of more than one month *in vivo* in device (which ensures long-term delivery); (6) the encapsulated cells should deliver an efficacious quantity of a useful biological product (which ensures effectiveness of the treatment); (7) the cells should have a low level of host immune reaction (which ensures the longevity of the graft); and (8) the cells should be nontumorigenic (to provide added safety to the host, in case of device leakage).

The ARPE-19 cell line (*see* Dunn et al., 62 Exp. Eye Res. 155-69 (1996), Dunn et al., 39 Invest. Ophthalmol. Vis. Sci. 2744-9 (1998), Finnemann et al., 94 Proc. Natl. Acad. Sci. USA 12932-7 (1997), Handa et al., 66 Exp. Eye. 411-9 (1998), Holtkamp et al., 112 Clin. Exp. Immunol. 34-43 (1998), Maidji et al., 70 J. Virol. 8402-10 (1996); United States Patent No. 6,361,771) demonstrates all of the characteristics of a successful platform cell for an encapsulated cell-based delivery system. The ARPE-19 cell line is available from the American Type Culture Collection (ATCC Number CRL-2302). ARPE-19 cells are normal retinal pigmented epithelial (RPE) cells and express the retinal pigmentary epithelial cell-specific markers CRALBP and RPE-65. ARPE-19 cells form stable monolayers, which exhibit morphological and functional polarity.

When the devices of the invention are used, preferably between 10^2 and 10^8 engineered ARPE-19 cells, most preferably 5×10^2 to 3×10^5 ARPE-19 cells that have been genetically engineered to secrete one or more VEGF receptor constructs described herein are encapsulated in each device. Dosage may be controlled by implanting a fewer or greater number of capsules, preferably between 1 and 50 capsules per patient. The devices described

herein are capable of delivering between about 0.1 pg and 1000 µg of the soluble VEGF receptor construct(s) per eye per patient per day.

Techniques and procedures for isolating cells or tissues which produce a selected product are known to those skilled in the art, or can be adapted from known procedures with no more than routine experimentation.

If the cells to be isolated are replicating cells or cell lines adapted to growth *in vitro*, it is particularly advantageous to generate a cell bank of these cells. A particular advantage of a cell bank is that it is a source of cells prepared from the same culture or batch of cells. That is, all cells originated from the same source of cells and have been exposed to the same conditions and stresses. Therefore, the vials can be treated as homogenous culture. In the transplantation context, this greatly facilitates the production of identical or replacement devices. It also allows simplified testing protocols, which insure that implanted cells are free of retroviruses and the like. It may also allow for parallel monitoring of vehicles *in vivo* and *in vitro*, thus allowing investigation of effects or factors unique to residence *in vivo*.

As used herein, the terms "individual" or "recipient" or "host" are used interchangeably to refer to a human or an animal subject.

A "biologically active molecule" ("BAM") is a substance that is capable of exerting a biologically useful effect upon the body of an individual in whom a device of the present invention is implanted. For example, the VEGF receptor constructs described herein are examples of BAMs.

The terms "capsule" and "device" and "vehicle" are used interchangeably herein to refer to the ECT devices of the invention.

Unless otherwise specified, the term "cells" means cells in any form, including but not limited to cells retained in tissue, cell clusters, and individually isolated cells.

As used herein a "biocompatible capsule" or "biocompatible device" or "biocompatible vehicle" means that the capsule or device or vehicle, upon implantation in an individual, does not elicit a detrimental host response sufficient to result in the rejection of the capsule or to render it inoperable, for example through degradation.

As used herein an "immunoisulatory capsule" or "immunoisulatory device" or "immunoisulatory vehicle" means that the capsule upon implantation into an individual, minimizes the deleterious effects of the host's immune system on the cells within its core.

As used herein "long-term, stable expression of a biologically active molecule" means the continued production of a biologically active molecule at a level sufficient to maintain its

useful biological activity for periods greater than one month, preferably greater than three months and most preferably greater than six months. Implants of the devices and the contents thereof are able to retain functionality for greater than three months *in vivo* and in many cases for longer than a year.

5 The "semi-permeable" nature of the jacket membrane surrounding the core permits molecules produced by the cells (*e.g.*, metabolites, nutrients and/or therapeutic substances) to diffuse from the device into the surrounding host eye tissue, but is sufficiently impermeable to protect the cells in the core from detrimental immunological attack by the host.

 The exclusion of IgG from the core of the vehicle is not the touchstone of
10 immunoisolation, because in most cases IgG alone is insufficient to produce cytolysis of the target cells or tissues. Thus, for immunoisulatory capsules, jacket nominal molecular weight cutoff (MWCO) values up to 1000 kD are contemplated. Preferably, the MWCO is between 50-700 kD. Most preferably, the MWCO is between 70-300 kD. *See, e.g.*, WO 92/19195.

 The instant invention also relates to biocompatible, optionally immunoisulatory,
15 devices for the delivery of one or more of the soluble VEGF receptors described herein to the eye. Such devices contain a core containing living cells that produce or secrete the VEGF receptor and a biocompatible jacket surrounding the core, wherein the jacket has a molecular weight cut off ("MWCO") that allows the diffusion of the VEGF receptor into the eye and to the central nervous system, including the brain, ventricle, spinal cord.

20 A variety of biocompatible capsules are suitable for delivery of molecules according to this invention. Useful biocompatible polymer capsules comprise (a) a core which contains a cell or cells, either suspended in a liquid medium or immobilized within a biocompatible matrix, and (b) a surrounding jacket comprising a membrane which does not contain isolated cells, which is biocompatible, and permits diffusion of the cell-produced biologically active
25 molecule into the eye.

 Many transformed cells or cell lines are advantageously isolated within a capsule having a liquid core, comprising, *e.g.*, a nutrient medium, and optionally containing a source of additional factors to sustain cell viability and function. The core of the devices of the invention can function as a reservoir for growth factors (*e.g.*, prolactin, or insulin-like growth
30 factor 2), growth regulatory substances such as transforming growth factor β (TGF- β) or the retinoblastoma gene protein or nutrient-transport enhancers (*e.g.*, perfluorocarbons, which can enhance the concentration of dissolved oxygen in the core). Certain of these substances are also appropriate for inclusion in liquid media.

In addition, the instant devices can also be used as a reservoir for the controlled delivery of needed drugs or biotherapeutics. In such cases, the core contains a high concentration of the selected drug or biotherapeutic (alone or in combination with cells or tissues). In addition, satellite vehicles containing substances which prepare or create a hospitable environment in the area of the body in which a device according to the invention is implanted can also be implanted into a recipient. In such instances, the devices containing immunoisolated cells are implanted in the region along with satellite vehicles releasing controlled amounts of, for example, a substance which down-modulates or inhibits an inflammatory response from the recipient (*e.g.*, anti-inflammatory steroids), or a substance which stimulates the ingrowth of capillary beds (*e.g.*, an angiogenic factor).

Alternatively, the core may comprise a biocompatible matrix of a hydrogel or other biocompatible material (*e.g.*, extracellular matrix components) which stabilizes the position of the cells. The term "hydrogel" herein refers to a three dimensional network of cross-linked hydrophilic polymers. The network is in the form of a gel, substantially composed of water, preferably gels being greater than 90% water. Compositions which form hydrogels fall into three classes. The first class carries a net negative charge (*e.g.*, alginate). The second class carries a net positive charge (*e.g.*, collagen and laminin). Examples of commercially available extracellular matrix components include MatrigelTM and VitrogenTM. The third class is net neutral in charge (*e.g.*, highly crosslinked polyethylene oxide, or polyvinylalcohol).

Any suitable matrix or spacer may be employed within the core, including precipitated chitosan, synthetic polymers and polymer blends, microcarriers and the like, depending upon the growth characteristics of the cells to be encapsulated.

Alternatively, the capsule may have an internal scaffold. The scaffold may prevent cells from aggregating and improve cellular distribution within the device. (*See* PCT publication no. WO 96/02646). The scaffold defines the microenvironment for the encapsulated cells and keeps the cells well distributed within the core. The optimal internal scaffold for a particular device is highly dependent on the cell type to be used. In the absence of such a scaffold, adherent cells aggregate to form clusters.

For example, the internal scaffold may be a yarn or a mesh. The filaments used to form a yarn or mesh internal scaffold are formed of any suitable biocompatible, substantially non-degradable material. (*See* United States Patent Nos. 6,303,136 and 6,627,422, which are herein incorporated by reference). Preferably, the capsule of this invention will be similar to

those described by PCT International patent applications WO 92/19195 or WO 95/05452, incorporated by reference; or U.S. Pat. Nos. 5,639,275; 5,653,975; 4,892,538; 5,156,844; 5,283,187; or 5,550,050, incorporated by reference. Materials useful in forming yarns or woven meshes include any biocompatible polymers that are able to be formed into fibers such as, for example, acrylic, polyester, polyethylene, polypropylene, polyacrylonitrile, polyethylene terephthalate, nylon, polyamides, polyurethanes, polybutester, or natural fibers such as cotton, silk, chitin or carbon. Any suitable thermoplastic polymer, thermoplastic elastomer, or other synthetic or natural material having fiber-forming properties may be inserted into a pre-fabricated hollow fiber membrane or a hollow cylinder formed from a flat membrane sheet. For example, silk, PET or nylon filaments used for suture materials or in the manufacture of vascular grafts are highly conducive to this type of application. In other embodiments, metal ribbon or wire may be used and woven. Each of these filament materials has well-controlled surface and geometric properties, may be mass produced, and has a long history of implant use. In certain embodiments, the filaments may be "texturized" to provide rough surfaces and "hand-holds" onto which cell projections may attach. The filaments may be coated with extracellular matrix molecules or surface-treated (*e.g.* plasma irradiation) to enhance cellular adhesion to the filaments.

In some embodiments, the filaments, preferably organized in a non-random unidirectional orientation, are twisted in bundles to form yarns of varying thickness and void volume. Void volume is defined as the spaces existing between filaments. The void volume in the yarn should vary between 20-95%, but is preferably between 50-95%. The preferred void space between the filaments is between 20-200 μm , sufficient to allow the scaffold to be seeded with cells along the length of the yarn, and to allow the cells to attach to the filaments. The preferred diameter of the filaments comprising the yarn is between 5-100 μm . These filaments should have sufficient mechanical strength to allow twisting into a bundle to comprise a yarn. The filament cross-sectional shape can vary, with circular, rectangular, elliptical, triangular, and star-shaped cross-section being preferred.

Alternatively, the filaments or yarns can be woven into a mesh. The mesh can be produced on a braider using carriers, similar to bobbins, containing monofilaments or multifilaments, which serve to feed either the yarn or filaments into the mesh during weaving. The number of carriers is adjustable and may be wound with the same filaments or a combination of filaments with different compositions and structures. The angle of the braid, defined by the pick count, is controlled by the rotational speed of the carriers and the

production speed. In one embodiment, a mandrel is used to produce a hollow tube of mesh. In certain embodiments, the braid is constructed as a single layer, in other embodiments it is a multi-layered structure. The tensile strength of the braid is the linear summation of the tensile strengths of the individual filaments.

5 In other embodiments, a tubular braid is constructed. The braid can be inserted into a hollow fiber membrane upon which the cells are seeded. Alternatively, the cells can be allowed to infiltrate the wall of the mesh tube to maximize the surface area available for cell attachment. When such cell infiltration occurs, the braid serves both as a cell scaffold matrix and as an inner support for the device. The increase in tensile strength for the braid-
10 supported device is significantly higher than in alternative approaches.

As noted, for implant sites that are not immunologically privileged, such as periorcular sites, and other areas outside the anterior chamber (aqueous) and the posterior chamber (vitreous), the capsules are preferably immunoisulatory. Components of the biocompatible material may include a surrounding semipermeable membrane and the internal cell-
15 supporting scaffolding. The transformed cells are preferably seeded onto the scaffolding, which is encapsulated by the permselective membrane, which is described above. Also, bonded fiber structures can be used for cell implantation. (See U.S. Pat. No. 5,512,600, incorporated by reference). Biodegradable polymers include those comprised of poly(lactic acid) PLA, poly(lactic-coglycolic acid) PLGA, and poly(glycolic acid) PGA and their
20 equivalents. Foam scaffolds have been used to provide surfaces onto which transplanted cells may adhere (PCT International patent application Ser. No. 98/05304, incorporated by reference). Woven mesh tubes have been used as vascular grafts (PCT International patent application WO 99/52573, incorporated by reference). Additionally, the core can be composed of an immobilizing matrix formed from a hydrogel, which stabilizes the position of
25 the cells. A hydrogel is a 3-dimensional network of cross-linked hydrophilic polymers in the form of a gel, substantially composed of water.

Various polymers and polymer blends can be used to manufacture the surrounding semipermeable membrane, including polyacrylates (including acrylic copolymers), polyvinylidenes, polyvinyl chloride copolymers, polyurethanes, polystyrenes, polyamides,
30 cellulose acetates, cellulose nitrates, polysulfones (including polyether sulfones), polyphosphazenes, polyacrylonitriles, poly(acrylonitrile/covinyl chloride), as well as derivatives, copolymers and mixtures thereof. Preferably, the surrounding semipermeable membrane is a biocompatible semipermeable hollow fiber membrane. Such membranes, and

methods of making them are disclosed by U.S. Pat. Nos. 5,284,761 and 5,158,881, incorporated by reference. The surrounding semipermeable membrane is formed from a polyether sulfone hollow fiber, such as those described by U.S. Pat. No. 4,976,859 or U.S. Pat. No. 4,968,733, incorporated by reference. An alternate surrounding semipermeable membrane material is polysulfone.

The capsule can be any configuration appropriate for maintaining biological activity and providing access for delivery of the product or function, including for example, cylindrical, rectangular, disk-shaped, patch-shaped, ovoid, stellate, or spherical. Moreover, the capsule can be coiled or wrapped into a mesh-like or nested structure. If the capsule is to be retrieved after it is implanted, configurations which tend to lead to migration of the capsules from the site of implantation, such as spherical capsules small enough to travel in the recipient host's blood vessels, are not preferred. Certain shapes, such as rectangles, patches, disks, cylinders, and flat sheets offer greater structural integrity and are preferable where retrieval is desired.

Preferably the device has a tether that aids in maintaining device placement during implant, and aids in retrieval. Such a tether may have any suitable shape that is adapted to secure the capsule in place. For example, the suture may be a loop, a disk, or a suture. In some embodiments, the tether is shaped like an eyelet, so that suture may be used to secure the tether (and thus the device) to the sclera, or other suitable ocular structure. In another embodiment, the tether is continuous with the capsule at one end, and forms a pre-threaded suture needle at the other end. In one preferred embodiment, the tether is an anchor loop that is adapted for anchoring the capsule to an ocular structure. The tether may be constructed of a shape memory metal and/or any other suitable medical grade material known in the art.

In a hollow fiber configuration, the fiber will have an inside diameter of less than 1000 microns, preferably less than 750 microns. We also contemplate devices having an outside diameter less than 300-600 microns. For implantation in the eye, in a hollow fiber configuration the capsule will preferably be between 0.4 cm to 1.5 cm in length, most preferably between 0.4 to 1.0 cm in length. Longer devices may be accommodated in the eye, however, a curved or arcuate shape may be required for secure and appropriate placement. The hollow fiber configuration is preferred for intraocular placement.

For periocular placement, either a hollow fiber configuration (with dimensions substantially as above) or a flat sheet configuration is contemplated. The upper limit

contemplated for a flat sheet is approximately 5 mm x 5 mm--assuming a square shape. Other shapes with approximately the same surface area are also contemplated.

Microdevices manufactured for delivery of soluble VEGFR may have a length of between 1 and 2.5 millimeters, with an inner diameter of between 300 and 500 microns and an outer diameter of between 450 and 700 microns. In such micronized devices, an inner scaffolding containing between 10 and 60 monofilaments of PET can be utilized. The molecular weight cut off ranges from these micronized devices are between 100 and 2000 kDa. In contrast, passive diffusion of a 70 kDa dextran ranges between 100 and 2000 $\times 10^{-10}$ cm²/s. While any suitable membrane material(s) described herein may be used in these micronized devices, two preferred materials are polyethersulfone and/or polysulfone. Moreover, microdevices can be manufactured with and without anchors made of a suitable material (e.g., nitinol). For a complete discussion of micronized devices, see WO2007/078922, which is herein incorporated by reference.

The open membrane contemplated for use with the VEGFR constructs described herein has an upper hydraulic permeability value of approximately 3000 mls/min/m²/mmHg. Alternatively, if a very open membrane is not utilized, a more "immunoisulatory" membrane will be used. For such an immunoisulatory membrane, the hydraulic permeability will typically be in the range of 0.4-170 mls/min/m²/mmHg, for example, 0.5-100 mls/min/m²/mmHg, preferably in the range of 25 to 70 mls/min/m²/mmHg. The glucose mass transfer coefficient of the capsule, defined, measured and calculated as described by Dionne et al., ASAIO Abstracts, p. 99 (1993), and Colton et al., The Kidney, eds., Brenner B M and Rector F C, pp. 2425-89 (1981) will be greater than 10⁻⁶ cm/sec, preferably greater than 10⁻⁴ cm/sec.

The surrounding or peripheral region (jacket), which surrounds the core of the instant devices can be permselective, biocompatible, and/or immunoisulatory. It is produced in such a manner that it is free of isolated cells, and completely surrounds (*i.e.*, isolates) the core, thereby preventing contact between any cells in the core and the recipient's body. Biocompatible semi-permeable hollow fiber membranes, and methods of making them are disclosed in U.S. Pat. Nos. 5,284,761 and 5,158,881 (*See also*, WO 95/05452), each of which incorporated herein by reference in its entirety. For example, the capsule jacket can be formed from a polyether sulfone hollow fiber, such as those described in U.S. Pat. Nos. 4,976,859 and 4,968,733, and 5,762,798, each incorporated herein by reference.

To be permselective, the jacket is formed in such a manner that it has a molecular

weight cut off ("MWCO") range appropriate both to the type and extent of immunological reaction anticipated to be encountered after the device is implanted and to the molecular size of the largest substance whose passage into and out of the device into the eye is desirable.

The type and extent of immunological attacks which may be mounted by the recipient

5 following implantation of the device depend in part upon the type(s) of moiety isolated within it and in part upon the identity of the recipient (*i.e.*, how closely the recipient is genetically related to the source of the BAM). When the implanted tissue or cells are allogeneic to the recipient, immunological rejection may proceed largely through cell-mediated attack by the recipient's immune cells against the implanted cells. When the tissue or cells are xenogeneic
10 to the recipient, molecular attack through assembly of the recipient's cytolytic complement attack complex may predominate, as well as the antibody interaction with complement.

The jacket allows passage into the eye of substances up to a predetermined size, but prevents the passage of larger substances. More specifically, the surrounding or peripheral region is produced in such a manner that it has pores or voids of a predetermined range of
15 sizes, and, as a result, the device is permselective. The MWCO of the surrounding jacket must be sufficiently low to prevent access of the substances required to carry out immunological attacks to the core, yet sufficiently high to allow delivery of the VEGF receptor to the recipient's eye. Preferably, when truncated VEGF receptors are used, the MWCO of the biocompatible jacket of the devices of the instant invention is from about 1 kD
20 to about 150 kD. However, if delivery of a non-truncated receptor is desired, an open membrane with a MWCO greater than 200 kD should be used.

As used herein with respect to the jacket of the device, the term "biocompatible" refers collectively to both the device and its contents. Specifically, it refers to the capability of the implanted intact device and its contents to avoid the detrimental effects of the body's
25 various protective systems and to remain functional for a significant period of time. As used herein, the term "protective systems" refers to the types of immunological attack which can be mounted by the immune system of an individual in whom the instant vehicle is implanted, and to other rejection mechanisms, such as the fibrotic response, foreign body response and other types of inflammatory response which can be induced by the presence of a foreign
30 object in the individuals' body. In addition to the avoidance of protective responses from the immune system or foreign body fibrotic response, the term "biocompatible", as used herein, also implies that no specific undesirable cytotoxic or systemic effects are caused by the

vehicle and its contents such as those that would interfere with the desired functioning of the vehicle or its contents.

The external surface of the device can be selected or designed in such a manner that it is particularly suitable for implantation at a selected site. For example, the external surface
5 can be smooth, stippled or rough, depending on whether attachment by cells of the surrounding tissue is desirable. The shape or configuration can also be selected or designed to be particularly appropriate for the implantation site chosen.

The biocompatibility of the surrounding or peripheral region (jacket) of the device is produced by a combination of factors. Important for biocompatibility and continued
10 functionality are device morphology, hydrophobicity and the absence of undesirable substances either on the surface of, or leachable from, the device itself. Thus, brush surfaces, folds, interlayers or other shapes or structures eliciting a foreign body response are avoided. Moreover, the device-forming materials are sufficiently pure to insure that unwanted substances do not leach out from the device materials themselves. Additionally, following
15 device preparation, the treatment of the external surface of the device with fluids or materials (e.g. serum) which may adhere to or be absorbed by the device and subsequently impair device biocompatibility is avoided.

First, the materials used to form the device jacket are substances selected based upon their ability to be compatible with, and accepted by, the tissues of the recipient of the
20 implanted device. Substances are used which are not harmful to the recipient or to the isolated cells. Preferred substances include polymer materials, *i.e.*, thermoplastic polymers. Particularly preferred thermoplastic polymer substances are those which are modestly hydrophobic, *i.e.* those having a solubility parameter as defined in Brandrup J., et al. Polymer Handbook 3rd Ed., John Wiley & Sons, NY (1989), between 8 and 15, or more preferably,
25 between 9 and 14 (Joules/m³)^{1/2}. The polymer substances are chosen to have a solubility parameter low enough so that they are soluble in organic solvents and still high enough so that they will partition to form a proper membrane. Such polymer substances should be substantially free of labile nucleophilic moieties and be highly resistant to oxidants and enzymes even in the absence of stabilizing agents. The period of residence *in vivo* which is
30 contemplated for the particular vehicle must also be considered: substances must be chosen which are adequately stable when exposed to physiological conditions and stresses. Many thermoplastics are known which are sufficiently stable, even for extended periods of residence *in vivo*, such as periods in excess of one or two years. The choice of materials used

to construct the device is determined by a number of factors as described in detail in Dionne WO 92/19195, herein incorporated by reference. Briefly, various polymers and polymer blends can be used to manufacture the capsule jacket. Polymeric membranes forming the device and the growth surfaces therein may include polyacrylates (including acrylic
5 copolymers), polyvinylidenes, polyvinyl chloride copolymers, polyurethanes, polystyrenes, polyamides, polymethylmethacrylate, polyvinylid difluoride, polyolefins, cellulose acetates, cellulose nitrates, polysulfones, polyphosphazenes, polyacrylonitriles, poly(acrylonitrile/covinyl chloride), as well as derivatives, copolymers and mixtures thereof.

A preferred membrane casting solution comprises a either polysulfone dissolved in
10 the water-miscible solvent dimethylacetamide (DMACSO) or polyethersulfone dissolved in the water-miscible solvent butyrolactone. This casting solution can optionally comprise hydrophilic or hydrophobic additives which affect the permeability characteristics of the finished membrane. A preferred hydrophilic additive for the polysulfone or polyethersulfone is polyvinylpyrrolidone (PVP). Other suitable polymers comprise polyacrylonitrile (PAN),
15 polymethylmethacrylate (PMMA), polyvinylid difluoride (PVDF), polyethylene oxide, polyolefins (*e.g.*, polyisobutylene or polypropylene), polyacrylonitrile/polyvinyl chloride (PAN/PVC), and/or cellulose derivatives (*e.g.*, cellulose acetate or cellulose butyrate). Compatible water-miscible solvents for these and other suitable polymers and copolymers are found in the teachings of U.S. Pat. No. 3,615,024.

20 Second, substances used in preparing the biocompatible jacket of the device are either free of leachable pyrogenic or otherwise harmful, irritating, or immunogenic substances or are exhaustively purified to remove such harmful substances. Thereafter, and throughout the manufacture and maintenance of the device prior to implantation, great care is taken to prevent the adulteration or contamination of the device or jacket with substances, which
25 would adversely affect its biocompatibility.

Third, the exterior configuration of the device, including its texture, is formed in such a manner that it provides an optimal interface with the eye of the recipient after implantation. Certain device geometries have also been found to specifically elicit foreign body fibrotic responses and should be avoided. Thus, devices should not contain structures having
30 interlayers such as brush surfaces or folds. In general, opposing vehicle surfaces or edges either from the same or adjacent vehicles should be at least 1 mm apart, preferably greater than 2 mm and most preferably greater than 5 mm. Preferred embodiments include cylinders having an outer diameter of between about 200 and 350 μm and a length between about 0.4

and 6 mm. Preferably, the core of the devices of the invention has a volume of approximately 2.5 μ l. However, those skilled in the art will recognize that it is also possible to use "micronized" devices having a core volume of less than 0.5 μ l (*e.g.*, about 0.3 μ l).

The surrounding jacket of the biocompatible devices can optionally include substances which decrease or deter local inflammatory response to the implanted vehicle and/or generate or foster a suitable local environment for the implanted cells or tissues. For example antibodies to one or more mediators of the immune response could be included. Available potentially useful antibodies such as antibodies to the lymphokines tumor necrosis factor (TNF), and to interferons (IFN) can be included in the matrix precursor solution.

Similarly, an anti-inflammatory steroid can be included. *See* Christenson, L., et al., J. Biomed. Mat. Res., 23, pp. 705-718 (1989); Christenson, L., Ph.D. thesis, Brown University, 1989, herein incorporated by reference. Alternatively, a substance which stimulates angiogenesis (ingrowth of capillary beds) can be included.

In some embodiments, the jacket of the present device is immunoisulatory. That is, it protects cells in the core of the device from the immune system of the individual in whom the device is implanted. It does so (1) by preventing harmful substances of the individual's body from entering the core, (2) by minimizing contact between the individual and inflammatory, antigenic, or otherwise harmful materials which may be present in the core and (3) by providing a spatial and physical barrier sufficient to prevent immunological contact between the isolated moiety and detrimental portions of the individual's immune system.

In some embodiments, the external jacket may be either an ultrafiltration membrane or a microporous membrane. Those skilled in the art will recognize that ultrafiltration membranes are those having a pore size range of from about 1 to about 100 nanometers while a microporous membrane has a range of between about 0.05 to about 10 microns.

The thickness of this physical barrier can vary, but it will always be sufficiently thick to prevent direct contact between the cells and/or substances on either side of the barrier. The thickness of this region generally ranges between 5 and 200 microns; thicknesses of 10 to 100 microns are preferred, and thickness of 20 to 50 or 20 to 75 microns are particularly preferred. Types of immunological attack which can be prevented or minimized by the use of the instant device include attack by macrophages, neutrophils, cellular immune responses (*e.g.* natural killer cells and antibody-dependent T cell-mediated cytotoxicity (ADCC)), and humoral response (*e.g.* antibody-dependent complement mediated cytotoxicity).

The capsule jacket may be manufactured from various polymers and polymer blends including polyacrylates (including acrylic copolymers), polyvinylidenes, polyvinyl chloride copolymers, polyurethanes, polystyrenes, polyamides, cellulose acetates, cellulose nitrates, polysulfones (including polyether sulfones), polyphosphazenes, polyacrylonitriles, poly(acrylonitrile/covinyl chloride), as well as derivatives, copolymers and mixtures thereof. Capsules manufactured from such materials are described, *e.g.*, in U.S. Pat. Nos. 5,284,761 and 5,158,881, incorporated herein by reference. Capsules formed from a polyether sulfone (PES) fiber, such as those described in U.S. Pat. Nos. 4,976,859 and 4,968,733, incorporated herein by reference, may also be used.

Depending on the outer surface morphology, capsules have been categorized as Type 1 (T1), Type 2 (T2), Type 1/2 (T1/2), or Type 4 (T4). Such membranes are described, *e.g.*, in Lacy et al., "Maintenance Of Normoglycemia In Diabetic Mice By Subcutaneous Xenografts Of Encapsulated Islets", *Science*, 254, pp. 1782-84 (1991), Dionne et al., WO 92/19195 and Baetge, WO 95/05452. A smooth outer surface morphology is preferred.

Those skilled in the art will recognize that capsule jackets with permselective, immunoisulatory membranes are preferable for sites that are not immunologically privileged. In contrast, microporous membranes or permselective membranes may be suitable for immunologically privileged sites. For implantation into immunologically privileged sites, capsules made from the PES or PS membranes are preferred.

Any suitable method of sealing the capsules known in the art may be used, including the employment of polymer adhesives and/or crimping, knotting and heat sealing. In addition, any suitable "dry" sealing method can also be used. In such methods, a substantially non-porous fitting is provided through which the cell-containing solution is introduced. Subsequent to filling, the capsule is sealed. Such methods are described in, *e.g.*, United States Patent Nos. 5,653,688; 5,713,887; 5,738,673; 6,653,687; 5,932,460; and 6,123,700, which are herein incorporated by reference.

According to the methods of this invention, other molecules may be co-delivered in addition to the VEGF receptors described herein. For example, it may be preferable to deliver a trophic factor(s) with an anti-angiogenic factor.

Co-delivery can be accomplished in a number of ways. First, cells may be transfected with separate constructs containing the genes encoding the described molecules. Second, cells may be transfected with a single construct containing two or more genes as well as the necessary control elements. Third, two or more separately engineered cell lines can be either

co-encapsulated or more than one device can be implanted at the site of interest.

Multiple gene expression from a single transcript is preferred over expression from multiple transcription units. *See, e.g.*, Macejak, *Nature*, 353, pp. 90-94 (1991); WO 94/24870; Mountford and Smith, *Trends Genet.*, 11, pp. 179-84 (1995); Dirks et al., *Gene*, 5 128, pp. 247-49 (1993); Martinez-Salas et al., *J. Virology*, 67, pp. 3748-55 (1993) and Mountford et al., *Proc. Natl. Acad. Sci. USA*, 91, pp. 4303-07 (1994).

For some indications, it may be preferable to deliver BAMs to two different sites in the eye concurrently. For example, it may be desirable to deliver a neurotrophic factor to the vitreous to supply the neural retina (ganglion cells to the RPE) and to deliver an anti- 10 angiogenic factor (such as one or more of the VEGF receptors of the invention) via the sub-Tenon's space to supply the choroidal vasculature.

This invention also contemplates use of different cell types during the course of the treatment regime. For example, a patient may be implanted with a capsule device containing a first cell type (*e.g.*, BHK cells). If after time, the patient develops an immune response to 15 that cell type, the capsule can be retrieved, or explanted, and a second capsule can be implanted containing a second cell type (*e.g.*, CHO cells). In this manner, continuous provision of the therapeutic molecule is possible, even if the patient develops an immune response to one of the encapsulated cell types.

The methods and devices of this invention are intended for use in a primate, 20 preferably human host, recipient, patient, subject or individual. A number of different implantation sites are contemplated for the devices and methods of this invention. Suitable implantation sites include, but are not limited to, the aqueous and vitreous humors of the eye, the periocular space, the anterior chamber, and/or the Subtenon's capsule.

The type and extent of immunological response by the recipient to the implanted 25 device will be influenced by the relationship of the recipient to the isolated cells within the core. For example, if core contains syngeneic cells, these will not cause a vigorous immunological reaction, unless the recipient suffers from an autoimmunity with respect to the particular cell or tissue type within the device. Syngeneic cells or tissue are rarely available. In many cases, allogeneic or xenogeneic cells or tissue (*i.e.*, from donors of the same species 30 as, or from a different species than, the prospective recipient) may be available. The use of immunoisulatory devices allows the implantation of allogeneic or xenogeneic cells or tissue, without a concomitant need to immunosuppress the recipient. Use of immunoisulatory capsules also allows the use of unmatched cells (alographs). Therefore, the instant device

makes it possible to treat many more individuals than can be treated by conventional transplantation techniques.

The type and vigor of an immune response to xenografted tissue is expected to differ from the response encountered when syngeneic or allogeneic tissue is implanted into a recipient. This rejection may proceed primarily by cell-mediated, or by complement-mediated attack. The exclusion of IgG from the core of the vehicle is not the touchstone of immunoprotection, because in most cases IgG alone is insufficient to produce cytolysis of the target cells or tissues. Using immunoisulatory devices, it is possible to deliver needed high molecular weight products or to provide metabolic functions pertaining to high molecular weight substances, provided that critical substances necessary to the mediation of immunological attack are excluded from the immunoisulatory capsule. These substances may comprise the complement attack complex component C₃, or they may comprise phagocytic or cytotoxic cells. Use of immunoisulatory capsules provides a protective barrier between these harmful substances and the isolated cells.

While the devices of the present invention are macrocapsules, those skilled in the art will recognize that microcapsules such as, for example those described in Rha, Lim, and Sun may also be used. (*See*, Rha, C. K. et al., U.S. Pat. No. 4,744,933; *Methods in Enzymology* 137, pp. 575-579 (1988); U.S. Patent No. 4,652,833; U.S. Patent No. 4,409,331). In general, microcapsules differ from macrocapsules by (1) the complete exclusion of cells from the outer layer of the device, and (2) the thickness of the outer layer of the device. Typically, microcapsules have a volume on the order of 1 μ l and contain fewer than 10⁴ cells. More specifically, microencapsulation encapsulates approximately 500-50,000 cells, generally, per capsule.

Capsules with a lower MWCO may be used to further prevent interaction of molecules of the patient's immune system with the encapsulated cells.

Any of the devices used in accordance with the methods described herein must provide, in at least one dimension, sufficiently close proximity of any isolated cells in the core to the surrounding eye tissues of the recipient in order to maintain the viability and function of the isolated cells. However, the diffusional limitations of the materials used to form the device do not in all cases solely prescribe its configurational limits. Certain additives can be used which alter or enhance the diffusional properties, or nutrient or oxygen transport properties, of the basic vehicle. For example, the internal medium of the core can be supplemented with oxygen-saturated perfluorocarbons, thus reducing the needs for

immediate contact with blood-borne oxygen. This will allow isolated cells or tissues to remain viable while, for instance, a gradient of angiotensin is released from the vehicle into the surrounding tissues, stimulating ingrowth of capillaries. References and methods for use of perfluorocarbons are given by Faithful, N. S. *Anaesthesia*, 42, pp. 234-242 (1987) and NASA Tech Briefs MSC-21480, U.S. Govt. Printing Office, Washington, D.C. 20402, incorporated herein by reference. Alternatively for clonal cell lines such as PC12 cells, genetically engineered hemoglobin sequences may be introduced into the cell lines to produce superior oxygen storage. See NPO-17517 NASA Tech Briefs, 15, p. 54.

The thickness of the device jacket should be sufficient to prevent an immunoresponse by the patient to the presence of the devices. For that purpose, the devices preferably have a minimum thickness of 1 μm or more and are free of the cells.

Additionally, reinforcing structural elements can also be incorporated into the devices. For example, these structural elements can be made in such a fashion that they are impermeable and are appropriately configured to allow tethering or suturing of the device to the eye tissues of the recipient. In certain circumstances, these elements can act to securely seal the jacket (*e.g.*, at the ends of the cylinder), thereby completing isolation of the core materials (*e.g.*, a molded thermoplastic clip). In many embodiments, it is desirable that these structural elements should not occlude a significant area of the permselective jacket.

The device of the present invention is of a sufficient size and durability for complete retrieval after implantation. One preferred device of the present invention has a core of a volume of approximately 1-3 μL . The internal geometry of micronized devices has a volume of approximately 0.05-0.1 μL .

Along with the soluble VEGF receptors described herein, at least one additional BAM can also be delivered from the device to the eye. For example, the at least one additional BAM can be provided from a cellular or a noncellular source. When the at least one additional BAM is provided from a noncellular source, the additional BAM(s) may be encapsulated in, dispersed within, or attached to one or more components of the cell system including, but not limited to: (a) sealant; (b) scaffold; (c) jacket membrane; (d) tether anchor; and/or (e) core media. In such embodiment, co-delivery of the BAM from a noncellular source may occur from the same device as the BAM from the cellular source.

Alternatively, two or more encapsulated cell systems can be used. For example, the least one additional biologically active molecule can be a nucleic acid, a nucleic acid fragment, a peptide, a polypeptide, a peptidomimetic, a carbohydrate, a lipid, an organic

molecule, an inorganic molecule, a therapeutic agent, or any combinations thereof.

Specifically, the therapeutic agents may be an anti-angiogenic drug, a steroidal and non-steroidal anti-inflammatory drug, an anti-mitotic drug, an anti-tumor drug, an anti-parasitic drug, an IOP reducer, a peptide drug, and/or any other biologically active molecule drugs

5 approved for ophthalmologic use.

Suitable excipients include, but are not limited to, any non-degradable or biodegradable polymers, hydrogels, solubility enhancers, hydrophobic molecules, proteins, salts, or other complexing agents approved for formulations.

Non-cellular dosages can be varied by any suitable method known in the art such as
10 varying the concentration of the therapeutic agent, and/or the number of devices per eye, and/or modifying the composition of the encapsulating excipient. Cellular dosage can be varied by changing (1) the number of cells per device, (2) the number of devices per eye, and/or (3) the level of BAM production per cell. Cellular production can be varied by changing, for example, the copy number of the gene for the BAM in the transduced cell, or
15 the efficiency of the promoter driving expression of the BAM. Suitable dosages from non-cellular sources may range from about 1 pg to about 1000 ng per day.

The instant invention also relates to methods for making the macrocapsular devices described herein. Devices may be formed by any suitable method known in the art. (*See, e.g.,* United States Patent Nos. 6,361,771; 5,639,275; 5,653,975; 4,892,538; 5,156,844;
20 5,283,138; and 5,550,050, each of which is incorporated herein by reference).

Membranes used can also be tailored to control the diffusion of molecules, such as soluble VEGF receptor, based on their molecular weight. (*See* Lysaght et al., 56 J. Cell Biochem. 196 (1996), Colton, 14 Trends Biotechnol. 158 (1996)). Using encapsulation techniques, cells can be transplanted into a host without immune rejection, either with or
25 without use of immunosuppressive drugs. The capsule can be made from a biocompatible material that, after implantation in a host, does not elicit a detrimental host response sufficient to result in the rejection of the capsule or to render it inoperable, for example through degradation. The biocompatible material is relatively impermeable to large molecules, such as components of the host's immune system, but is permeable to small molecules, such as
30 insulin, growth factors, and nutrients, while allowing metabolic waste to be removed. A variety of biocompatible materials are suitable for delivery of growth factors by the composition of the invention. Numerous biocompatible materials are known, having various outer surface morphologies and other mechanical and structural characteristics.

If a device with a jacket of thermoplastic or polymer membrane is desired, the pore size range and distribution can be determined by varying the solids content of the solution of precursor material (the casting solution), the chemical composition of the water-miscible solvent, or optionally including a hydrophilic or hydrophobic additive to the casting solution, as taught by U.S. Pat. No. 3,615,024. The pore size may also be adjusted by varying the hydrophobicity of the coagulant and/or of the bath.

Typically, the casting solution will comprise a polar organic solvent containing a dissolved, water-insoluble polymer or copolymer. This polymer or copolymer precipitates upon contact with a solvent-miscible aqueous phase, forming a permselective membrane at the site of interface. The size of pores in the membrane depends upon the rate of diffusion of the aqueous phase into the solvent phase; the hydrophilic or hydrophobic additives affect pore size by altering this rate of diffusion. As the aqueous phase diffuses farther into the solvent, the remainder of the polymer or copolymer is precipitated to form a trabecular support which confers mechanical strength to the finished device.

The external surface of the device is similarly determined by the conditions under which the dissolved polymer or copolymer is precipitated (*i.e.*, exposed to the air, which generates an open, trabecular or sponge-like outer skin, immersed in an aqueous precipitation bath, which results in a smooth permselective membrane bilayer, or exposed to air saturated with water vapor, which results in an intermediate structure).

The surface texture of the device is dependent in part on whether the extrusion nozzle is positioned above, or immersed in, the bath: if the nozzle is placed above the surface of the bath a roughened outer skin will be formed, whereas if the nozzle is immersed in the bath a smooth external surface is formed.

The surrounding or peripheral matrix or membrane can be preformed, filled with the materials which will form the core (for instance, using a syringe), and subsequently sealed in such a manner that the core materials are completely enclosed. The device can then be exposed to conditions which bring about the formation of a core matrix if a matrix precursor material is present in the core.

The devices of the invention can provide for the implantation of diverse cell or tissue types, including fully-differentiated, anchorage-dependent, fetal or neonatal, or transformed, anchorage-independent cells or tissue. The cells to be isolated are prepared either from a donor (*i.e.*, primary cells or tissues, including adult, neonatal, and fetal cells or tissues) or from cells which replicate *in vitro* (*i.e.*, immortalized cells or cell lines, including genetically

modified cells). In all cases, a sufficient quantity of cells to produce effective levels of the needed product or to supply an effective level of the needed metabolic function is prepared, generally under sterile conditions, and maintained appropriately (*e.g.* in a balanced salt solution such as Hank's salts, or in a nutrient medium, such as Ham's F12) prior to isolation.

5 The ECT devices of the invention are of a shape which tends to reduce the distance between the center of the device and the nearest portion of the jacket for purposes of permitting easy access of nutrients from the patient into the cell or of entry of the patient's proteins into the cell to be acted upon by the cell to provide a metabolic function. In that regard, a non-spherical shape, such as a cylinder, is preferred.

10 Four important factors that influence the number of cells or amount of tissue to be placed within the core of the device (*i.e.*, loading density) of the instant invention are: (1) device size and geometry; (2) mitotic activity within the device; (3) viscosity requirements for core preparation and or loading; and (4) pre-implantation assay and qualification requirements.

15 With respect to the first of these factors, (device size and geometry), the diffusion of critical nutrients and metabolic requirements into the cells as well as diffusion of metabolites away from the cell are critical to the continued viability of the cells. In the case of RPE cells such as ARPE-19 cells, the neighboring cells are able to phagocytize the dying cells and use the debris as an energy source.

20 Among the metabolic requirements met by diffusion of substances into the device is the requirement for oxygen. The oxygen requirements of the specific cells must be determined for the cell of choice. *See* Methods and references for determination of oxygen metabolism are given in Wilson D. F. et al., J. Biol. Chem., 263, pp. 2712-2718, (1988).

25 With respect to the second factor (cell division), if the cells selected are expected to be actively dividing while in the device, then they will continue to divide until they fill the available space, or until phenomena such as contact inhibition limit further division. For replicating cells, the geometry and size of the device will be chosen so that complete filling of the device core will not lead to deprivation of critical nutrients due to diffusional limitations.

30 With respect to the third factor (viscosity of core materials) cells in densities occupying up to 70% of the device volume can be viable, but cell solutions in this concentration range would have considerable viscosity. Introduction of cells in a very viscous solution into the device could be prohibitively difficult. In general, for both two step

and coextrusion strategies, cell loading densities of higher than 30% will seldom be useful, and in general optimal loading densities will be 20% and below. For example, for fragments of tissues, it is important, in order to preserve the viability of interior cells, to observe the same general guidelines as above and tissue fragments should not exceed 250 microns in diameter with the interior cells having less than 15, preferably less than 10 cells between them and the nearest diffusional surface.

Finally, with respect to the fourth factor (preimplantation and assay requirements), in many cases, a certain amount of time will be required between device preparation and implantation. For instance, it may be important to qualify the device in terms of its biological activity. Thus, in the case of mitotically active cells, preferred loading density will also consider the number of cells which must be present in order to perform the qualification assay.

In most cases, prior to implantation *in vivo*, it will be important to use *in vitro* assays to establish the efficacy of the BAM (*e.g.*, the VEGF receptor) within the device. Devices can be constructed and analyzed using model systems in order to allow the determination of the efficacy of the vehicle on a per cell or unit volume basis.

Following these guidelines for device loading and for determination of device efficacy, the actual device size for implantation will then be determined by the amount of biological activity required for the particular application. The number of devices and device size should be sufficient to produce a therapeutic effect upon implantation and is determined by the amount of biological activity required for the particular application. In the case of secretory cells releasing therapeutic substances, standard dosage considerations and criteria known to the art will be used to determine the amount of secretory substance required.

Factors to be considered include the size and weight of the recipient; the productivity or functional level of the cells; and, where appropriate, the normal productivity or metabolic activity of the organ or tissue whose function is being replaced or augmented. It is also important to consider that a fraction of the cells may not survive the immunoisolation and implantation procedures. Moreover, whether the recipient has a preexisting condition which can interfere with the efficacy of the implant must also be considered. Devices of the instant invention can easily be manufactured which contain many thousands of cells. For example, current clinical devices contain between 200,000 and 750,000 cells, whereas micronized devices would contain between 10,000 and 100,000 cells.

Encapsulated cell therapy is based on the concept of isolating cells from the recipient host's immune system by surrounding the cells with a semipermeable biocompatible material before implantation within the host. For example, the invention includes a device in which genetically engineered ARPE-19 cells are encapsulated in an immunoisulatory capsule, which, upon implantation into a recipient host, minimizes the deleterious effects of the host's immune system on the ARPE-19 cells in the core of the device. ARPE-19 cells are immunoisolated from the host by enclosing them within implantable polymeric capsules formed by a microporous membrane. This approach prevents the cell-to-cell contact between the host and implanted tissues, thereby eliminating antigen recognition through direct presentation.

The VEGF receptors described herein can be delivered intraocularly (*e.g.*, in the anterior chamber and the vitreous cavity) or periocularly (*e.g.*, within or beneath Tenon's capsule), or both. The devices of the invention may also be used to provide controlled and sustained release of the receptors to treat various ophthalmic disorders, ophthalmic diseases and/or diseases which have ocular effects.

Intraocular (preferably in the vitreous) or per ocular (preferably in the sub-Tenon's space or region) delivery of an anti-angiogenic factor, such as any of the soluble VEGF receptors described herein, in a dosage range of 0.1 pg and 1000 µg (*e.g.*, between 0.1 pg and 500 µg; between 0.1 pg and 250 µg; between 0.1 pg and 100 µg; between 0.1 pg and 50 µg; between 0.1 pg and 25 µg; between 0.1 pg and 10 µg; between 0.1 pg and 5 µg; between 0.1 pg and 100 ng; between 0.1 pg and 50 ng; between 0.1 pg and 25 ng; between 0.1 pg and 10 ng; or between 0.1 pg and 5 ng) per eye per patient per day is contemplated.

Ophthalmic disorders that may be treated by various embodiments of the present invention include, but are not limited to diabetic retinopathies, diabetic macular edema, proliferative retinopathies, retinal vascular diseases, vascular anomalies, age-related macular degeneration and other acquired disorders, endophthalmitis, infectious diseases, inflammatory but non-infectious diseases, AIDS-related disorders, ocular ischemia syndrome, pregnancy-related disorders, peripheral retinal degenerations, retinal degenerations, toxic retinopathies, retinal tumors, choroidal tumors, choroidal disorders, vitreous disorders, retinal detachment and proliferative vitreoretinopathy, non-penetrating trauma, penetrating trauma, post-cataract complications, and inflammatory optic neuropathies.

Those skilled in the art will recognized that age-related macular degeneration includes, but is not limited to, wet and dry age-related macular degeneration, exudative age-

related macular degeneration, and myopic degeneration.

In some preferred embodiments, the disorder to be treated is the wet form of age-related macular degeneration or diabetic retinopathy. The present invention may also be useful for the treatment of ocular neovascularization, a condition associated with many ocular diseases and disorders. For example, retinal ischemia-associated ocular neovascularization is a major cause of blindness in diabetes and many other diseases.

The cell lines and devices of the present invention may also be used to treat ocular symptoms resulting from diseases or conditions that have both ocular and non-ocular symptoms. Some examples include cytomegalovirus retinitis in AIDS as well as other conditions and vitreous disorders; hypertensive changes in the retina as a result of pregnancy; and ocular effects of various infectious diseases such as tuberculosis, syphilis, lyme disease, parasitic disease, toxocara canis, ophthalmomyiasis, cyst cercosis and fungal infections.

The devices and cell lines may also be used to treat conditions relating to other intraocular neovascularization-based diseases. For example, such neovascularization can occur in diseases such as diabetic retinopathy, central retinal vein occlusion and, possibly, age-related macular degeneration. Corneal neovascularization is a major problem because it interferes with vision and predisposes patients to corneal graft failure. A majority of severe visual loss is associated with disorders that result in ocular neovascularization.

The invention also relates to methods and the delivery of soluble VEGF receptors in order to treat cell proliferative disorders, such as, hematologic disorders, atherosclerosis, inflammation, increased vascular permeability, and malignancy.

The use of the devices and techniques described herein provide several advantages over other delivery routes: the VEGF receptors can be delivered to the eye directly, which reduces or minimizes unwanted peripheral side effects and very small doses of the receptor (*i.e.*, nanogram or low microgram quantities rather than milligrams) can be delivered compared with topical applications, thereby also potentially lessening side effects. Moreover, since viable cells continuously produce newly synthesized receptors, these techniques should be superior to injection delivery of VEGF receptor, where the dose fluctuates greatly between injections and the receptor is continuously degraded but not continuously replenished.

Living cells and cell lines genetically engineered to secrete the soluble VEGF receptors of the invention can be encapsulated in the device of the invention and surgically inserted (under retrobulbar anesthesia) into any appropriate anatomical structure of the eye. For example, the devices can be surgically inserted into the vitreous of the eye, where they

are preferably tethered to the sclera to aid in removal. Devices can remain in the vitreous as long as necessary to achieve the desired prophylaxis or therapy. For example, the desired therapy may include promotion of neuron or photoreceptor survival or repair, or inhibition and/or reversal of retinal or choroidal neovascularization, as well as inhibition of uveal, retinal and optic nerve inflammation. With vitreal placement, the VEGF receptor, may be delivered to the retina or the retinal pigment epithelium (RPE).

In other embodiments, cell-loaded devices are implanted periorcularly, within or beneath the space known as Tenon's capsule, which is less invasive than implantation into the vitreous. Therefore, complications such as vitreal hemorrhage and/or retinal detachment are potentially eliminated. This route of administration also permits delivery of the soluble VEGF receptors described herein to the RPE or the retina. Periorcular implantation is especially preferred for treating choroidal neovascularization and inflammation of the optic nerve and uveal tract. In general, delivery from periorcular implantation sites will permit circulation of the soluble VEGF receptors to the choroidal vasculature, retinal vasculature, and the optic nerve.

Delivery of anti-angiogenic factors, such as the soluble VEGF receptors of the invention, directly to the choroidal vasculature (periorcularly) or to the vitreous (intraocularly) using the devices and methods described herein may reduce or alleviate the problems associated with prior art treatment methods and devices and may permit the treatment of poorly defined or occult choroidal neovascularization as well as provide a way of reducing or preventing recurrent choroidal neovascularization via adjunctive or maintenance therapy.

Implantation of the biocompatible devices of the invention is performed under sterile conditions. The device can be implanted using a syringe or any other method known to those skilled in the art. Generally, the device is implanted at a site in the recipient's body which will allow appropriate delivery of the secreted product or function to the recipient and of nutrients to the implanted cells or tissue, and will also allow access to the device for retrieval and/or replacement. A number of different implantation sites are contemplated. These include, *e.g.*, the aqueous humor, the vitreous humor, the sub-Tenon's capsule, the periorcular space, and the anterior chamber. Preferably, for implant sites that are not immunologically privileged, such as periorcular sites, and other areas outside the anterior chamber (aqueous) and the posterior chamber (vitreous), the capsules are immunoisulatory.

It is preferable to verify that the cells immobilized within the device function properly both before and after implantation. Any assays or diagnostic tests well known in the art can

be used for these purposes. For example, an ELISA (enzyme-linked immunosorbent assay), chromatographic or enzymatic assay, or bioassay specific for the secreted product can be used. If desired, secretory function of an implant can be monitored over time by collecting appropriate samples (*e.g.*, serum) from the recipient and assaying them.

5 The use of many of the prior art devices and surgical techniques resulted in a large number of retinal detachments. The occurrence of this complication is lessened because the devices and methods of this invention are less invasive compared to several other therapies.

Modified, truncated and/or mutein forms of the VEGF receptors described herein can also be used in accordance with this invention. Further, the use of active fragments of these
10 receptors (*i.e.*, those fragments having biological activity sufficient to achieve a therapeutic effect) is also contemplated. Also contemplated are receptor molecules modified by attachment of one or more polyethylene glycol (PEG) or other repeating polymeric moieties as well as combinations of these proteins and polycistronic versions thereof.

Treatment of many conditions according to the methods described herein will require
15 only one or at most less than 50 implanted devices per eye to supply an appropriate therapeutic dose. Therapeutic dosages may be between about 0.1 pg and 1000 µg per eye per patient per day (*e.g.*, between 0.1 pg and 500 µg; between 0.1 pg and 250 µg, between 0.1 pg and 100 µg; between 0.1 pg and 50 µg; between 0.1 pg and 25 µg; between 0.1 pg and 10 µg; between 0.1 pg and 5 µg; between 0.1 pg and 100 ng; between 0.1 pg and 50 ng; between 0.1
20 pg and 25 ng; between 0.1 pg and 10 ng; or between 0.1 pg and 5 ng per eye per patient per day). Each of the devices of the present invention is capable of storing between about 1,000 and about 750,000 cells, in individual or cluster form, depending on their type.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

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EXAMPLES

Example 1: Sub-cloning

Various fragments of secretory VEGF receptor 1 ("VR1") were expressed in NTC-200 cells. These fragments include domain 2 ("D2"), which is the VEGF binding domain
30 (VBD) of VR1. D2 was fused in frame with combinations of domain 1, 3 or 4 (referred to herein as "D1", "D3" and "D4", respectively). In some cases, domain 3 of VEGF receptor 2 ("VR2 D3") was used in place of VR1 D3 to create a VR1/VR2 chimeric molecule. In

another embodiment, dimers of domains 2 and 3 (referred to herein as “D2” and “D3”) of VEGF receptor 2 were used.

To achieve secretion of the protein, the constructs contained either the native or IgSP murine immunoglobulin leader sequence, the latter of which was used previously to direct secretion of CNTF from NTC-201 cells. Nucleotide and amino acid sequences of intended proteins and corresponding nucleic acid sequences, are described herein as follows:

- 1) sVR1 D1-3
- 2) sVR1 D2-3
- 3) sVR1 D1-4
- 4) sVR1 D2-4
- 5) sVR D1-3 R1/R2 chimera dimer
- 6) sVR D2-3 R1/R2 chimera dimer
- 7) sVR2 D2-3 dimer

As used herein, sVR1 is secretory VEGF receptor 1, sVR2 is secretory VEGF receptor 2, D1 is Domain 1, D2 is Domain 2, D3 is Domain 3, D4 is Domain 4 and the sVR chimeras comprise fragments of sVR1 and sVR2.

Gene fragments were amplified from sVEGFR1 (hFlt) or sVEGFR2 (hKDR) cDNA (GenBank Accession Nos. U01134 and AF063658, respectively) by PCR using oligonucleotide primer pairs specific for the desired product. Amplified products were digested with the appropriate restriction endonuclease and ligated into Neurotech mammalian expression vector pKAN3, a schematic of which is shown in Figure 3. The pKAN3 backbone is based on the pNUT-IgSP-hCNTF expression plasmid used to create the ARPE-19-hCNTF cell lines.

The nucleotide sequence of pKAN3 is shown below:

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1   CTGTTGTTTTT AAAACCAGCC TGGAGTAGAG CAGATGGGTT AAGGTGAGTG ACCCCTCAGC
61  CCTGGACATT CTAGATGAG CCCCTCAGG AGTAGAGAAT AATGTTGAGA TGAGTTCTGT
121 TGGCTAAAAAT AATCAAGGCT AGTCTTTATA AACTGTCTC CTCTTCTCCT AGCTTCGATC
181 CAGAGAGAGA CCTGGGCGGA GCTGGTCGCT GCTCAGGAAC TCCAGGAAAG GAGAAGCTGA
241 GGTACCACG CTGCGAATGG GTTACGGAG ATAGCTGGCT TTCCGGGGTG AGTTCTCGTA
301 AACTCCAGAG CAGCGATAGG CCGTAATATC GGGGAAAGCA CTATAGGAC ATGATGTTCC
361 ACACGTCACA TGGGTCGTCC TATCCGAGCC AGTCGTGCCA AAGGGGCGGT CCCGCTGTGC
421 AACTGGCGC TCCAGGGAGC TCTGCACTCC GCCGAAAAG TGCCTCGGC TCTGCCAAGG
481 ACGCGGGGCG CGTGAATATG CGTGGGCTGG AGCAACCGCC TGCTGGGTGC AAACCCCTTG
541 CGCCCGGACT CGTCCAACGA CTATAAGAG GGCAGGCTGT CCTCTAAGCG TCACCCCTAG
601 AGTCGAGCTG TGACGGTCCT TACAGTCGAG GGCTCGCATC TCTCCTTCAC GCGCCCGCCG
661 CCCTACCTGA GGCCTCCATC CACGCCGGTT GAGTCGCGTT CTGCCGCTC CCGCCTGTGG
721 TGCCTCCTGA ACTGCGTCCG CCGTCTAGGT AAGTTTAAAG CTCAGGTCGA GACCGGGCCT
781 TTGTCCGGCG CTCCCTTGA GCCTACCTAG ACTCAGCCGG CTCTCCACGC TTTGCCTGAC
841 CCTGCTTGCT CAACTCTACG TCTTTGTTTC GTTTTCTGTT CTGCGCGGTT ACAGATCCAA
901 GCTGTGACCG GCGCCTACCT CGAGACCGGT GCGGCCGCAT TTAAATACTA GTCCGGGTGG
961 CATCCCTGTG ACCCCTCCCC AGTGCCTCTC CTGGCCCTGG AAGTTGCCAC TCCAGTCCCC
1021 ACCAGCCTTG TCCTAATAAA ATTAAGTTGC ATCATTTTGT CTGACTAGGT GTCCTTCTAT

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1081. AATATTATGG GGTGGAGGGG GGTGGTATGG AGCAAGGGGC AAGTTGGGAA GACAACCTGT
1141. AGGGCCTGCG GGGTCTATTG GGAACCAAGC TGGAGTGCAG TGGCACAATC TTGGCTCACT
1201. GCAATCTCCG CCTCCTGGGT TCAAGCGATT CTCTGCCTC AGCCTCCCGA CGGCCGTAAT
1261. TCGTAATCAT GTCATAGCTG TTTCTGTGT GAAATTGTTA TCCGCTCACA ATTCCACACA
1321. ACATACGAGC CGGAAGCATA AAGTGTAAG CCTGGGGTGC CTAATGAGTG AGCTAACTCA
1381. CATTAATTGC GTTGCCTCA CTGCCCGCTT TCCAGTCGGG AAACCTGTCTG TGCCAGCTGC
1441. ATTAATGAAT CGGCCAACGC GCGGGAGAG GCGGTTTTCG TATTGGGCGC TCTTCGCTT
1501. CCTCGCTCAC TGA CTGCTCGGTCG TTCGGCTGCG GCGAGCGGTA TCAGCTCACT
1561. CAAAGGCGGT AATACGGTTA TCCACAGAAT CAGGGGATAA CGCAGGAAAG AACATGTGAG
1621. CAAAAGGCCA GCAAAGGCC AGGAACCGTA AAAAGGCCGC GTTGCTGGCG TTTTCCATA
1681. GGCTCCGCC CCCTGACGAG CATCACAAA ATCGACGCTC AAGTCAGAG TGCGGAAACC
1741. CGACAGGACT ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG CGCTCTCCTG
1801. TTCCGACCCT GCCGCTTACC GGATACCTGT CCGCCTTTCT CCCTTCGGGA AGCGTGGCGC
1861. TTTCTCATAG CTCACGCTGT AGGTATCTCA GTTCGGTGTA GGTCTGTCG TCCAAGCTGG
1921. GCTGTGTGCA CGAACCCCG GTTCAGCCCG ACCGCTGCGC CTTATCTGTC AACTATGCTC
1981. TTGAGTCCAA CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT GGTAAACAGGA
2041. TTAGCCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG GCCTAACTAC
2101. GGCTACACTA GAAGAACAGT ATTTGGTATC TGCGCTCTGC TGAAGCCAGT TACCTTCGGA
2161. AAAAGAGTTG GTAGCTCTTG ATCCGGCAA CAAACCACCG CTGGTAGCGG TGGTTTTTTT
2221. GTTTGCAAGC AGCAGATTAC GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT
2281. TCTACGGGGT CTGACGCTCA GTGGAACGAA AACTCACGTT AAGGGATTTT GGTATGAGA
2341. TTATCAAAAA GGATCTTCAC CTAATCCTT TTAATTAATA AATGAAGTTT TAAATCAATC
2401. TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTCTAAGAA ACCATTATTA TCATGACATT
2461. AACCTATAAA ATAGGCGTA TCACGAGGCC CTTTCGTCTC GCGCGTTTCG GTGATGACGG
2521. TGAACACCTC TGACACATGC AGCTCCCGGA GACGGTCACA GCTTGTCTGT AAGCGGATGC
2581. CGGGAGCAGA CAAGCCCGTC AGGGCGCGTC AGCGGGTGT TGGCGGTGTC GGGGCTGGCT
2641. TAACTATGCG GCATCAGAGC AGATTGTACT GAGAGTGCAC CGATCCCCC GGTACCCGAT
2701. CCAGACATGA TAAGATACAT TGATGAGTTT GGACAAACCA CAACTAGAAAT GCGAAGCAAA
2761. AAATGCTTTA TTTGTGAAAT TTGTGATGCT ATTGCTTTAT TTGTAACCAT TATAAGCTGC
2821. AATAACAAG TTAACAACAA CAATTGCATT CATTTTATGT TTCAGGTTC AAGGGGAGGTG
2881. TGGGAGGTTT TTTAAAGCAA GTAAACCTC TACAAATGTG GTATGGCTGA TTATGATCAT
2941. GAACAGACTG TGAGGACTGA GGGGCTGAA ATGAGCCTTG GGACTGTGAA TCTAAATAC
3001. ACAACAATT AGAATCAGTA GTTAAACACA TTATACACTT AAAAATTTTA TATTTACCTT
3061. AGAGCTTTAA ATCTCTGTAG GTAGTTTGTG CAATTATGTC ACACCACAGA AGTAAGGTTT
3121. CTTCAACAAG ATCCCAAGTC GAACCCAGGA GTCCCGCTCA GAAGAACTCG TCAAGAAGGC
3181. GATAGAAGGC GATGCGCTGC GAATCGGGAG CCGCGATACC GTAAAGCAAT AGCAAGCGGT
3241. CAGCCCATTC GCCGCCAAGC TCTTCAGCAA TATCACGGGT AGCCAACGCT ATGTCCTGAT
3301. AGCGGTCCGC CACACCCAGC CGGCCACAGT CGATGAATCC AGAAAAGCGG CCATTTTCCA
3361. CCATGATATT CGGCAAGCAG GCATCGCCAT GGGTCACGAC GAGATCCTCG CCGTCGGGCA
3421. TGCGCGCCTT AGCCTGGCG AACAGTTGCG CTGGCGCGAG CCCCTGATGC TCTTCGTTCA
3481. GATCATCCTG ATCGACAAGA CCGGCTTCCA TCCGAGTACG TGCTCGCTCG ATGCGATGTT
3541. TCGCTTGGTG GTCGAATGGG CAGGTAGCCG GATCAAGCGT ATGCGAGCGC CGCATTCAT
3601. CAGCCATGAT GGATACTTTC TCGGCAGGAG CAAGGTGAGA TGACAGGAGA TCCTGCCCGC
3661. GCACTTCGCC CAATAGCAGC CAGTCCCTTC CCGCTTCAGT GACAACGTCG AGCACAGCTG
3721. CGCAAGGAAC GCCCGTCGTG GCCAGCCACG ATAGCCGCGC TGCCTCGTCC TGCAGTTCAT
3781. TCAGGGCACC GGACAGGTCG GTCTTGACAA AAAGAACCAG GCGCCCCTGC GCTGACAGCC
3841. GGAACACGGC GGCATCAGAG CAGCCGATTG TCTGTTGTGC CCAGTCATAG CCGAATAGCC
3901. TCTCCACCA AGCGGCCGGA GAACCTGCGT GCAATCCATC TTGTTCAATC ATGCGAAACG
3961. ATCCTCATCC TGTCTCTTGA TCAGATCCCA AGCTGGGGAT CTGCAGGAAT CGATATCAAG
4021. CTTATCGATA AGCTTTTTCG AAAAGCCTAG GCCTCCAAAA AAGCCTCCTC ACTACTTCTG
4081. GAATAGCTCA GAGGCCGAGG CGGCCTCGGC CTCTGCATAA ATAAAAAAA TTAGTCAGCC
4141. ATGGGGCGGA GAATGGGCGG AACTGGGCGG AGTTAGGGGC GGGATGGGC GAGTTAGGGG
4201. CGGGACTATG GTTGCTGACT AATTGAGATG CATGCTTTGC ATACTTCTGC CTGCTGGGGA
4261. GCCTGGGGAC TTTCCACACC TGGTTGCTGA CTAATTGAGA TGCATGCTTT GCATACTTCT
4321. GCCTGCTGGG GAGCCTGGGG ACTTTCCACA CCCTAATGA CACACATTCC ACAGCTGGTT
4381. CTTTCCGCTT CAGAAGGTAC ACTCTTCTT TTTCAATATT ATTGAAGCAT TTATCAGGGT
4441. TATTGTCTCA TGAGCGGATA CATATTTGAA TGTATTTAGA AAAATAAACA AATAGGGGTT
4501. CCGCGCACAT TTCCCCGAAA AGTGCCACCT GACGGCGGCC (SEQ ID NO:15)

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In SEQ ID NO:15, nucleotides 1-595 are pMT1; nucleotides 631-918 are U5 5' UTR, nucleotides 919-953 are MCS; nucleotides 954-1250 are hGH pA; nucleotides 1258-2680 are pUC 18; nucleotides 2698-2992 are SV40 pA; nucleotides 2988-3133 are SV40 Intron E19S; nucleotides 3158-3952 are NeoR; nucleotides 4030-4400 are SV40 promoter; and 4401-4534 are AmpR promoter.

Similarly, the complementary nucleic acid sequence for the pKAN3 vector is shown in SEQ ID NO:16:

	GAACCAAAAA	TTTTGGTCCG	ACCTCATCTC	GTCTACCCAA	TTCCACTCAC	TGGGGAGTCG
	GGACCTGTAA	GAATCTACTC	GGGGGAGTCC	TCATCTCTTA	TTACAACCTCT	ACTCAAGACA
5	ACCGATTTTA	TTAGTTCGGA	TCAGAAATAT	TTTGACAGAG	GAGAAGAGGA	TCGAAGCTAG
	GTCTCTCTCT	GGACCCGCCT	CGACCAGCGA	CGAGTCCCTTG	AGGTCCCTTTC	CTCTTCGACT
	CCAATGGTGC	GACGCTTACC	CAAATGCCTC	TATCGACCGA	AAGGCCCCAC	TCAAGAGCAT
	TTGAGGTCTC	GTCGCTATCC	GGCATTATAG	CCCTTTTCGT	GATATCCCTG	TACTACAAGG
	TGTGCAGTGT	ACCCAGCAGG	ATAGGCTCGG	TCAGCACGGT	TTCCCCGCCA	GGGCGACACG
10	TGTGACCGCG	AGGTCCCTCG	AGACGTGAGG	CGGGCTTTTC	ACGCGAGCCG	AGACGGTTCC
	TGCGCCCCGC	GCACTGATAC	GCACCCGACC	TCGTTGGCGG	ACGACCCACG	TTTGGGAAAC
	GCGGGCCTGA	GCAGGTTGCT	GATATTTCTC	CCGTCCGACA	GGAGATTCCG	AGTGGGGATC
	TCAGCTCGAC	ACTGCCAGGA	ATGTCAGCTC	CCGAGCGTAG	AGAGGAAGTG	CGCGGGCGGC
	GGGATGGACT	CCGGCGGTAG	GTGCGGCCAA	CTCAGCGCAA	GACGGCGGAG	GGCGGACACC
15	ACGGAGGACT	TGACGCAGGC	GGCAGATCCA	TTCAAATTTT	GAGTCCAGCT	CTGGCCCCGA
	AACAGGCCGC	GAGGGAACCT	CGGATGGATC	TGAGTCGGCC	GAGAGGTGCG	AAACGGACTG
	GGACGAACGA	GTTGAGATGC	AGAAACAAAG	CAAAAGACAA	GACGCGGCAA	TGTCTAGGTT
	CGACACTGGC	CGCGGATGGA	GCTCTGGCCA	CGCCGGCGTA	AATTTATGAT	CAGGCCACCC
	GTAGGGACAC	TGGGGAGGGG	TCACGGAGAG	GACCGGGACC	TTCAACCGTG	AGGTCACGGG
20	TGGTCGGAAC	AGGATTATTT	TAATTCAACG	TAGTAAACAA	GACTGATCCA	CAGGAAGATA
	TTATAATACC	CCACCTCCCC	CCACCATACC	TCGTTCCCCG	TTCAACCCCT	CTGTTGGACA
	TCCCGGACGC	CCCAGATAAC	CCTTGGTTTC	ACCTCACGTC	ACCGTGTTAG	AACCGAGTGA
	CGTTAGAGGC	GGAGGACCCA	AGTTTCGCTAA	GAGGACGGAG	TCGGAGGGGT	GCCGGCATTAA
	AGCATTAGTA	CAGTATCGAC	AAAGGACACA	CTTTAACAAT	AGGCGAGTGT	TAAGGTGTGT
25	TGTATGCTCG	GCCCTTCGTAT	TTCAACATTC	GGACCCACAG	GATTACTCAC	TCGATTGAGT
	GTAATTAACG	CAACGCGAGT	GACGGGCGAA	AGGTCAGCCC	TTTGGACAGC	ACGGTCGACG
	TAATTACTTA	GCCGGTTGCG	CGCCCCCTCT	CGCCAAACGC	ATAACCCGCG	AGAAGGCGAA
	GGAGCGAGTG	ACTGAGCGAC	GCGAGCCAGC	AAGCCGACGC	CGCTCGCCAT	AGTCGAGTGA
30	GTTTCCGCCA	TTATGCCAAT	AGGTGTCTTA	GTCCCTTATT	GCGTCCCTTT	TTGATCACTC
	GTTTTCGGGT	CGTTTTCGGG	TCCTTGGCAT	TTTTCCGGCG	CAACGACCCG	AAAAAGGTAT
	CCGAGGCGGG	GGGACTGCTC	GTAGTGTTTT	TAGCTGCGAG	TTCACTCTCC	ACCGCTTTGG
	GCTGTCTCTGA	TATTTCTATG	GTCCGCAAAG	GGGGACCTTC	GAGGGAGCAC	GCGAGAGGAC
	AAGGCTGGGA	CGCGGAATGG	CCTATGGACA	GGCGGAAAGA	GGGAAGCCCT	TCGCAACGCG
35	AAAGAGTATC	GAGTGCGACA	TCCATAGAGT	CAAGCCACAT	CCAGCAAGCG	AGGTTTCGACC
	CGACACACGT	GCTTGGGGGG	CAAGTCCGGC	TGGCGACGCG	GAATAGGCCA	TTGATAGCAG
	AACTCAGGTT	GGGCCATTCT	GTGCTGAATA	GCGGTGACCG	TCGTCCGTGA	CCATTGTCTT
	AATCGGTCTC	GCTCCATACA	TCCGCCACGA	TGCTCAAGA	ACTTCACCA	CCGATTGATG
	CCGATGTGAT	CTTCTTGTC	TAAACCATAG	ACGCGAGACG	ACTTCGGTCA	ATGGAAGCCT
40	TTTTCTCAAC	CATCGAGAAC	TAGGCCGTTT	GTTTGGTGGC	GACCATCGCC	ACCAAAAAAA
	CAAACGTTTC	TCGTCTAATG	CGCGTCTTTT	TTTCCTAGAG	TTCTTCTAGG	AAACTAGAAA
	AGATGCCCCA	GACTGCGAGT	CACCTTGCTT	TTGAGTGCAA	TTCCCTAATA	CCAGTACTCT
	AATAGTTTTT	CCTAGAAGTG	GATTTAGGAA	AATTTAATTT	TTACTTCAAA	ATTTAGTTAG
	ATTTTCATATA	TACTCATTTC	AACCAGACTG	TCAGATTCTT	TGGTAATAAT	AGTACTGTAA
45	TTGGATATTT	TTATCCGCAT	AGTGCTCCGG	GAAAGCAGAG	CGCGCAAAGC	CCTACTTGCC
	ACTTTTGGAG	ACTGTGTACG	TCGAGGGCCT	CTGCCAGTGT	CGAACCAAGC	TTCGCCTACG
	GCCCTCGTCT	GTTCCGGCAG	TCCCGCGCAG	TCGCCCACAA	CCGCCCACAG	CCCCGACCGA
	ATTGATACGC	CGTAGTCTCG	TCTAACATGA	CTCTCACGTG	GCTAGGGGGG	CCATGGGCTA
	GGTCTGTACT	ATTCTATGTA	ACTACTCAAA	CCTGTTTGGT	GTTGATCTTA	CGTCACTTTT
50	TTTACGAAAT	AAACACTTTA	AACACTACGA	TAACGAAATA	AACATTGGTA	ATATTTCGACG
	TTATTTGTTC	AATTGTTGTT	GTTAACGTAA	GTAAATACAA	AAGTCCAAGT	CCCCCTCCAC
	ACCTTCCAAA	AAATTTTCGT	CATTTTGGAG	ATGTTTACAC	CATACCGACT	AATACTAGTA
	CTTGTCTGAC	ACTCCTGACT	CCCCGGACTT	TACTCGGAAC	CCTGACACTT	AGATTTTATG
	TGTTTGTAA	TC'TTAGTCAT	CAAATTTGTT	AATATGTGAA	TTTTTTAAAT	ATAAATGGAA
55	TCTCGAAATT	TAGAGACATC	CATCAAACAG	GTTAATACAG	TGTGGTGTCT	TCATTCCAAG
	GAAGTGTTTC	TAGGGTTCAG	CTTGGGGTCT	CAGGGCGAGT	CTTCTTGAGC	AGTTCTTCCG
	CTATCTTCCG	CTACGCGACG	CTTAGCCCTC	GCCGCTATGG	CATTTTCGTG	TCCTTCGCCA
	GTCGGGTAAG	CGGCGGTTTC	AGAAGTCGTT	ATAGTGCCCA	TCGGTTGCGA	TACAGACTAA
	TCGCCAGGCG	GTGTGGGTTC	GCCGGTGTCA	GCTACTTAGG	TCTTTTTCGCC	GGTAAAAGGT
60	GGTACTATAA	GCCGTTTCGT	CGTAGCGGTA	CCCAGTGCTG	CTCTAGGAGC	GGCAGCCCGT
	ACGCGCGGAA	CTCGGACCGC	TTGTCAAGCC	GACCGCGCTC	GGGGACTACG	AGAAGCAGGT
	CTAGTAGGAC	TAGCTGTTCT	GGCCGAAGGT	AGGCTCATGC	ACGAGCGAGT	TACGCTACAA
	AGCGAACCAC	CAGCTTACCC	GTCCATCGGC	CTAGTTCGCA	TACGTCGGCG	GCGTAACGTA
	GTCGGTACTA	CCTATGAAAG	AGCCGTCTCT	GTTCACACTC	ACTGTCTCTC	AGGACGGGGC
65	CGTGAAGCGG	GTTATCGTTC	GTCAGGGAAG	GGCGAAGTCA	CTGTTGCGAG	TCGTGTCTGAC
	GCGTTTCCTT	CGGGCAGCAC	CGGTTCGGTG	TATCGGCGCG	ACGGAAGCAG	ACGCTCAAGTA
	AGTCCCGTGG	CCTGTCCAGC	CAGAACTGTT	TTTCTTGGCC	CGCGGGGACG	CGACTGTCGG
	CCTTGTGCGG	CCGTAGTCTC	GTCGGCTAAC	AGACAACACG	GGTCAGTATC	GGCTTATCGG
	AGAGGTGGGT	TCGCCGGCCT	CTTGGACGCA	CGTTAGGTAG	AACAAGTTAG	TACGCTTTTG
70	TAGGAGTAGG	ACAGAGAACT	AGTCTAGGGT	TCGACCCCTA	GACGCTCTTA	GCTATAGTTT
	GAATAGCTAT	TCGAAAAACG	TTTTCGGATC	CGGAGGTTTT	TTTCGGAGGAG	TGATGAAGAC
	CTTATCGAGT	CTCCGGCTCC	GCCGGAGCCG	GAGACGTATT	TATTTTTTTT	AATCAGTCGG
	TACCCCGCCT	CTTACCCGCC	TTGACCCGCC	TCAATCCCCG	CCCTACCCCG	CTCAATCCCC
	GCCCTGATAC	CAACGACTGA	TTAACTCTAC	GTACGAAACG	TATGAAGACG	GACGACCCCT
75	CGGACCCCTG	AAAGGTGTGG	ACCAACGACT	GATTAACCTC	ACGTACGAAA	CGTATGAAGA
	CGGACGACCC	CTCGGACCCC	TGAAAGGTGT	GGGATTGACT	GTGTGTAAGG	TGTCGACCAA
	GAAAGGCGGA	GTCTTCCATG	TGAGAAGGAA	AAAGTTATAA	TAACCTTCGTA	AATAGTCCCA

ATAACAGAGT ACTCGCCTAT GTATAAACTT ACATAAATCT TTTTATTGT TTATCCCCAA
GGCGCGTGTA AAGGGGCTTT TCACGGTGGA CTGCCGCCGG (SEQ ID NO:16)

Transformed recombinant clones were selected with kanamycin, and purified
miniprep plasmid DNA was analyzed by restriction digestion and agarose gel electrophoresis
analysis. Putative plasmid clones containing an appropriate insert were verified by
automated dideoxy sequencing followed by alignment analysis using Vector NTI v7.0
sequence analysis software (Invitrogen Corp, Carlsbad, CA).

Example 2: Cell Line Construction

Verified plasmid clones were used to transfect NTC-200 cells to obtain stable
polyclonal cell lines. Briefly, 200-300K cells, plated 18 hours previously, were transfected
with 3.0 ug of plasmid DNA using 6.0 ul of Fugene 6 transfection reagent (Roche Applied
Science, Indianapolis IN) according to the manufacturer's recommendations. Transfections
were performed in 3.0 ml of DMEM/F12 with 10% FBS, Endothelial SFM or Optimem
media (Invitrogen Corp, Carlsbad, CA). Twenty four to 48 hours later cells were either fed
with fresh media containing 1.0 ug/ul of G418 or passaged to a T-25 tissue culture flask
containing G418. Cell lines were passaged under selection for 14-21 days until normal
growth resumed, after which time drug was removed and cells were allowed to recover (~ 1
week) prior to characterization.

Expression stability of the recombinant protein from these cell lines was measure over
the course of several weeks using the Human sVEGF R1/Flt1 Quantikine ELISA Kit (R&D
Systems, Minneapolis, MN). Briefly, 50K cells, previously plated into 12 well tissue culture
plates in DMEM/F12 with 10% FBS, were washed twice in HBSS (Invitrogen Corp,
Carlsbad, CA) then pulsed for 2 hours with 1.0 ml of Endothelial SFM (Invitrogen Corp,
Carlsbad, CA). Pulse media was stored at -20 C and assayed within one week of collection
as per the manufacturer's protocol.

Stable cell lines secreting the following truncated VEGF receptors were successfully
created:

- 1) sVR1 D1-3
- 2) sVR1 D2-3
- 3) sVR1 D1-4
- 4) sVR1 D2-4
- 5) sVR D1-3 R1/R2 chimera dimer

6) sVR D2-3 R1/R2 chimera dimer

7) sVR2 D2-3 dimer

Example 3: Protein Characterization

5 Saturation binding studies of VEGF using conditioned media from transfected cell lines were performed. Briefly, titrated amounts of conditioned media were incubated overnight at room temperature in the presence of VEGF. Free VEGF was measured using a sensitive EIA (R&D Systems, Minneapolis, MN) and non-linear regression analysis was performed (GraphPad Software Inc., San Diego, CA). The results of these studies are
10 presented in Figures 1A-1C. Figures 1A and 1B depict typical VEGF binding curve for the various VR truncation forms disclosed herein. Figure 1C presents the dissociation constant, K_d , of the receptor fragments relative to control (VR1 D1-3 Fc). These data demonstrate that the recombinant VEGF receptor proteins secreted from these cell lines bind VEGF with high affinity. As a negative control, it was shown that conditioned media from mock transfected
15 parental cells (WT) did not bind VEGF.

 The bioactivity of conditioned media from transfected cells was measured using a human umbilical vein endothelial cell (HUVEC) growth inhibition assay. These data are summarized in Figures 2A-2C. The results of these assays demonstrate that the recombinant protein from these cell lines can efficiently inhibit endothelial cell proliferation similar to
20 control protein (VR1 D1-3-Fc). Again, conditioned media from mock transfected parental cells (WT) did not inhibit HUVEC growth.

EQUIVALENTS

The details of one or more embodiments of the invention are set forth in the accompanying description above. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. Other features, objects, and advantages of the invention will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms include plural referents unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All patents and publications cited in this specification are incorporated by reference.

The foregoing description has been presented only for the purposes of illustration and is not intended to limit the invention to the precise form disclosed, but by the claims appended hereto.

CLAIMS

We claim:

1. An isolated nucleic acid molecule encoding a polypeptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14.
2. An isolated nucleic acid molecule encoding a Vascular Endothelial Growth Factor (VEGF) receptor wherein said nucleic acid comprises a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, and SEQ ID NO:13.
3. An isolated polypeptide encoded by the nucleic acid of claim 1 or claim 2.
4. The isolated polypeptide of claim 3, wherein the polypeptide is a soluble VEGF receptor.
5. The isolated polypeptide of claim 4, wherein the soluble VEGF receptor preferentially binds to VEGF.
6. The isolated polypeptide of claim 5, wherein binding of the soluble VEGF receptor to VEGF inhibits endothelial cell proliferation and vascular permeability.
7. A vector comprising the nucleic acid molecule of claim 1 or claim 2.
8. A host cell comprising the vector of claim 7 or the nucleic acid molecule of claim 1 or claim 2.
9. The host cell of claim 8, wherein the host cell is an ARPE-19 cell.

10. A cell line comprising the vector of claim 7 or the isolated nucleic acid molecule of claim 1 or claim 2.
11. A cell line comprising an ARPE-19 cell genetically engineered to produce a soluble VEGF receptor, wherein the soluble VEGF receptor comprises, or optionally consists of, a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, and SEQ ID NO:13.
12. A cell line comprising an ARPE-19 cell genetically engineered to produce a soluble VEGF receptor comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14.
13. An implantable cell culture device, the device comprising:
- a) a core comprising one or more ARPE-19 cells that are genetically engineered to produce the polypeptide of any one of claims 4 to 6; and
 - b) a semipermeable membrane surrounding the core, wherein the membrane permits the diffusion of the soluble VEGF receptor there through.
14. The device of claim 13, wherein the core further comprises a matrix disposed within the semipermeable membrane.
15. The device of claim 14, wherein the matrix comprises a hydrogel or extracellular matrix components.
16. The device of claim 15, wherein the hydrogel comprises alginate cross-linked with a multivalent ion.
17. The device of claim 14, wherein the matrix comprises a plurality of monofilaments, wherein said monofilaments are
- a) twisted into a yarn or woven into a mesh or
 - b) twisted into a yarn that is in non-woven strands,
- and wherein the cells or tissue are distributed thereon.

18. The device of claim 17, wherein the filamentous cell-supporting matrix comprises a biocompatible material selected from the group consisting of acrylic, polyester, polyethylene, polypropylene polyacetonitrile, polyethylene terephthalate, nylon, polyamides, polyurethanes, polybutester, silk, cotton, chitin, carbon, and biocompatible metals.
19. The device of any one of claims 13 to 18, wherein the device further comprises a tether anchor.
20. The device of claim 19, wherein the tether anchor comprises an anchor loop.
21. The device of claim 20, wherein the anchor loop is adapted for anchoring the capsule to an ocular structure.
22. The device of claim 21, wherein the capsule is implanted into the eye.
23. The device of claim 22, wherein the capsule is implanted in the vitreous, the aqueous humor, the Subtenon's space, the periorbital space, the posterior chamber, or the anterior chamber of the eye.
24. The device of any one of claims 13 to 23, wherein the jacket comprises a permselective, immunoisulatory membrane.
25. The device of any one of claims 13 to 24, wherein the jacket comprises an ultrafiltration membrane or a microfiltration membrane.
26. The device of any one of claims 13 to 23, wherein the jacket comprises a non-porous membrane material.
27. The device of claim 26, wherein the non-porous membrane material is a hydrogel or a polyurethane.

28. The device of any one of claims 13 to 27, wherein the capsule is configured as a hollow fiber or a flat sheet.
29. The device of any one of claims 13 to 28, wherein at least one additional biologically active molecule is co-delivered from the device.
30. The device of claim 29, wherein the at least one additional biologically active molecule is from a non-cellular source.
31. The device of claim 29, wherein the at least one additional biologically active molecule is from a cellular source.
32. The device of claim 31, wherein the at least one additional biologically active molecule is produced by one or more genetically engineered ARPE-19 cell in the core.
33. A method for treating ophthalmic disorders, comprising implanting the implantable cell culture device of any one of claims 13 to 32 into the eye of a patient and allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye, thereby treating the ophthalmic disorder.
34. The method of claim 33, wherein the ophthalmic disorder is selected from the group consisting of retinopathy of prematurity, diabetic macular edema, diabetic retinopathy, age-related macular degeneration, glaucoma, retinitis pigmentosa, cataract formation, retinoblastoma and retinal ischemia.
35. The method of claim 34, wherein age-related macular degeneration is wet form age-related macular degeneration.
36. The method of claim 34, wherein the ophthalmic disorder is diabetic retinopathy.
37. A method for inhibiting endothelial cell proliferation, comprising implanting the implantable cell culture device of any one of claims 13 to 32 into a patient suffering from a cell proliferation disorder and allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF, wherein the binding inhibits endothelial cell proliferation in the patient.

38. The method of claim 37, wherein the disorder is selected from the group consisting of hematologic disorders, atherosclerosis, inflammation, increased vascular permeability and malignancy.

39. A method of delivering a soluble VEGF receptor to a recipient host, comprising implanting the implantable cell culture device of any one of claims 13 to 32 into a target region of the recipient host, wherein the encapsulated one or more ARPE-19 cells secrete the soluble VEGF receptor at the target region.

40. The method of claim 39, wherein the target region is selected from the group consisting of the central nervous system, including the brain, ventricle, spinal cord, and the aqueous and vitreous humors of the eye.

41. A method for making the implantable cell culture device of any one of claims 13 to 32, comprising

a) genetically engineering at least one ARPE-19 cell to secrete a soluble VEGF receptor encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, and SEQ ID NO: 13 and

b) encapsulating said genetically modified ARPE-19 cells within a semipermeable membrane, wherein said membrane allows the diffusion of the soluble VEGF receptor therethrough.

42. A method for making the implantable cell culture device of any one of claims 13 to 32, comprising

a) genetically engineering at least one ARPE-19 cell to secrete a soluble VEGF receptor comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14, and

b) encapsulating said genetically modified ARPE-19 cells within a semipermeable membrane, wherein said membrane allows the diffusion of the soluble VEGF

receptor therethrough.

43. Use of one or more ARPE-19 cells that are genetically engineered to produce the polypeptide of any one of claims 4 to 6 in the manufacture of an implantable cell culture device according to any one of claims 13 to 32 for treating ophthalmic disorders by implantation of the device into the eye of a patient and by allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye.

44. An implantable cell culture device according to any one of claims 13 to 32 for treating ophthalmic disorders by implantation of the device into the eye of a patient and by allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye.

45. One or more ARPE-19 cells that are genetically engineered to produce the polypeptide of any one of claims 4 to 6 for treating ophthalmic disorders by implantation of an implantable cell culture device according to any one of claims 13 to 32 into the eye of a patient and by allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye.

46. Use of an isolated nucleic acid molecule according to claim 1 or 2 in the manufacture of one or more ARPE-19 cells that are genetically engineered to produce the polypeptide of any one of claims 4 to 6 for treating ophthalmic disorders by implantation into the eye of a patient an implantable cell culture device according to any one of claims 13 to 32 and by allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye.

47. An isolated nucleic acid molecule according to claim 1 or 2 for treating ophthalmic disorders, said treating comprising implanting into the eye of a patient an implantable cell culture device according to any one of claims 13 to 32 and allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye, and wherein said one or more ARPE-19 cells in said device have been genetically engineered with said isolated nucleic acid molecule to thereby produce the polypeptide of any one of claims 4 to 6.

48. The use of claim 43 or 46, an implantable cell device according to claim 44, the one or more ARPE-19 cells according to claim 45 or the isolated nucleic acid molecule according

to claim 47, wherein the ophthalmic disorder is selected from the group consisting of retinopathy of prematurity, diabetic macular edema, diabetic retinopathy, age-related macular degeneration (e.g. wet form age-related macular degeneration), glaucoma, retinitis pigmentosa, cataract formation, retinoblastoma and retinal ischemia.

49. Use of one or more ARPE-19 cells that are genetically engineered to produce the polypeptide of any one of claims 4 to 6 in the manufacture of an implantable cell culture device according to any one of claims 13 to 32 for inhibiting endothelial cell proliferation by implantation of the device into the eye of a patient suffering from a cell proliferation disorder and by allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye and to thereby inhibit endothelial cell proliferation in said patient.

50. An implantable cell culture device according to any one of claims 13 to 32 for inhibiting endothelial cell proliferation by implantation of the device into the eye of a patient suffering from a cell proliferation disorder and by allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye and to thereby inhibit endothelial cell proliferation in said patient.

51. One or more ARPE-19 cells that are genetically engineered to produce the polypeptide of any one of claims 4 to 6 for inhibiting endothelial cell proliferation by implantation of an implantable cell culture device according to any one of claims 13 to 32 into the eye of a patient suffering from a cell proliferation disorder and by allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye and to thereby inhibit endothelial cell proliferation in said patient.

52. Use of an isolated nucleic acid molecule according to claim 1 or 2 in the manufacture of one or more ARPE-19 cells that are genetically engineered to produce the polypeptide of any one of claims 4 to 6 for inhibiting endothelial cell proliferation by implantation into the eye of a patient suffering from a cell proliferation disorder an implantable cell culture device according to any one of claims 13 to 32 and by allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye and to thereby inhibit endothelial cell proliferation in said patient.

53. An isolated nucleic acid molecule according to claim 1 or 2 for inhibiting endothelial cell proliferation, said treating comprising implanting into the eye of a patient suffering from a cell proliferation disorder an implantable cell culture device according to any one of claims 13 to 32 and allowing the soluble VEGF receptor to diffuse from the device and bind to VEGF in the eye and to thereby inhibit endothelial cell proliferation in said patient, and wherein said one or more ARPE-19 cells in said device have been genetically engineered with said isolated nucleic acid molecule to thereby produce the polypeptide of any one of claims 4 to 6.

54. The use of claim 49 or 52, an implantable cell device according to claim 50, the one or more ARPE-19 cells according to claim 51 or the isolated nucleic acid molecule according to claim 53, wherein the disorder is selected from the group consisting of hematologic disorders, atherosclerosis, inflammation, increased vascular permeability and malignancy.

55. Use of one or more ARPE-19 cells that are genetically engineered to produce the polypeptide of any one of claims 4 to 6 in the manufacture of an implantable cell culture device according to any one of claims 13 to 32 for delivering a soluble VEGF receptor to a recipient host by implantation of the device into a target region of the recipient host and wherein the encapsulated one or more ARPE-19 cells secrete the soluble VEGF receptor at the target region.

56. An implantable cell culture device according to any one of claims 13 to 32 for delivering a soluble VEGF receptor to a recipient host by implantation of the device into a target region of the recipient host and wherein the encapsulated one or more ARPE-19 cells secrete the soluble VEGF receptor at the target region.

57. One or more ARPE-19 cells that are genetically engineered to produce the polypeptide of any one of claims 4 to 6 for delivering a soluble VEGF receptor to a recipient host by implantation of an implantable cell culture device according to any one of claims 13 to 32 into a target region of the recipient host and wherein the encapsulated one or more ARPE-19 cells secrete the soluble VEGF receptor at the target region.

58. Use of an isolated nucleic acid molecule according to claim 1 or 2 in the manufacture of one or more ARPE-19 cells that are genetically engineered to produce the polypeptide of any one of claims 4 to 6 for delivering a soluble VEGF receptor to a recipient host by implantation into a target region of the recipient host an implantable cell culture device according to any one of claims 13 to 32 and wherein the encapsulated one or more ARPE-19 cells secrete the soluble VEGF receptor at the target region.

59. An isolated nucleic acid molecule according to claim 1 or 2 for delivering a soluble VEGF receptor to a recipient host, said delivering comprising implanting into a target region of the recipient host an implantable cell culture device according to any one of claims 13 to 32, wherein the encapsulated one or more ARPE-19 cells secrete the soluble VEGF receptor at the target region, and wherein said one or more ARPE-19 cells in said device have been genetically engineered with said isolated nucleic acid molecule to thereby produce the polypeptide of any one of claims 4 to 6.

60. The use of claim 55 or 58, an implantable cell device according to claim 56, the one or more ARPE-19 cells according to claim 57, or the isolated nucleic acid molecule according to claim 59, wherein the target region is selected from the group consisting of the central nervous system, including the brain, ventricle, spinal cord, and the aqueous and vitreous humors of the eye.

61. A method of producing an isolated polypeptide, the method comprising expressing the isolated nucleic acid molecule of claim 1 or claim 2 and harvesting the expressed polypeptide.

62. The method of claim 33, wherein between 0.1 pg and 1000 µg per eye per patient per day of the soluble VEGF receptor diffuse into the eye.

63. The method of claim 38, wherein between 0.1 pg and 1000 µg per patient per day of the soluble VEGF receptor diffuses from the device.

64. The method of claim 42, wherein between 0.1 pg and 1000 µg per patient per day of the soluble VEGF receptor diffuse into the target region.

FIGURE 1

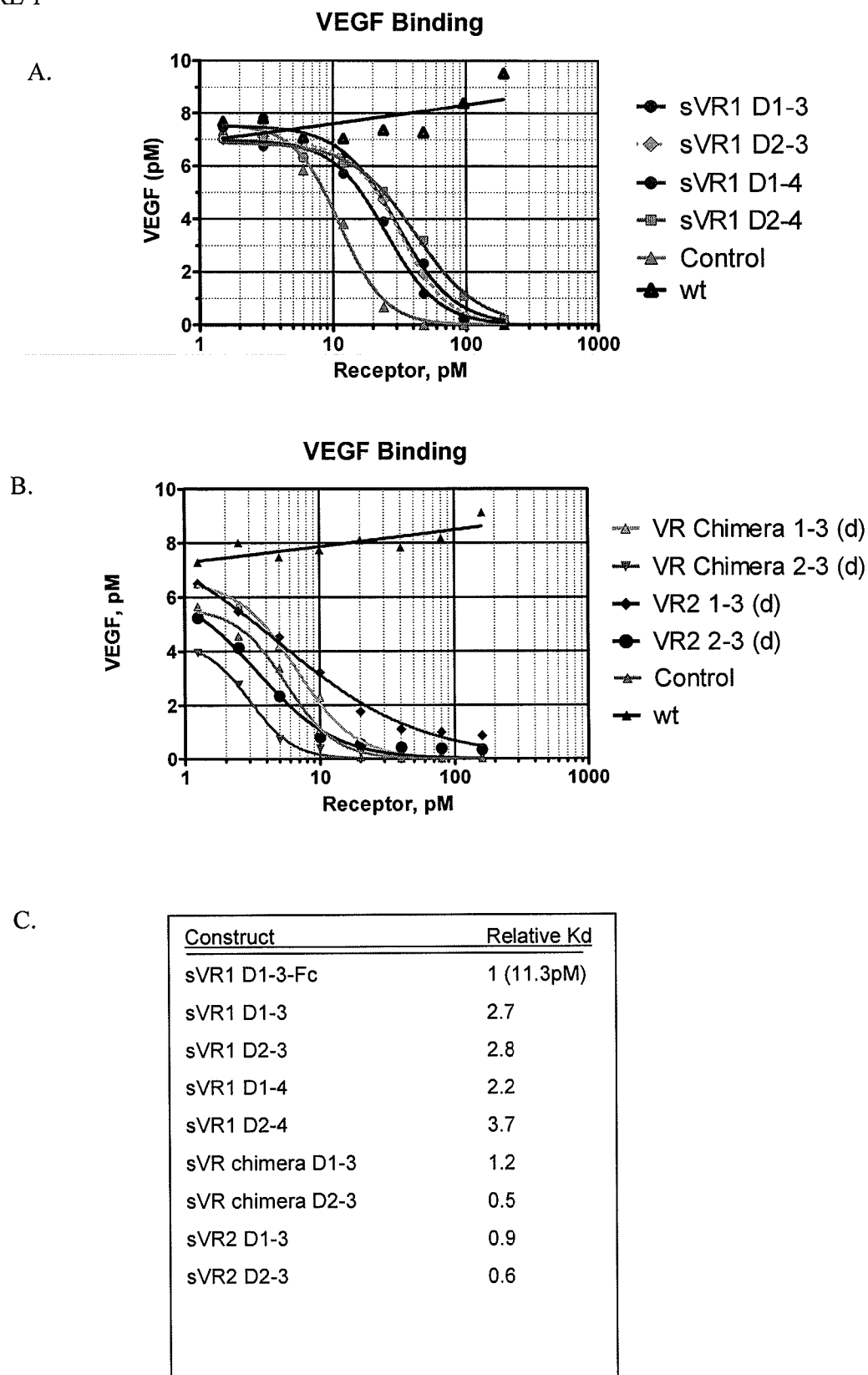
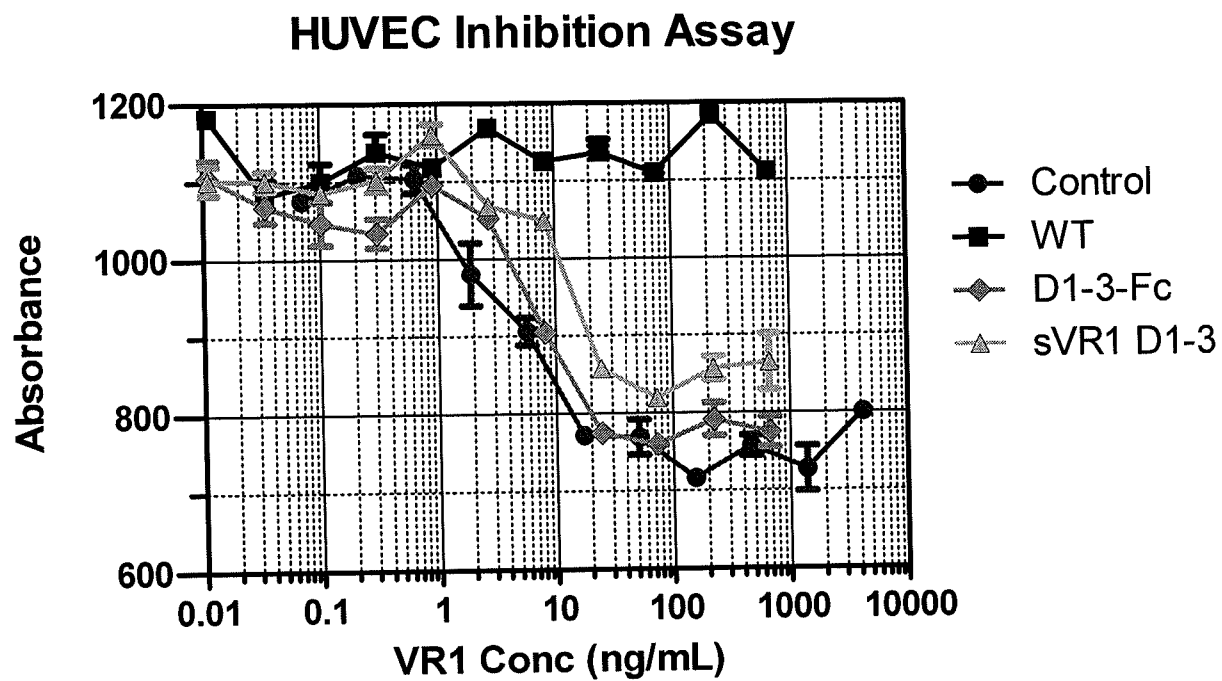


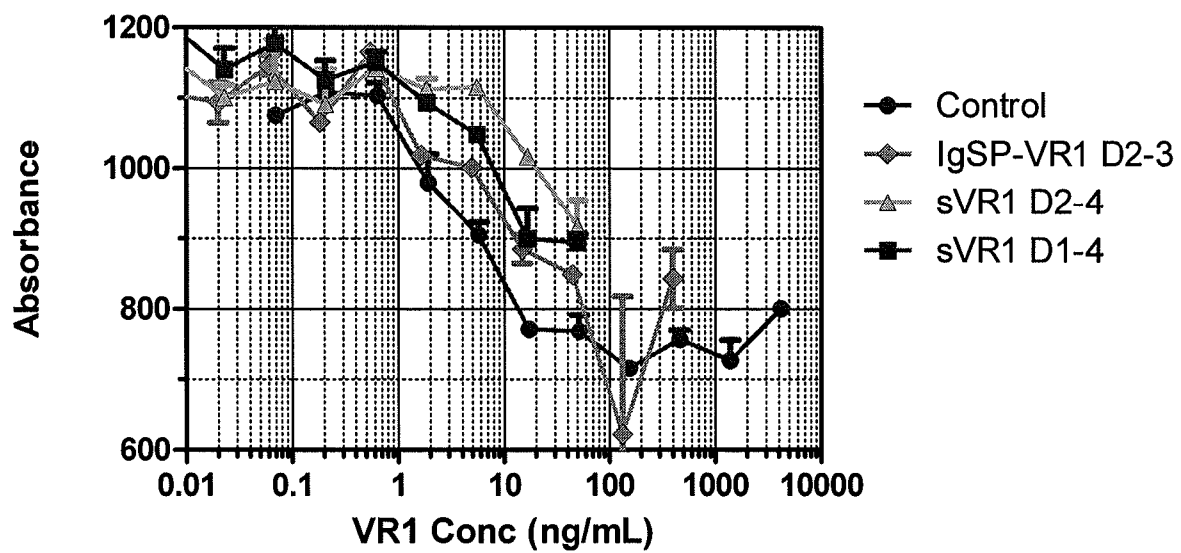
FIGURE 2

A.



B.

HUVEC Inhibition Assay



C.

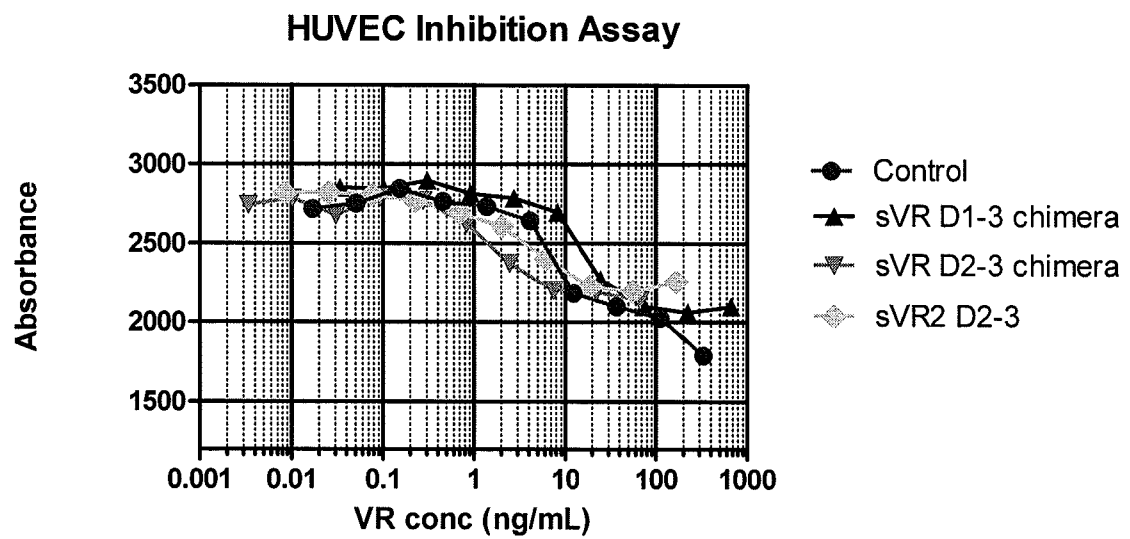
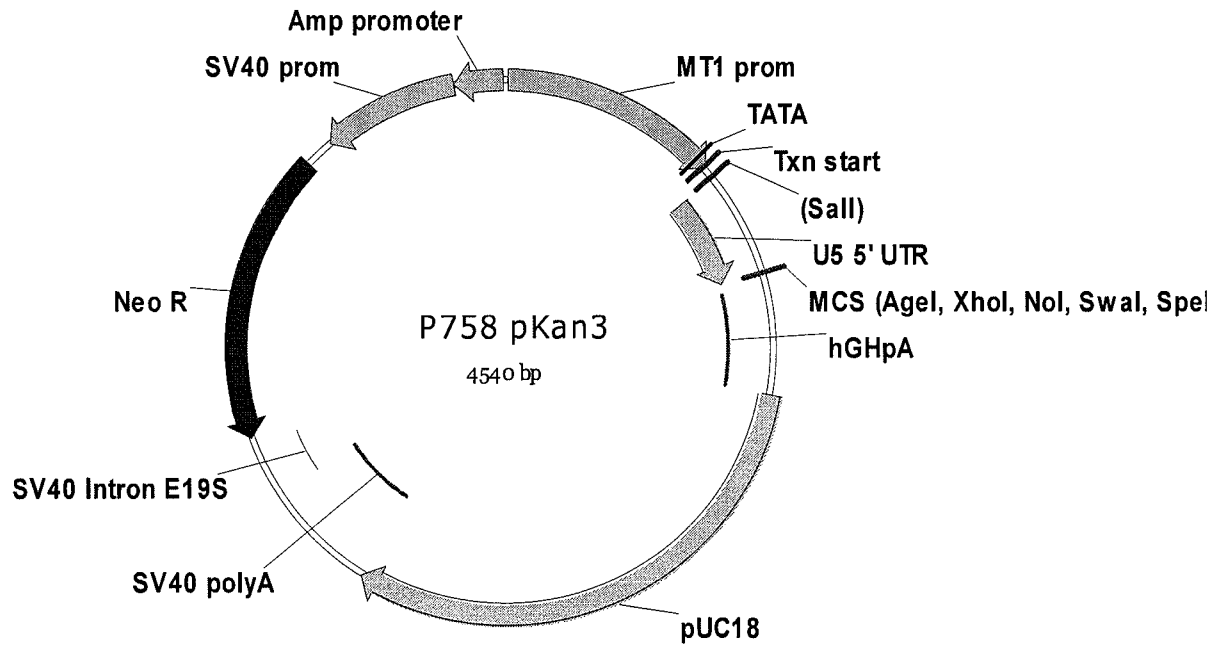


FIGURE 3



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