(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 June 2005 (16.06.2005)

(10) International Publication Number
WO 2005/054436 A2

(51) International Patent Classification:
C12N

(21) International Application Number:
PCT/US2004/039857

(22) International Filing Date:
24 November 2004 (24.11.2004)

(25) Filing Language:
English

(26) Publication Language:
English

(30) Priority Data:
60/525,903 26 November 2003 (26.11.2003) US

(71) Applicant (for all designated States except US):

(72) Inventors:

(75) Inventors/Applicants (for US only):


(54) Title: COMPOSITIONS AND METHODS FOR EX VIVO PRESERVATION OF BLOOD VESSELS FOR VASCULAR GRAFTS USING INHIBITORS OF TYPE III AND/OR TYPE IV PHOSPHODIESTERASES

(57) Abstract: The present invention relates to ex vivo methods for preserving/maintaining blood vessels that are to be used as vascular grafts by specifically inhibiting Type III and/or Type IV phosphodiesterase. The present invention also relates to compositions comprising a specific inhibitor of a Type III and/or Type IV phosphodiesterase for use in the methods of the invention.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BL, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published: without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.
COMPOSITIONS AND METHODS FOR EX VIVO PRESERVATION 
OF BLOOD VESSELS FOR VASCULAR GRAFTS USING 
INHIBITORS OF TYPE III AND/OR TYPE IV PHOSPHODIESTERASES

This application claims benefit under 35 U.S.C. § 119(e) of United States 
Provisional Application Serial No. 60/525,903 filed November 26, 2003, which is 
incorporated herein by reference in its entirety.

1. FIELD OF THE INVENTION

The present invention provides methods for the specific inhibition of Type III and/or 
Type IV phosphodiesterases in blood vessels, such as arteries or veins, as a means for 
preservation/maintenance of the blood vessel for use as a vascular graft prior to attachment 
to the recipient blood vessel in vascular surgical procedures. The present invention also 
provides compositions comprising a specific inhibitor of Type III and/or Type IV 
phosphodiesterases for use in ex vivo preservation/maintenance of blood vessels useful as 
vascular grafts.

2. BACKGROUND OF THE INVENTION

2.1 Organ Preservation Solutions

Adequate preservation of organs intended for transplantation is critical to the proper 
functioning of the organ following implantation. Long preservation times are desired to 
enable cross-matching of donor and recipient to improve subsequent survival, as well as to 
allow for coast to coast and international transportation of organs to expand the donor and 
recipient pools. Organ preservation requires preservation of both the structure and function 
of the organ, including the specialized cells of the organ as well as the blood vessels and 
other cells found in the organ that together are responsible for its function.

Many different organ preservation solutions have been designed, as investigators 
have sought to lengthen the time that an organ may remain extra-corporeally, as well as to 
maximize function of the organ following implantation. Several of the key solutions that 
have been used over the years include: 1) the Stanford University solution [see, e.g., 
Swanson et al., 1988, Journal of Heart Transplantation, 7(6):456-467]; 2) a modified 
Collins solution [see, e.g., Maurer et al., 1990, Transplantation Proceedings, 22(2):548-550; 
Swanson et al., supra]; and 3) the University of Wisconsin solution (U.S. Patent No. 
4,798,824, issued to Belzer et al.). Further, U.S. Patent Nos. 5,552,267 and 5,370,989 to
Stern et al. and a publication by Kayano et al., 1999, *J. Thoracic Cardiovascular Surg.* 118:135-144 describe an organ preservation solution known as the Columbia University solution.

In addition to the composition of the organ preservation or maintenance solution, the method of organ preservation also affects the success of preservation. Several methods of cardiac preservation have been studied in numerous publications: 1) warm arrest/cold ischemia; 2) cold arrest/macroperfusion; 3) cold arrest/microperfusion; and 4) cold arrest/cold ischemia. The first method involves arresting the heart with a warm cardioplegic solution prior to explantation and cold preservation, but this method fails because of the rapid depletion of myocardial energy store during the warm period. The second method, which involves arresting the heart with a cold preservation solution, is better; but continuous perfusion of the heart with preservation solution during the storage period fails because of the generation of toxic oxygen radicals. In addition, the procedure of the second method is cumbersome and does not lend itself to easy clinical use. The third method, called "trickle perfusion", is better but also cumbersome. The fourth method of preservation is that of a cold cardioplegic arrest followed by a period of cold immersion of the heart. The fourth method is currently the standard method of cardiac preservation. This fourth method reliably preserves hearts for periods of up to six hours, but less than four hours is considered ideal for this method.

2.2 Coronary Artery Bypass Graft (CABG)

Coronary artery disease is a major medical problem in throughout the world. Coronary arteries, as well as other blood vessels, frequently become clogged with plaque, impairing the efficiency of the heart's pumping action, and inhibiting blood flow which can lead to heart attack and death. In certain instances, these arteries can be unblocked through relatively noninvasive techniques such as balloon angioplasty. In other cases, a bypass of the blocked vessel is necessary.

A coronary artery bypass graft ("CABG") involves performing an anastomosis on a diseased coronary artery to reestablish blood flow to an ischemic portion of the heart muscle. Improved long-term survival has been demonstrated bypassing the left anterior descending artery with a left internal mammary artery and this encouraged surgeons to extend revascularization with arterial grafts to all coronary arteries.

Since the internal mammary artery can only be used for two CABG procedures (using right and left internal mammary arteries, respectively), where multiple-vessels need
to be bypassed, other arteries or veins have to be used. Such other arteries or veins that have been used include the right gastroepiploic artery, the inferior epigastric artery, the internal mammary artery (also known as the internal thoracic artery), the radial artery, and the saphenous vein. The internal mammary artery is the most common arterial conduit used for CABG; yet, despite its widespread use and superior patency when compared to the saphenous vein (Grondin et al., 1984, Circulation, 70 (suppl I): I-208-212; Camerson et al., 1996, N Engl J Med, 334: 216-219), the saphenous vein continues to be one of the most popular conduits for CABG (Roubos et al., 1995, Circulation, 92 (9 Suppl) II31-6).

During a typical coronary artery bypass graft procedure using the saphenous vein, a section of the saphenous vein is surgically removed from the leg and the graft is retained ex vivo (out of the body) for a length of time prior to attachment to another blood vessel within the body (Angelini and Jeremy, 2002, Bioengineering, 39 (3-4): 491-499). In a bypass operation involving such a venous graft, the graft is harvested by a surgically invasive procedure from the leg of the patient and then stored for up to about four hours ex vivo as the heart surgery is conducted. Although there are variations in methodology in surgical preparation of the heart, the first part of the procedure typically requires an incision through the patient's sternum (sternotomy), and in one technique, the patient is then placed on a bypass pump so that the heart can be operated on while not beating. In alternative techniques, the heart is not stopped during the procedure. Having harvested and stored the saphenous vein or arterial blood vessel conduit and upon completion of the surgery to prepare the heart for grafting, the bypass procedure is performed. A precise surgical procedure is required to attach the bypass graft to the coronary artery (anastomosis), with the graft being inserted between the aorta and the coronary artery. The inserted venous/arterial segments/transplants act as a bypass of the blocked portion of the coronary artery and thus provide for a free or unobstructed flow of blood to the heart. More than 500,000 bypass procedures are performed in the United States every year.

The overall short and long term success of the CABG procedure is dependent on several factors including the condition of the graft that is to be inserted which itself depends on any form of damage during the removal of the graft from the body or deterioration or damage of the graft due to storage conditions. In such circumstances, the short term detrimental effect can be potentially lethal thrombotic disease as a result of contact of flowing blood with a changed phenotype of the graft due to its deterioration or damage during the removal or storage stage. Possible long term detrimental effects include the vein graft itself becoming diseased, stenosed, or occluded, similar to the original bypassed
vessel. In this case, the diseased or occluded saphenous vein grafts are associated with acute ischemic syndromes necessitating some form of intervention. It is, therefore, of critical importance not only that care be taken in the surgical procedure to remove the blood vessel to be used as the graft in surgical bypass procedures including CABG, but, also that no deterioration or damage occurs in the storage period of the graft prior to attachment to another blood vessel and the resumption of blood flow in that vessel.

2.3 Phosphodiesterases

Phosphodiesterases are a class of intracellular enzymes involved in the metabolism of the second messenger nucleotides cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Numerous phosphodiesterase inhibitors have been described in the literature for a variety of therapeutic uses, including treatment of obstructive lung disease, allergies, hypertension, angina, congestive heart failure and depression. The phosphodiesterases have been classified into eleven major families, Types I-XI, based on amino acid or DNA sequences. For a general review of phosphodiesterases and their classification, see Maurice et al., 2003, Molecular Pharmacology 64:533-546. The members of the family vary in their tissue, cellular and subcellular distribution, as well as their links to cAMP and cGMP pathways. For example, phosphodiesterases stimulated by calcium/calmodulin are classified as Type I, and phosphodiesterases stimulated by cGMP are classified as Type II. Type III phosphodiesterases, of which there are known to be two genes, are cAMP-specific and cGMP inhibitable, and Type IV, of which there are known to be 4 genes, are high affinity, high-specificity cAMP-specific. Type V phosphodiesterases are cGMP-specific, and Type VI phosphodiesterases are photoreceptor cGMP-specific.

In order to decrease the likeliness of short and long term detrimental consequences of grafting blood vessels in surgical procedures, including coronary arterial bypass grafting (CABG), and consequently to improve the overall outcome of patients undergoing such procedures, there is a need for improved storing conditions for vascular grafts during the time period from harvesting of the graft to attaching the graft to another blood vessel in the patient.

Citation or identification of any reference in Section 2 or in any other section of this application shall not be construed as an admission that such reference is available as prior art to the present invention.
3. SUMMARY OF THE INVENTION

The present invention is directed to a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase for preserving a blood vessel or a functional portion thereof, removed from an individual. The blood vessel or portion thereof can be used as a vascular graft in the same or different individual. It is believed that the use of a specific Type III and/or Type IV phosphodiesterase inhibitor enhances the viability of the blood vessel and will result in less damage or phenotypic change of the blood vessel as a result of storage conditions. Thus, it is believed that blood vessels so treated will improve short and long term outcomes of vascular bypass procedures involving blood vessel grafts, including, but not limited to, coronary artery bypass, abdominal aneurysm repair, carotid endarterectomy, deep vein occlusion, or popliteal aneurysm repair.

Accordingly, the present invention provides a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase in a solution comprising heparinized blood. Alternatively, the present invention provides a solution consisting of a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase in heparinized blood. The present invention also provides a method of preserving a blood vessel comprising contacting an isolated blood vessel or portion thereof \textit{ex vivo} with a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase in a solution comprising heparinized blood. The present invention also provides a method of preserving a blood vessel or a functional portion thereof comprising contacting an isolated blood vessel or portion thereof \textit{ex vivo} with a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase, at a temperature in the range of about 0.5°C to about 10°C.

As used herein, a specific inhibitor of a specific type phosphodiesterase is an inhibitor that, at at least one concentration, inhibits the activity of the phosphodiesterase of the specific type by at least 50% but does not inhibit the activity of a phosphodiesterase of another type by more than 25%. In a specific embodiment, the inhibitor is a specific inhibitor of Type III phosphodiesterase, which, at least one concentration, inhibits at least 50% of Type III phosphodiesterase activity but does not inhibit phosphodiesterase activity of another type, \textit{e.g.}, Type I, II, IV, V, VI, VII, VIII, IX, X or XI, by more than 25%.
Preferably, the inhibitor does not inhibit Type V phosphodiesterase activity by more than 25%. More preferably, the inhibitor does not inhibit each of Type I, II and V phosphodiesterase activity by more than 25%. Most preferably, the inhibitor does not inhibit each of Type I, II, IV, V, VI, VII, VIII, IX, X and XI phosphodiesterase activity by more than 25%.

In another specific embodiment, the inhibitor is a specific inhibitor of Type IV phosphodiesterase, which, at at least one concentration, inhibits at least 50% of Type IV phosphodiesterase activity but does not inhibit phosphodiesterase activity of another type, e.g., Type I, II, III, V, VI, VII, VIII, IX, X or XI, by more than 25%. Preferably, the inhibitor does not inhibit Type V phosphodiesterase activity by more than 25%. More preferably, the inhibitor does not inhibit each of Type I, II and V phosphodiesterase activity by more than 25%. Most preferably, the inhibitor does not inhibit each of Type I, II, III, V, VI, VII, VIII, IX, X and XI phosphodiesterase activity by more than 25%. In yet another specific embodiment, the inhibitor is a specific inhibitor of Type III and Type IV phosphodiesterases, which, at at least one concentration, inhibits at least 50% of Type III and Type IV phosphodiesterase activity but does not inhibit phosphodiesterase activity of another type, e.g., Type I, II, V, VI, VII, VIII, IX, X or XI, by more than 25%. Preferably, the inhibitor does not inhibit Type V phosphodiesterase activity by more than 25%. More preferably, the inhibitor does not inhibit each of Type I, II and V phosphodiesterase activity by more than 25%. Most preferably, the inhibitor does not inhibit each of Type I, II, V, VI, VII, VIII, IX, X and XI phosphodiesterase activity by more than 25%.

In other embodiments of the present invention, an isolated *ex vivo* blood vessel or functional portion thereof in contact with a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase, at a temperature in the range of about 0.5°C to about 10°C is provided. The present invention also provides an isolated *ex vivo* isolated blood vessel or functional portion thereof in contact with a solution comprising heparinized blood and a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase. As used herein, a functional portion of a blood vessel is a portion of a blood vessel of sufficient size as to be able to act as a vascular graft.

The present invention also provides a container containing a blood vessel or functional portion thereof in contact with a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase, at a temperature in the range of about 0.5°C to about 10°C. Alternatively, the container contains the blood vessel or portion
thereof in contact with a solution comprising heparinized blood and a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase.

Further, the present invention provides a method of using a blood vessel as a vascular graft comprising contacting an isolated blood vessel or functional portion thereof \textit{ex vivo} with a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase, and inserting the blood vessel into a patient so as to form a vascular graft in the patient. The present invention also provides a method for performing a coronary artery bypass graft in a patient comprising removing from contact with a blood vessel or functional portion thereof a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase; and grafting the blood vessel or functional portion thereof into the patient so as to serve as a coronary bypass graft.

4. \textbf{DETAILED DESCRIPTION OF THE INVENTION}

It is believed that the use of a specific Type III and/or Type IV phosphodiesterase inhibitor enhances the viability of a blood vessel and results in less damage or phenotypic change of the blood vessel as a result of storage conditions. Thus, blood vessels so treated should improve short and long term outcomes of vascular bypass procedures involving blood vessel grafts, including coronary artery bypass grafts, abdominal aneurysm repair, carotid endarterectomy, deep vein occlusion, and popliteal aneurysm repair. Exemplary blood vessels that can be so isolated include, but are not limited to, a saphenous vein, a mammary artery, a renal artery, and a radial artery.

4.1 Preservation Solutions

The present invention is directed to use of a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase. In specific embodiments, the solution can contain more than one specific inhibitor of Type III and/or Type IV phosphodiesterase. The solution can be an aqueous solution or a semi-solid gel. In a preferred embodiment, the solution is an aqueous solution. Any physiologic solution to which the inhibitors are added can be used in the present invention so long as the solution does not damage the blood vessel graft tissue that is placed in it. For example, the solution comprising the inhibitor can be saline, buffered saline, phosphate buffered saline. The solution can also be Hank’s Balanced Salt solution (HBSS), which typically comprises 1.26 mM CaCl$_2$, 5.36 mM KCl, 0.44 mM KH$_2$PO$_4$, 0.81 mM MgSO$_4$, 136 mM NaCl, 0.42 mM Na$_2$HPO$_4$, 6.1 mM glucose, 20 mM HEPES-NaOH, at pH 7.4 (Herreros et al., 2000, \textit{J.}
Neurochem 74(5):1941-1950) or modified HBSS, which typically comprises 143 mM NaCl, 5.6 mM KCl, 2 mM MgCl₂, 10 mM HEPES, 10 mM glucose, 0.2 mM CaCl₂, and 0.4% BSA, at pH 7.2 (Briddon et al., 1999, Blood 93:38 47-3855). The solution can also be Ringer’s Lactate, which typically comprises 155 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 2 mM NaH₂PO₄, 10 mM HEPES, and 10 mM glucose, at pH 7.2 (Sturgill-Koszycki and Swanson, 2000, J Exp. Med. 192:1261-1272). The solution can also be Tyrodes buffer, which typically comprises 137 mM NaCl, 12 mM NaHCO₃, 26 mM KCl, 5.5 mM glucose, 0.1% BSA, and 5.0 mM Hepes at pH 7.35 (Kasirer-Friede et al., 2002, J. Biol. Chem., 277:11949-11956). The solution can also be Kreb’s buffer, which typically comprises 119 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.17 MgSO₄, 25 mM NaHCO₃, 1.18 mM KH₂PO₄, 0.026 mM EDTA, and 5.5 mM glucose (Knock et al., 2002, J. Physiology 538:879-890).

The solutions of the present invention can also contain a variety of additional additives, such as vasodilators, as detailed infra. In a preferred embodiment, the solution comprises heparinized blood and the specific inhibitor of Type III and/or Type IV phosphodiesterase activity. Alternatively, the solution consists of a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase in heparinized blood. In a preferred embodiment, the solution is sterilized.

In an embodiment of the present invention, the preservation solution further comprises a vasodilator in an amount sufficient to maintain vascular homeostasis.

Preferably, the vasodilator is cell membrane permeable. The vasodilator can be selected from the group including, but not limited to, of adenosine 3',5'-cyclic monophosphate (cyclic adenosine monophosphate, cyclic-AMP, or cAMP), analogues of adenosine 3',5'-cyclic monophosphate (cyclic adenosine monophosphate, cyclic-AMP, or cAMP), guanosine 3',5'-cyclic monophosphate (cyclic guanosine monophosphate, cyclic-GMP, or cGMP), analogues of guanosine 3',5'-cyclic monophosphate (cyclic guanosine monophosphate, cyclic-GMP, or cGMP), nitroglycerin, adenosine, and pertussis toxin. Suitable combinations of the vasodilators may be used. The analogues of adenosine 3',5'-cyclic monophosphate can be selected from the group including, but not limited to, dibutyryl adenosine 3',5'-cyclic monophosphate (dB cAMP), and 8-bromo-adenosine 3',5'-cyclic monophosphate. The analogues of guanosine 3',5'-cyclic monophosphate can be selected from the group including, but not limited to, dibutyryl guanosine 3',5'-cyclic monophosphate (dB cGMP), 8-(4-chlorophenylthio)-guanosine 3',5'-cyclic monophosphate (8-CPT cGMP) and 8-bromo-guanosine 3',5'-cyclic monophosphate (8-bromo cGMP). Other suitable analogues of cAMP and/or cGMP may also be used. Preferably, the analog
is cell membrane-permeable. The optimal concentration of db cAMP is believed to be about 2 mM, although in specific embodiments, db cAMP concentrations of about 1 mM, or of about 2 to 4 mM can be used. It is known that db cAMP concentrations higher than about 4 mM become toxic to endothelial cells. Hence, 2 mM is considered to be the optimal concentration of db cAMP. In a preferred embodiment, the concentration of dibutyryl adenosine 3',5'-cyclic monophosphate (db cAMP) ranges from about 1 mM to about 4 mM. The term "about" as used herein is intended to cover the range of experimental variation.

A preferred solution of the invention contains a vasodilator, wherein the vasodilator is dibutyryl adenosine 3',5'-cyclic monophosphate (db cAMP), alone or in combination with nitroglycerin and adenosine. In another preferred solution, the vasodilator is 8-bromo-adenosine 3',5'-cyclic monophosphate (8-bromo-cAMP), alone or in combination with nitroglycerin and adenosine. In a preferred embodiment, the concentration of nitroglycerin ranges from about 0.05 g/l to about 0.2 g/l. In another preferred embodiment, the concentration of adenosine ranges from about 3 mM to about 20 mM.

In certain embodiments, the solutions of the present invention also comprise a sugar, for example, D-glucose, e.g., in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics. In a preferred embodiment, the concentration of the sugar ranges from about 50 mM to about 80 mM. The solutions can also comprise magnesium ions, e.g., in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics. In a preferred embodiment, the concentration of magnesium ions ranges from about 2 mM to about 10 mM. In particular, the magnesium ions can be derived from magnesium sulfate, magnesium gluconate, or magnesium phosphate, or suitable combinations thereof. The magnesium ions can also be derived from some other suitable magnesium containing compound. D-Glucose, adenosine, and magnesium ions are substrates for adenosine triphosphate (ATP) synthesis. Metabolic substrates such as D-glucose and perhaps adenosine for ATP formation are probably important for maintaining the small degree of anaerobic metabolism that occurs during ex vivo preservation of blood vessels. Basal energy metabolism (even during hypothermia) can be supported by the anaerobic metabolism of D-glucose. The presence of magnesium ions allows for the proper functioning of the enzymes needed for adenosine triphosphate (ATP) synthesis. In general, substrates for ATP synthesis are helpful to allow intracellular function and maintenance of cellular bioenergetics.

In other embodiments, the preservation solution also comprises a macromolecule of molecular weight greater than 20,000 daltons, e.g., in an amount sufficient to maintain
endothelial integrity and cellular viability. In a preferred embodiment, the macromolecule of molecular weight greater than 20,000 daltons is a macromolecule having a molecular weight greater than about 100,000 daltons, a polysaccharide, or a polyethylene glycol. Other suitable macromolecules can be used. The macromolecule of molecular weight greater than 20,000 daltons can be a colloid. In a preferred embodiment, the polysaccharide is a dextran. Furthermore, in a preferred combination, the dextran is a dextran molecule having a molecular weight of 308,000 daltons. Macromolecules of molecular weight greater than 20,000 daltons are believed to be helpful in reducing trans-endothelial leakage and subsequent intracellular and interstitial edema in the reperfusion period, by serving to plug small endothelial leaks which may occur. Macromolecules may thus also prevent the extravasation of intravascular contents into the pericellular space, thus helping to prevent cellular swelling and rupture during the preservation and recovery periods.

The osmolarity of the preservation solution of the invention is also a factor in helping to prevent cellular swelling and rupture. The osmolarity of the solution should be greater than the cellular osmolarity. Cellular osmolarity is about 290 mOSm/l. In a preferred embodiment, the osmolarity ranges from about 315 mOSm/l to about 340 mOSm/l.

The preservation solution also optionally comprises potassium ions, preferably in a concentration greater than about 110 mM. The potassium ions can be derived from potassium sulfate, potassium gluconate, monopotassium phosphate (KH₂PO₄), or suitable combinations thereof. The potassium ions can also be derived from some other suitable potassium containing compound. In a preferred embodiment, the concentration of potassium ions ranges from about 110 mM to about 140 mM.

In other embodiments, the preservation solution also comprises a buffer in an amount sufficient to maintain the average pH of the solution during the period of blood vessel preservation at about the physiologic pH value. In a preferred embodiment, the buffer is monopotassium phosphate (KH₂PO₄). However, other suitable buffers may be used. The buffering capacity should be adequate to buffer the organic acids that accrue during ischemia. Because basal metabolism results in the generation of acid, preferably a buffering system is used. The pH of the solution can decline during prolonged storage times that can be employed with this solution. In a preferred embodiment, the initial pH of the preservation solution is adjusted to the alkaline side of normal physiologic pH because then the average pH during storage of the blood vessel in the preservation solution remains physiologic. Normal physiologic pH is about 7.4. A preferred embodiment of the
preservation solution has a pH range of about 7.3 to about 7.6. The pH may be adjusted to
the desired value with the addition of a suitable base, such as potassium hydroxide (KOH).
Hence, during the period of preservation, the pH of the preservation solution starts on the
alkaline side of physiologic pH, and may drift slowly down to the acidic side of physiologic
pH. But the average pH of the preservation solution during the period of preservation is
preferably about the physiologic value.

In other embodiments, the preservation solution may further comprise impermeant
anions, e.g., in an amount sufficient to help maintain endothelial integrity and cellular
viability. The impermeant anion can be the gluconate anion or the lactobionate anion.

Other suitable impermeant anions can be used. In a preferred embodiment, the
concentration of the gluconate anion ranges from about 85 mM to about 105 mM. The
gluconate anion can be derived from potassium gluconate or magnesium gluconate. The
gluconate anion can also be derived from some other suitable gluconate containing
compound. Impermeant anions are large anions that cannot cross cell membranes, so that
sodium is at least in part prevented from diffusing down its concentration gradient into the
cell during the preservation period. Impermeant anions thus help to prevent cellular edema.

The preservation solution may further comprise an anticoagulant, e.g., in an amount
sufficient to help prevent clotting of blood within the capillary bed of the blood vessel. The
anticoagulant can be heparin or hirudin. Other suitable anticoagulants may be used. In a
preferred embodiment, the concentration of heparin ranges from about 1000 units/l to about
100,000 units/l. Anticoagulants are believed to help in preventing clotting of blood within
the capillary bed of the preserved blood vessel. Specifically, anticoagulants are believed to
help prevent a total no-reflow phenomenon at the level of the microcirculation, which
would be undesirable following re-implantation and could result in graft failure.

Anticoagulants are believed to be helpful in ensuring that thrombosis does not occur during
or after preservation, so that nutrient delivery and toxin removal can proceed.

In yet another embodiment, the preservation solution may further comprise an
antioxidant, e.g., in an amount sufficient to help decrease reperfusion injury secondary to
oxygen free radicals. The antioxidant can be butylated hydroxyanisole (BHA), butylated
hydroxytoluene (BHT), Vitamin C, Vitamin E, or suitable combinations thereof. Other
suitable antioxidants can be used. In a preferred embodiment, the antioxidant is butylated
hydroxyanisole (BHA) at a concentration range from about 25 µM to about 100 µM, alone
or in combination with butylated hydroxytoluene (BHT) at a concentration range from
about 25 µM to about 100 µM. The preservation solution can further comprise a reducing
agent, e.g., in an amount sufficient to help decrease reperfusion injury secondary to oxygen free radicals. Any suitable reducing agent can be used.

Optionally, the preservation solution may further comprise N-acetylcysteine in an amount sufficient to help cells produce glutathione. In a preferred embodiment, the concentration of N-acetylcysteine ranges from about 0.1 mM to about 5 mM. N-acetylcysteine is an agent which can enter cells and is believed to play a role in helping cells to produce glutathione, which is a reducing agent. During blood vessel preservation, glutathione is lost. Simply adding glutathione to the preservation solution, however, would likely be of little to no help, because it is now known that glutathione in solution does not enter easily into the cell.

In another optional embodiment, the preservation solution may further comprise an agent that helps prevent calcium entry into cells in an amount sufficient to help prevent calcium entry into cells. Agents that help prevent calcium entry into cells include so-called calcium channel blockers, as well as other agents that serve the described function. An agent that helps prevent calcium entry into cells that can be used is verapamil. Other suitable agents that help prevent calcium entry into cells may be used. In a preferred embodiment, the concentration of verapamil ranges from about 2 μM to about 25 μM. Agents that help prevent calcium entry into cells are believed to play a role in preventing calcium overload.

Optionally, the solution does not contain sodium. The absence of sodium in the solution is preferred, since any sodium which may enter the cells during the period of preservation (when energy currency is low and the normal trans-cellular gradient may not be well maintained) may 1) lead to cellular swelling, 2) cause calcium entry by facilitated diffusion (following re-implantation), and 3) sodium load the cell, such that a high amount of energy is required following reestablishment of blood flow before a normal membrane potential can be re-established.

In other embodiments, the preservation solution can further comprise a bacteriostat, in an amount sufficient to help inhibit the growth of, or destroy, bacteria. The bacteriostat can be cefazolin or penicillin. Other suitable bacteriostats or antibiotics can be used. In a specific embodiment, the concentration of cefazolin ranges from about 0.25 g/l to about 1 g/l. The addition of an antibiotic to the solution is a surgical consideration, due in one embodiment to the practical inability of sterilizing the solution completely, as the high molecular weight solutes would not pass through a 0.2 micron membrane filter which may
be used in the preparation of the preservation solution. It is believed that gamma irradiation may be used to better sterilize the solution.

In other embodiments, the solution can be any organ preservation solution known in the art in combination with the specific Type III and/or Type IV phosphodiesterase inhibitor(s). Illustrative examples of organ preservation solutions include, but are not limited to, the Euro-Collins solution, the University of Wisconsin solution, the low-potassium dextran glucose solution (Perfadex™), the Celsior™ solution, and the Columbia University solution. In general, these solutions contain electrolytes and, optionally, sugars. One illustrative solution comprises a sugar in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics, e.g., glucose, D-glucose; magnesium ions in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics; potassium ions; and a buffer, e.g., a monopotassium phosphate or bicarbonate buffer, in an amount sufficient to maintain the average pH of the solution at about the physiologic pH value, i.e., about pH 7.3 to about pH 7.6. The specific composition of certain known solutions is listed below.

The Euro-Collins solution is described in Maurer et al., 1990, Transplantation Proceedings 22:548-550 and in Swanson et al., 1988, J. Heart Transplantation 7(6):456-467, and typically comprises 115 mM potassium, 10 mM sodium, 8 mM magnesium, 10 mM bicarbonate, 100 mM phosphate, and 120 mM glucose, at a pH of about 7.4 and with an osmolality of about 452 mOsm/L.

The University of Wisconsin solution is described in U.S. Patent No. 4,798,824 to Belzer et al., and typically comprises 125 mM potassium, 30 mM sodium, 5 mM magnesium, 25 mM phosphate, 5 mM sulfate, 100 mM lactobionate, 50 mM hydroxyethyl starch, 5 mM adenosine and 1 mM allopurinol, at a pH of about 7.4 and with an osmolality of about 327 mOsm/L.

The low-potassium dextran glucose solution (Perfadex™, commercially available from VitroLife, Gothenberg, Sweden), and typically comprises 4 mM potassium, 165 mM sodium, 2 mM magnesium, 101 mM chloride, 34 mM phosphate, 2 mM sulfate, 20 mM Dextran-40, and 56 mM glucose, at a pH of about 7.4 and with an osmolality of about 335 mOsm/L.

The Celsior™ solution is commercially available from Sangstat Medical Corporation, Freemont, CA, and typically comprises 60 mM mannitol, 80 mM lactobionic acid, 20 mM glutamic acid, 30 mM histidine, 0.25 mM calcium, 15 mM potassium, 13 mM
magnesium, 100 mM sodium hydroxide, and 3 mM reduced glutathione, at a pH of about 7.3 and with an osmolality of about 320-360 mOsm/L.

The Columbia University solution is described in U.S. Patent Nos. 5,370,989 and 5,552,267 to Stern et al., and typically comprises 120 mM potassium, 5 mM magnesium, 25 mM phosphate, 5 mM sulfate, 95 mM gluconate, 50 mM Dextran 50, 67 mM glucose, 5 mM adenosine, 2 mM dibutyryl adenosine 3',5'-cyclic monophosphate (dBCAMP), 0.1 mg/ml nitroglycerin, 50 μM butylated hydroxyanisole, 50 μM butylated hydroxytoluene, 0.5 mM N-acetylcysteine, 10 U/ml heparin, and 10 μM verapamil, at a pH of about 7.6 and with an osmolality of about 325 mOsm/L.

In a specific embodiment, a preservation solution of the present invention comprises, or alternatively consists of, a specific inhibitor of a Type III and/or Type IV phosphodiesterase; a vasodilator in an amount sufficient to maintain vascular homeostasis, wherein the vasodilator is selected from the group consisting of: adenosine 3',5'-cyclic monophosphate analogues, guanosine 3',5'-cyclic monophosphate analogues, nitroglycerin, and pertussis toxin; a sugar in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics; magnesium ions in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics; a macromolecule of molecular weight greater than 20,000 daltons in an amount sufficient to maintain endothelial integrity and cellular viability; potassium ions in a concentration greater than about 110 mM; and a buffer in an amount sufficient to maintain the average pH of the blood vessel or portion thereof during said contacting step at about the physiologic pH value.

In another specific embodiment, a preservation solution of the present invention comprises, or alternatively consists of, a specific inhibitor of a Type III and/or Type IV phosphodiesterase; 67.4 mM D-glucose; 5 mM magnesium sulfate (MgSO₄); 25 mM monopotassium phosphate (KH₂PO₄); 50 g/l dextran (molecular weight 308,000 daltons); 95 mM potassium gluconate (K-gluconate); 50 μM butylated hydroxyanisole (BHA); 50 μM butylated hydroxytoluene (BHT); 0.5 mM N-acetylcysteine; 5 mM adenosine; 0.1 g/l nitroglycerin; 10 μM verapamil; 2 mM dibutyryl adenosine 3',5'-cyclic monophosphate (dBCAMP); 10,000 units heparin; and 0.5 g/l cefazolin. The pH is adjusted to 7.6 with potassium hydroxide.

The amount of the preservation solution required in a surgical procedure, such as a cardiac arterial bypass graft (CABG) would be clear to one who is skilled in such surgical procedures, and depends, inter alia, upon the particular container used to hold the blood vessel and solution.
The preservation solution is suitable for use at the low temperatures that may be required or desired during vascular bypass, e.g., CABG, or other surgical procedure. For instance, temperatures of about 0.5 to about 10 degrees Centigrade, preferably 4°C, may be used during CABG or other surgical procedure.

The present invention is also directed to a container containing a preservation solution of the present invention. In a preferred embodiment, the container is one of certain dimensions useful in preserving a blood vessel. In another embodiment, the container also contains the blood vessel or functional portion thereof, intended for use as a vascular graft, in contact with the solution.

4.2 Phosphodiesterase Inhibitors

The preservation solutions of the present invention comprise a specific inhibitor of a Type III and/or Type IV phosphodiesterase. As used herein, a specific inhibitor of a type phosphodiesterase is an inhibitor that, at least one concentration of the inhibitor, inhibits the activity of the phosphodiesterase of the specific type by at least 50% but does not inhibit the activity of a phosphodiesterase of another type by more than 25%. In certain embodiments, the specific inhibitor, at least one concentration, inhibits the activity of the phosphodiesterase of the certain type by at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% but does not inhibit the activity of a phosphodiesterase of another type by more than 20%, 15%, 10%, 5%, 2%, or 1%. Any method known in the art can be used to measure the activity of the phosphodiesterases. Illustrative methods for measuring phosphodiesterase activity are described in Huraux et al., 1998, Anesthesiology 88:1654-1659; Tsuru et al., 1990, Eur. J. Pharmacol. 191:447-455; and Salmenpera and Levy, 1996, Anesth. Analg. 82:954-957.

In a specific embodiment, the inhibitor is present in the solution in a concentration range of about 0.05 μM to about 250 μM, preferably in a range of about 0.1 μM to about 100 μM. In an embodiment, the concentration range refers to the concentration of a single inhibitor in the solution. For example, in one embodiment wherein the solution comprises a specific inhibitor of Type III phosphodiesterase and a specific inhibitor of Type IV phosphodiesterase, the concentration of the Type III inhibitor and the Type IV inhibitor is each about 250 μM in the solution. The optimal concentration of the inhibitor in the solution to achieve specific inhibition can be determined by standard techniques. It is noted that specific inhibitors of Type III or Type IV phosphodiesterase may, at high concentrations, be less specific for their particular type. For example, milrinone, a specific
phosphodiesterase Type III inhibitor, at high concentrations, also inhibits Type IV phosphodiesterase and Type V phosphodiesterase activity. See, Lugnier and Komas, 1993, *European Heart J* 14 (Suppl. I):141-148. However, as long as there is a concentration of milrinone at which Type III phosphodiesterase is inhibited by at least 50%, but other phosphodiesterases are not inhibited by more than 25% (which there are), milrinone meets the definition of a specific Type III phosphodiesterase inhibitor according to the present invention.

In a specific embodiment, the inhibitor is a specific inhibitor of Type III phosphodiesterase, which, at at least one concentration, inhibits at least 50% of Type III phosphodiesterase activity but does not inhibit phosphodiesterase activity of another type, e.g., Type I, II, IV, V, VI, VII, VIII, IX, X or XI, by more than 25%. Preferably, the inhibitor does not inhibit Type V phosphodiesterase activity by more than 25%. More preferably, the inhibitor does not inhibit each of Type I, II and V phosphodiesterase activity by more than 25%. Most preferably, the inhibitor does not inhibit each of Type I, II, IV, V, VI, VII, VIII, IX, X and XI phosphodiesterase activity by more than 25%. In certain aspects of this embodiment, the specific inhibitor of Type III phosphodiesterase inhibits the activity of Type III phosphodiesterase by at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% but does not inhibit the activity of Type V phosphodiesterase by more than 20%, 15%, 10%, 5%, 2%, or 1%. In other aspects of this embodiment, the specific inhibitor of Type III phosphodiesterase inhibits the activity of Type III phosphodiesterase by at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% but does not inhibit the activity of each of Type I, II and V phosphodiesterase by more than 20%, 15%, 10%, 5%, 2%, or 1%.

Specific inhibitors of Type III phosphodiesterase activity are known in the art, see, e.g., Salmenpera and Levy, 1996, *Anesth. Analg.* 82:954-957; Hirose et al., 1998, *Japan J. Pharmacol.* 124:229-37; Dent et al., 1998, *Pulm. Pharmacol. Ther.* 11:47-56; and Maurice et al., 2003, *Molecular Pharmacology* 64:533-546. Illustrative examples of specific inhibitors of Type III phosphodiesterase activity include, but are not limited to, Indolidan (LY 195115), Cilostamide (OPC 3689), Lixazinone (RS 82856), Y-590, Imazodan (CI 914), SKF 94120, Quazinone, ICI 153,110, Cilostazol (OPC 13013), Bemorand (RWJ 22867), Siguazodan (SK&F 94836), Adibendan (BM 14,478), Milrinone (WIN 47203), Enoximone (MDL 17043), Pimobendan (UD-CG 115, MCI-154), Saterinone (BDF 8634), Amirinone, Olprinone, NSP-153, Zardaverine, quinzazolines, benzafentrine, Sulmazole (ARL 115), ORG 9935, and a mixture of any two or more of the foregoing.
In a specific embodiment, the inhibitor is a specific inhibitor of Type IV phosphodiesterase, which, at at least one concentration, inhibits at least 50% of Type IV phosphodiesterase activity but does not inhibit phosphodiesterase activity of another type, e.g., Type I, II, III, V, VI, VII, VIII, IX, X or XI, by more than 25%. Preferably, the inhibitor does not inhibit Type V phosphodiesterase activity by more than 25%. More preferably, the inhibitor does not inhibit each of Type I, II and V phosphodiesterase activity by more than 25%. Most preferably, the inhibitor does not inhibit each of Type I, II, III, V, VI, VII, VIII, IX, X and XI phosphodiesterase activity by more than 25%. In certain aspects of this embodiment, the specific inhibitor of Type IV phosphodiesterase inhibits the activity of Type IV phosphodiesterase by at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% but does not inhibit the activity of Type V phosphodiesterase (and/or any other type) by more than 20%, 15%, 10%, 5%, 2%, or 1%. In other aspects of this embodiment, the specific inhibitor of Type IV phosphodiesterase inhibits the activity of Type IV phosphodiesterase by at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% but does not inhibit the activity of each of Type I, II and V phosphodiesterase by more than 20%, 15%, 10%, 5%, 2%, or 1%.

Mesopram, Atizoram, Zadaverine, quinazolines, benzaflentrine, Filamilast, C1-1018, C1-1044, D-4418, IC-485, L-791,943, CD-998, YM-976, YM-58997, V-11294A, CDC-801, CC-7075, R-P-73401, and a mixture of any two or more of the foregoing.

In yet another specific embodiment, the inhibitor is a specific inhibitor of Type III and Type IV phosphodiesterases, which, at at least one concentration, inhibits at least 50% of Type III and Type IV phosphodiesterase activity but does not inhibit phosphodiesterase activity of another type, e.g., Type I, II, V, VI, VII, VIII, IX, X or XI, by more than 25%. Preferably, the inhibitor does not inhibit Type V phosphodiesterase activity by more than 25%. More preferably, the inhibitor does not inhibit each of Type I, II and V phosphodiesterase activity by more than 25%. Most preferably, the inhibitor does not inhibit each of Type I, II, V, VI, VII, VIII, IX, X and XI phosphodiesterase activity by more than 25%. In certain aspects of this embodiment, the specific inhibitor of Type III and Type IV phosphodiesterase inhibits the activity of Type III and Type IV phosphodiesterase by at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% but does not inhibit the activity of Type V phosphodiesterase by more than 20%, 15%, 10%, 5%, 2%, or 1%. In other aspects of this embodiment, the specific inhibitor of Type III and Type IV phosphodiesterase inhibits the activity of Type III and Type IV phosphodiesterase by at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% but does not inhibit the activity of each of Type I, II and V phosphodiesterase by more than 20%, 15%, 10%, 5%, 2%, or 1%.

Specific inhibitors of Type III and Type IV phosphodiesterase activity are known in the art, see, e.g., Charpiot et al., 1998, Bioorg. Med. Chem. Lett. 8:2891-2896 and Banner et al., 1996, Br. J. Pharmacol. 119:1255-1261. Illustrative examples of a specific inhibitor of Type III and Type IV phosphodiesterase activity include, but are not limited to, Zadaverine, commercially available from Sigma Co. (St. Louis, MO), quinazolines, benzaflentrine, and a mixture of any two or more of the foregoing.

4.3 Methods of Preservation and Use of Preserved Blood Vessels

The invention also provides a method of preserving or maintaining a blood vessel comprising contacting the blood vessel with a solution of the present invention comprising a specific inhibitor of Type III and/or Type IV phosphodiesterase. The contacting comprises immersing, infusing, flushing, or perfusing. Other suitable procedures of contacting can be used. The method can be used wherein the blood vessel is intended for transplantation for a vascular bypass procedure, e.g., abdominal aneurysm repair, carotid endarterectomy, deep
vein occlusion, popliteal aneurysm repair, or for a coronary arterial bypass (CABG). Hence, the preservation solution may be used to preserve a blood vessel or functional portion thereof prior to use in such vascular transplantation procedures.

Any known blood vessel or a functional portion thereof can be preserved *ex vivo* in solution of the invention, preferably prior to use as a vascular graft. The blood vessel can be an artery or a vein. Exemplary blood vessels include, but are not limited to, the internal mammary artery (also known as the internal thoracic artery), the renal artery, the radial artery, the right gastroepiploic artery, the inferior epigastric artery and the saphenous vein, or a functional portion thereof. Preferably, the blood vessel is a saphenous vein or functional portion thereof. For example, where the blood vessel graft is for a coronary arterial bypass, the blood vessel can be the internal mammary artery (also known as the internal thoracic artery), the radial artery, the right gastroepiploic artery, the inferior epigastric artery and the saphenous vein, or a functional portion of the artery or vein. Preferably, the blood vessel for use as a graft is the saphenous vein or a functional portion thereof. In another example, where the graft is for abdominal aneurysm repair, carotid endarterectomy, deep vein occlusion, or popliteal aneurysm repair, the blood vessel is the renal artery or functional portion thereof, or the saphenous vein or a functional portion thereof. Preferably, the graft is isolated from the saphenous vein or a functional portion thereof.

As used herein, a “functional” portion of a blood vessel refers to a portion that is able to act as a vascular graft. The blood vessel can be isolated from and used as a vascular graft in, *e.g.*, any mammal including primates, pigs, dogs, cats. Preferably, the blood vessel is isolated from a human, *e.g.*, human child (less than 18 years old), or human adult (18 years or older). Preferably, the blood vessel is isolated from the patient in which it is subsequently used as a vascular graft.

One embodiment of the invention is directed to a method of preserving a blood vessel comprising contacting an isolated blood vessel or portion thereof *ex vivo* with a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase in a solution comprising heparinized blood. Another embodiment is directed to a method of preserving a blood vessel comprising contacting an isolated blood vessel or portion thereof *ex vivo* with a solution consisting of a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase in heparinized blood. In a preferred embodiment, the method of preserving a blood vessel comprising contacting an isolated blood vessel or portion thereof *ex vivo* with a solution comprising a specific inhibitor of
Type III phosphodiesterase and/or Type IV phosphodiesterase, wherein the solution is at a
temperature in the range of about 0.5°C to about 10°C, preferably at 4°C, during the
contacting step. In another preferred embodiment, the contacting is for a time period of not
longer than 4 hours, preferably not longer than 2 or 3 hours.

Optionally, prior to use of the blood vessel or functional portion thereof as a
vascular graft, the method further comprises a step of removing the solution from contact
with the blood vessel or portion thereof. Preferably, the removing of the solution comprises
flushing, immersing, infusing, or perfusing the blood vessel or portion thereof with a second
solution that lacks the specific phosphodiesterase inhibitor. Preferably, the second solution
is appropriate for maintaining cardiovascular homeostasis \textit{in vivo}, \textit{e.g.}, the solution lacks potassium. An exemplary solution is saline or Ringer's Lactate.

In another embodiment, the present invention is directed to an isolated \textit{ex vivo} blood
vessel or functional portion thereof in contact with a solution comprising a specific inhibitor
of Type III phosphodiesterase and/or Type IV phosphodiesterase, at a temperature in the
range of about 0.5°C to about 10°C, preferably 4°C. The present invention is also directed
to an isolated \textit{ex vivo} isolated blood vessel or functional portion thereof in contact with a
solution comprising, or alternatively consisting of, (a) heparinized blood and (b) a specific
inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase. Preferably, the
contacting is at a temperature in the range of about 0.5°C to about 10°C, and more
preferably at 4°C. Any known blood vessel or a functional portion thereof can be preserved
\textit{ex vivo} in solution of the invention, preferably prior to use as a vascular graft. Exemplary
blood vessels include, but are not limited to, the internal mammary artery (also known as
the internal thoracic artery), the radial artery, the right gastroepiploic artery, the inferior
epigastic artery and the saphenous vein. As used herein, a "functional" portion of a blood
vessel refers to a portion that is able to act as a vascular graft. The blood vessel can be
isolated from, \textit{e.g.}, any mammal including primates, pigs, dogs, cats. Preferably, the blood
vessel is isolated from a human, \textit{e.g.}, human child (less than 18 years old), or human adult
(18 years or older).

In another embodiment, the invention is directed to a container containing a solution
of the invention comprising the specific inhibitor of Type III and/or Type IV
phosphodiesterase and the blood vessel or functional portion thereof. Preferably, the blood
vessel or portion thereof is a human blood vessel or portion thereof.

In yet another embodiment, the present invention is directed to a method of using a
blood vessel as a vascular graft comprising contacting an isolated blood vessel or functional
portion thereof \textit{ex vivo} with a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase, and; inserting the blood vessel into a patient so as to form a vascular graft in the patient. In one embodiment, the solution further comprises heparinized blood. Preferably, the temperature of the solution ranges from about 0.5°C to about 10°C, and more preferably is about 4°C. Preferably, the contacting is for a time period not longer than four hours. The method can further comprise a step before inserting the vessel of removing the solution from contact with the blood vessel or portion thereof; wherein the removing step comprises flushing, immersing, infusing, or perfusing the blood vessel or portion thereof with a second solution that lacks the specific phosphodiesterase inhibitor. Preferably, the second solution is appropriate for maintaining cardiovascular homeostasis \textit{in vivo}, \textit{e.g.}, the solution lacks potassium. An exemplary solution is saline or Ringer’s Lactate.

Any known blood vessel or a functional portion thereof can be preserved \textit{ex vivo} in a solution of the invention, preferably prior to use as a vascular graft. Exemplary blood vessels include, but are not limited to, the internal mammary artery (also known as the internal thoracic artery), the renal artery, the radial artery, the right gastroepiploic artery, the inferior epigastric artery and the saphenous vein. Preferably, the blood vessel or functional portion thereof is a saphenous vein or functional portion thereof. As used herein, a “functional” portion of a blood vessel refers to a portion that is able to act as a vascular graft. The blood vessel can be isolated from, \textit{e.g.}, any mammal including primates, pigs, dogs, cats. Preferably, the blood vessel is isolated from a human, \textit{e.g.}, human child (less than 18 years old), or human adult (18 years or older). Further, the blood vessel or portion thereof is isolated from the same patient receiving the graft, \textit{i.e.}, the graft is autologous.

In a particular embodiment, the specific inhibitor is a specific inhibitor of Type III phosphodiesterase, \textit{e.g.}, Indolidan (LY 195115), Cilostamide (OPC 3689), Lixazinone (RS 82856), Y-590, Imazodan (CI 914), SKF 94120, Quazinone, ICI 153,110, Cilostazol (OPC 13013), Bemorandam (RWJ 22867), Sigauzodan (SK&F 94836), Adibendan (BM 14,478), Milrinone (WIN 47203), Enoximone (MDL 17043), Pimobendan (UD-CG 115, MCI-154), Saterinone (BDF 8634), Amirinone, Olprinone, NSP-153, Zardaverine, quinazolines, benzafentrine, Sulmazole (ARL 115), ORG 9935, and a mixture of any two or more of the foregoing. In another particular embodiment, the specific inhibitor is a specific inhibitor of Type IV phosphodiesterase, \textit{e.g.}, Rolipram (ZK 62711; Pyrrolidone), RO 20-1724 (Imidazoilidinone), SQ 64442 (Etazoiate), Denbufylline (BRL 30892), V11294, EMD 95832/3, SCH351591, KF19514, Thieno [3, 2-d] pyrimidines, RP73401, Cipamfylline,
Cilomilast, YM-10335, CDP840, SBZ07499 (ariflo), T-440, CP-80,633, 7-
methoxybenzofuran-4-carboxamides, Piclamilast, Roffumilast, Arofylline, BAY-19-8004,
Mesopram, Atizoram, Zardaverine, quinazolines, benzaftentrine, Filamilast, C1-1018, C1-
1044, D-4418, IC-485, L-791,943, CD-998, YM-976, YM-58997, V-11294A, CDC-801,
CC-7075, R-P-73401, and a mixture of any two or more of the foregoing.

In specific embodiments, the solution is the Columbia University solution further
comprising said specific inhibitor, or the Euro-Collins solution further comprising said
specific inhibitor, or the University of Wisconsin solution further comprising said specific
inhibitor, or the low-potassium dextran glucose solution further comprising said specific
inhibitor, or the Celsior™ solution further comprising said specific inhibitor. Optionally,
the solution further comprises a vasodilator. Exemplary vasodilators include, but are not
limited to an analog of adenosine 3',5'-cyclic monophosphate or an analog of guanosine
3',5'-cyclic monophosphate, such as dibutryl adenosine 3',5'-cyclic monophosphate (db
cAMP) or 8-bromo-adenosine 3',5'-cyclic monophosphate (8-bromo-cAMP). Preferably,
the analog is cell membrane-permeable. Other vasodilators include, but are not limited to
nitroglycerin, adenosine, pertussis toxin.

In yet another embodiment, the present invention is directed to a method for
performing a coronary artery bypass graft in a patient comprising, removing from contact
with a blood vessel or functional portion thereof a solution comprising a specific inhibitor
of Type III phosphodiesterase and/or Type IV phosphodiesterase; and grafting the blood
vessel or functional portion thereof into the patient so as to serve as a coronary bypass graft.
Preferably, the patient is a human patient and the blood vessel or portion thereof was
isolated from the same patient. Alternatively, the blood vessel is isolated from a non-human
animal.

Another embodiment of the invention is directed to a pharmaceutical pack or kit
comprising one or more containers filled with a solution of the invention comprising a
specific inhibitor of Type III and/or Type IV phosphodiesterase. For example, the kit can
comprise a container containing the low-potassium dextran glucose solution (Perfadex™)
further comprising a specific inhibitor of Type III phosphodiesterase. Optionally associated
with such container(s) can be instructions for use of the kit and/or a notice in the form
prescribed by a governmental agency regulating the manufacture, use or sale of
pharmaceuticals or biological products, which notice reflects approval by the agency of
manufacture, use or sale for human administration.
The following series of examples are presented by way of illustration and not by way of limitation on the scope of the present invention.

5. EXAMPLES

5.1 In carrying out a coronary bypass using a saphenous vein as the vascular graft, the patient is first anesthetized and a portion of the saphena is excised from either leg. The excised saphenous vein is placed in contact with a preservation solution comprising a specific inhibitor of Type III and/or Type IV phosphodiesterase in a kidney dish such that the solution is both inside and outside the vein and the dish is placed on ice. The excision in the leg is closed, and, concurrently, the chest is opened to allow access to the heart. The patient is placed on life support with a cardiac bypass machine and the heart is stopped. The saphenous vein is removed from the solution and is rinsed (flushed) with buffered saline lacking the specific phosphodiesterase inhibitor and potassium ions. The saphenous vein is cut to size for the bypass area and is grafted onto the cardiac tissue. The inserted venous segment acts as a bypass of the blocked portion of the coronary artery, and, thus, provides for a free or unobstructed flow of blood to the heart. The patient's heart is restarted and the chest is closed.

5.2 The following experimental vein graft procedure is used to assess the efficacy of a composition of the invention in ex vivo preservation of a blood vessel to be used as a vascular graft.

Vein graft procedure - intrapositional saphenous vein anastamosis

Female sheep, dogs or pigs are purchased from Charles River (Charles River, MA). The investigation will conform with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The surgical procedures used are standard; animals are anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). An incision is made on the left and right legs to expose the saphenous vein and femoral artery. A segment of the saphenous vein (19 cm long) is transected after ligation at both ends with 8-0 sutures. This segment is washed with saline solution containing 100 U/ml of heparin, and stored at 25°C for up to 2 hours in heparinized saline or in an experimental solution of heparinized saline containing a phosphodiesterase inhibitor of the invention, such as Milrinone.

A portion of the graft before these incubation is cut (0.5 cm) and placed in formaldehyde fixative (10%); after incubation for up to 2 hours, 0.5 cm sections are also cut
and placed in formaldehyde fixative (10%), both for later immunohistochemical analysis as described, *infra*. After incubation in control or experimental solutions as described, a segment of the femoral artery is temporally occluded at two places with a microvascular clamp (Roboz Surgical Instrument Co., Gaithersburg, MD), and a circular incision (of about the same size as the vein in diameter) is made. The anastomosis in loop is repaired by suturing the prepared vein into the clamped femoral artery with an 11-0 continuous suture around vein graft artery anastomosis. Contact between the instruments and the vein graft endothelium is avoided as much as possible throughout the procedure. After the vascular clamp is removed, the vein is inspected for adequacy of repair. Surgery is considered successful if strong pulsation is confirmed in both the graft and native artery without significant bleeding. If there is no pulsation or pulsations are diminished within a few minutes of restoration of blood flow, the procedure is considered a surgical failure. Cefazolin (50 mg/kg,) is administered and the skin incision is closed with a 6-0 nylon suture. Buprenorphine (2.5 mg/kg) is given subcutaneously for postoperative analgesia.

The duration of the entire procedure is approximately 30 minutes. One leg in each animal is for the experimental solutions; the contralateral leg is always used for the control solution. In order to verify intimal hyperplasia, both phorbol myristate acetate (PMA, Sigma, St. Louis, MO) and lipopolysaccaride (LPS, Sigma, St. Louis, MO) at 1.0 uM is used to incubate saphenous vein segments for up to 2 hours as positive controls.

**Morphology**

Animals are sacrificed at various time points after surgery and perfusion-fixed using 10% formaldehyde at physiological pressure. The grafts, together with a short segment of the native femoral artery, are harvested and cut at the center. The specimens are embedded in medium (OCT compound), and frozen at -80°C. The section (5 μm) at the mid portion of each composite graft is stained with hematoxylin and eosin (H&E) or Van Gieson's elastic stain (Sigma, St. Louis, MO), and the degree of neointimal expansion is analyzed quantitatively using a Zeiss microscope and image analysis system (Media Cybernetics, Silver Spring, MD). The consistency of neointimal formation in the central portion of the graft is histologically confirmed by analyzing serial sections from the center to the proximal and distal ends of the graft. The neointima of the vein graft is defined as the region between the lumen and the adventitia. Neointimal cell number is calculated by counting the number of nuclei visible in sections stained with H&E. The percentage of neointimal expansion is calculated as 100 x (neointimal area / neointimal area + luminal area). These
quantifications are performed by an observer blinded to the experimental circumstances. Masson Trichrome stain is performed according to the manufacture’s instructions (Sigma, St. Louis, MO).

5 En face immunofluorescence

The procedure used in this study is similar to that reported by Zou et al., 2000, Circ Res 86:434-440 and Dietrich et al., 2000, Arterioscler Thromb Vasc Biol 20:343-352. The vein patch is retrieved 24 hours after surgery and mounted onto a glass slide with endothelium side up, and air-dried for 1 hour at room temperature. The segments are fixed in cold acetone (-20°C) for 10 minutes and rinsed in PBS. The segments are then incubated with rat monoclonal antibody to MAC-1 (1:25, Pharmingen, San Diego, CA) for 30 minutes and visualized with FITC-labeled rabbit anti-rat IgG (1:25, Sigma, St. Louis, MO). MAC-1 positive cells are blindly counted at x 400 magnification in 10 fields of each segment.

15 Immunohistochemistry

Representative sections (5 mm) are immunostained with rat anti PECAM-1 (CD31) antibody (1:100, Pharmingen, San Diego, CA), rat anti-MAC-1 antibody (1:50, Pharmingen, San Diego, CA), and hamster anti-ICAM-1 antibody (1:100, Pharmingen, San Diego, CA). Sections are blocked with hydrogen peroxide (0.3%) in methanol for 10 min. Blocking is performed with goat serum (4%) and bovine serum albumin (1%) in PBS. Primary antibodies are added to slides, and incubated overnight at 4°C. Secondary antibodies (1:100; anti-hamster or rat IgG, Phamingen, San Diego, CA) are added for 30 min at room temperature. Sections are reacted with horseradish peroxidase conjugated streptavidin (1:100, Sigma, St. Louis, MO) for 30 min at room temperature and developed with 3,3’-diaminobenzidine (DAB substrate kit, Vector, Burlingame, CA).

Statistical analysis

All data are expressed as mean +/- SEM. Student’s unpaired t test for a comparison between two groups, or ANOVA with post hoc analysis using the Bonferroni/Dunn test for a comparison among more than two groups are used to determine significant difference. P values of less than 0.05 are considered statistically significant. All analyses are performed using the Statview statistical package, version J5.0 (Abacus Concepts Inc., Berkeley, CA).
6. REFERENCES CITED

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled. Such modifications are intended to fall within the scope of the appended claims.

All references, patent and non-patent, cited herein are incorporated herein by reference in their entireties and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.
WHAT IS CLAIMED IS:

1. A method of using a blood vessel as a vascular graft comprising:
   (a) contacting an isolated blood vessel or functional portion thereof ex vivo with a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase, and;
   (b) inserting the blood vessel into a patient so as to form a vascular graft in the patient.

2. The method of claim 1, wherein the temperature of the solution ranges from about 0.5°C to about 10°C.

3. The method of claim 1, wherein said contacting step is for a time period not longer than four hours.

4. The method of claim 1, which further comprises before step (b) a step of removing the solution from contact with the blood vessel or portion thereof.

5. The method of claim 4, wherein said removing step comprises flushing the blood vessel or portion thereof with a second solution lacking said specific inhibitor.

6. The method of claim 5, wherein said second solution is buffered saline or Ringer’s Lactate.

7. The method of claim 1, wherein the blood vessel is a saphenous vein, a mammary artery, or a radial artery; and the vascular graft is a coronary artery bypass graft.

8. The method of claim 7, wherein the blood vessel is a saphenous vein.

9. The method of claim 1, wherein said specific inhibitor is a specific inhibitor of Type III phosphodiesterase.

10. The method of claim 9, wherein said specific inhibitor is selected from the group consisting of Indolidan (LY 195115), Cilostamide (OPC 3689), Lixazinone (RS 82856), Y-590, Imazodan (CI 914), SKF 94120, Quazinone, ICI 153,110, Cilostazol (OPC 13013), Bemorandan (RWJ 22867), Siguazodan (SK&F 94836), Adibendan (BM 14,478), Milrinone (WIN 47203), Enoximone (MDL 17043), Pimobendan (UD-CG 115, MCI-154), Saterinone (BDF 8634), Amirinone, Olprinone, NSP-153, Zardaverine, quinazolines, benzafentrine, Sulmazole (ARL 115), ORG 9935, and a mixture of any two or more of the foregoing.

11. The method of claim 1, wherein said specific inhibitor is a specific inhibitor of Type IV phosphodiesterase.

12. The method of claim 11, wherein the specific inhibitor is selected from the group consisting of Rolipram (ZK 62711; Pyrrolidone), RO 20-1724 (Imidazoiidinone), SQ 64442 (Etazoiate), Denbufylline (BRL 30892), V11294, EMD 95832/3, SCH351591,
13. The method of claim 1, wherein said solution further comprises heparinized blood.

14. The method of claim 1, wherein said solution further comprises buffered saline.

15. The method of claim 1, wherein said solution is the Columbia University solution further comprising said specific inhibitor.

16. The method of claim 1, wherein said solution is the Euro-Collins solution further comprising said specific inhibitor.

17. The method of claim 1, wherein said solution is the University of Wisconsin solution further comprising said specific inhibitor.

18. The method of claim 1, wherein said solution is the low-potassium dextran glucose solution further comprising said specific inhibitor.

19. The method of claim 1, where said solution is the Celsior™ solution further comprising said specific inhibitor.

20. The method of claim 1, wherein said solution further comprises an analog of adenosine 3',5'-cyclic monophosphate or an analog of guanosine 3',5'-cyclic monophosphate.

21. The method of claim 1, wherein said solution further comprises dibutyryl adenosine 3',5'-cyclic monophosphate (dB cAMP).

22. The method of claim 1, wherein said solution further comprises 8-bromo-adenosine 3',5'-cyclic monophosphate (8-bromo-cAMP).

23. The method of claim 1, wherein said solution further comprises nitroglycerin.

24. The method of claim 1 wherein said solution further comprises:
   (a) a vasodilator in an amount sufficient to maintain vascular homeostasis, wherein the vasodilator is selected from the group consisting of: adenosine 3',5'-cyclic monophosphate analogues, guanosine 3',5'-cyclic monophosphate analogues, nitroglycerin, and pertussis toxin;
   (b) a sugar in an amount sufficient to support intracellular function and
maintenance of cellular bioenergetics;

(c) magnesium ions in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics;

(d) a macromolecule of molecular weight greater than 20,000 daltons in an amount sufficient to maintain endothelial integrity and cellular viability;

(e) potassium ions in a concentration greater than about 110 mM; and

(f) a buffer in an amount sufficient to maintain the average pH of the blood vessel or portion thereof during said contacting step at about the physiologic pH value.

25. The method of claim 9, wherein the concentration of the specific inhibitor of Type III phosphodiesterase ranges from about 0.1μM to 100μM.

26. The method of claim 11, wherein the concentration of the specific inhibitor of Type IV phosphodiesterase ranges from about 0.1μM to 100μM.

27. The method of claim 1, wherein said solution further comprises Phosphate Buffered Saline (PBS), Hanks’ Balanced Salt Solution (HBSS), HBSS (Modified), Ringer’s Lactate, Tyrodes buffer, Krebs buffer, Euro-Collins solution, University of Wisconsin solution, low-potassium dextran glucose solution, Celsior™ solution, or Columbia University solution.

28. The method of claim 1, wherein the patient is a human.

29. The method of claim 28, wherein the blood vessel or portion thereof is from the patient.

30. A solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase in a solution comprising heparinized blood.

31. The solution of claim 30, wherein said specific inhibitor is a specific inhibitor of Type III phosphodiesterase.

32. The solution of claim 31, wherein said specific inhibitor is selected from the group consisting of Indolidan (LY 195115), Cilostamide (OPC 3689), Lixazinone (RS 82856), Y-590, Imazodan (CI 914), SKF 94120, Quazinone, ICI 153,110, Cilostazol (OPC 13013), Bemorandanzan (RWJ 22887), Siguazodan (SK&F 94836), Adibendan (BM 14,478), Milrinone (WIN 47203), Enoximone (MDL 17043), Pimobendan (UD-CG 115, MCI-154), Saterinone (BDF 8634), Amirinone, Olprinone, NSP-153, Zardaverine, quinazolines, benzafentrine, Sulmazole (ARL 115), ORG 9935, and a mixture of any two or more of the foregoing.

33. The solution of claim 30, wherein said specific inhibitor is a specific inhibitor of Type IV phosphodiesterase.
34. The solution of claim 33, wherein the specific inhibitor is selected from the group consisting of Rolipram (ZK 62711; Pyrrolidone), RO 20-1724 (Imidazooi-dinone), SQ 64442 (Etazoiate), Denbufylline (BRL 30892), V11294, EMD 95832/3, SCH351591, KF19514, Thieno [3, 2-d] pyrimidines, RP73401, Cipamfylline, Cilomilast, YM-10335, CDP840, SBZ07499 (ariflo), T-440, CP-80,633, 7-methoxybenzofuran-4-carboxamides, Piclamilast, Roffumilast, Arofylline, BAY-19-8004, Mesopram, Atizoram, Zardaverine, quinazolines, benzafentrine, Filamilast, C1-1018, C1-1044, D-4418, IC-485, L-791,943, CD-998, YM-976, YM-58997, V-11294A, CDC-801, CC-7075, R-P-73401, and a mixture of any two or more of the foregoing.

35. The solution of claim 30, wherein said solution further comprises an analog of adenosine 3',5'-cyclic monophosphate or an analog of guanosine 3',5'-cyclic monophosphate.

36. The solution of claim 30, wherein said solution further comprises dibutryryl adenosine 3',5'-cyclic monophosphate (db cAMP).

37. The solution of claim 30, wherein said solution further comprises 8-bromo-adenosine 3',5'-cyclic monophosphate (8-bromo-cAMP).

38. A solution consisting of a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase in heparinized blood.

39. The solution of claim 38, wherein said specific inhibitor is a specific inhibitor of Type III phosphodiesterase.

40. The solution of claim 39 wherein said specific inhibitor is selected from the group consisting of Indolidan (LY 195115), Cilostamide (OPC 3689), Lixazinone (RS 82856), Y-590, Imazodan (CI 914), SKF 94120, Quazinone, ICI 153,110, Cilostazol (OPC 13013), Beemorand (RWJ 22867), Siguazodan (SK&F 94836), Adibendan (BM 14,478), Milrinone (WIN 47203), Enoximone (MDL 17043), Pimobendan (UD-CG 115, MCI-154), Saterinone (BDF 8634), Amirinine, Olprinoine, NSP-153, Zardaverine, quinazolines, benzafentrine, Sulmazole (ARL 115), ORG 9935, and a mixture of any two or more of the foregoing.

41. The solution of claim 38, wherein said specific inhibitor is a specific inhibitor of Type IV phosphodiesterase.

42. The solution of claim 41, wherein the specific inhibitor is selected from the group consisting of Rolipram (ZK 62711; Pyrrolidone), RO 20-1724 (Imidazooi-dinone), SQ 64442 (Etazoiate), Denbufylline (BRL 30892), V11294, EMD 95832/3, SCH351591, KF19514, Thieno [3, 2-d] pyrimidines, RP73401, Cipamfylline, Cilomilast, YM-10335, CDP840, SBZ07499 (ariflo), T-440, CP-80,633, 7-methoxybenzofuran-4-carboxamides,
43. The solution of claim 38, wherein said solution further comprises an analog of adenosine 3',5'-cyclic monophosphate or an analog of guanosine 3',5'-cyclic monophosphate.

44. The solution of claim 38, wherein said solution further comprises dibutyryl adenosine 3',5'-cyclic monophosphate (db cAMP).

45. The solution of claim 38, wherein said solution further comprises 8-bromo-adenosine 3',5'-cyclic monophosphate (8-bromo-cAMP).

46. An isolated ex vivo blood vessel or functional portion thereof in contact with a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase, at a temperature in the range of about 0.5°C to about 10°C.

47. The blood vessel or portion thereof of claim 46, wherein the blood vessel is a saphenous vein, a mammary artery, or a radial artery.

48. The blood vessel or portion thereof of claim 46, wherein the blood vessel is a saphenous vein.

49. The blood vessel or portion thereof of claim 46, wherein said specific inhibitor is a specific inhibitor of Type III phosphodiesterase.

50. The blood vessel or portion thereof of claim 49, wherein said specific inhibitor is selected from the group consisting of Indolidan (LY 195115), Cilostamide (OPC 3689), Lixazinone (RS 82856), Y-590, Imazodan (CI 914), SKF 94120, Quazinone, ICI 153,110, Cilostazol (OPC 13013), Bemorandan (RWJ 22867), Siguazodan (SK&F 94836), Adibendan (BM 14,478), Milrinone (WIN 47203), Enoximone (MDL 17043), Pimobendan (UD-CG 115, MCI-154), Saterinone (BDF 8634), Amirinone, Olprinone, NSP-153, Zardaverine, quinazolines, benzafentrine, Sulmazole (ARL 115), ORG 9935, and a mixture of any two or more of the foregoing.

51. The blood vessel or portion thereof of claim 46, wherein said specific inhibitor is a specific inhibitor of Type IV phosphodiesterase.

52. The blood vessel or portion thereof of claim 51, wherein the specific inhibitor is selected from the group consisting of Rolipram (ZK 62711; Pyrrolidone), RO 20-1724 (Imidazolidinone), SQ 64442 (Etazoiate), Denbufylline (BRL 30892), V11294, EMD 95832/3, SCH351591, KF19514, Thieno [3, 2-d] pyrimidines, RP73401, Cipamfylline, Cilomilast, YM-10335, CDP840, SBZ07499 (ariflo), T-440, CP-80,633, 7-
methoxybenzofuran-4-carboxamides, Piclamilast, Roflumilast, Aroylline, BAY-19-8004, Mesopram, Atizoram, Zardaverine, quinazolines, benzafentrine, Filamlast, C1-1018, C1-1044, D-4418, IC-485, L-791,943, CD-998, YM-976, YM-58997, V-11294A, CDC-801, CC-7075, R-P-73401, and a mixture of any two or more of the foregoing.

53. The blood vessel or portion thereof of claim 49, wherein the concentration of the specific inhibitor of Type III phosphodiesterase ranges from about 0.1μM to 100μM.

54. The blood vessel or portion thereof of claim 51, wherein the concentration of the specific inhibitor of Type IV phosphodiesterase ranges from about 0.1μM to 100μM.

55. The blood vessel or portion thereof of claim 46, wherein said solution comprises heparinized blood.

56. The blood vessel or portion thereof of claim 46, wherein said solution is buffered saline further comprising said specific inhibitor.

57. The blood vessel or portion thereof of claim 46, wherein said solution is the Columbia University solution further comprising said specific inhibitor.

58. The blood vessel or portion thereof of claim 46, wherein said solution is the Euro-Collins solution further comprising said specific inhibitor.

59. The blood vessel or portion thereof of claim 46, wherein said solution is the University of Wisconsin solution further comprising said specific inhibitor.

60. The blood vessel or portion thereof of claim 46, wherein said solution is the low-potassium dextran glucose solution further comprising said specific inhibitor.

61. The blood vessel or portion thereof of claim 46, wherein said solution is the Celsior™ solution further comprising said specific inhibitor.

62. The blood vessel or portion thereof of claim 46, wherein said solution further comprises an analog of adenosine 3',5'-cyclic monophosphate or an analog of guanosine 3',5'-cyclic monophosphate.

63. The blood vessel or portion thereof of claim 62, wherein said solution further comprises dibutyryl adenosine 3',5'-cyclic monophosphate (db cAMP).

64. The blood vessel or portion thereof of claim 62, wherein said solution further comprises 8-bromo-adenosine 3',5'-cyclic monophosphate (8-bromo-cAMP).

65. The blood vessel or portion thereof of claim 46, wherein said solution further comprises:

(a) a vasodilator in an amount sufficient to maintain vascular homeostasis, wherein the vasodilator is selected from the group consisting of: adenosine 3',5'-cyclic monophosphate analogues, guanosine 3',5'-cyclic monophosphate analogues, nitroglycerin, and pertussis toxin;
(b) a sugar in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics;

(c) magnesium ions in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics;

(d) a macromolecule of molecular weight greater than 20,000 daltons in an amount sufficient to maintain endothelial integrity and cellular viability;

(e) potassium ions in a concentration greater than about 110 mM; and

(f) a buffer in an amount sufficient to maintain the average pH of the blood vessel or portion thereof at about the physiologic pH value.

66. The blood vessel or portion thereof of claim 46, which is a human blood vessel or portion thereof.

67. An isolated *ex vivo* isolated blood vessel or functional portion thereof in contact with a solution comprising (a) heparinized blood and (b) a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase.

68. The blood vessel or portion thereof of claim 67, which is a human blood vessel or portion thereof.

69. A container containing the blood vessel or portion thereof of claim 46.

70. A container containing the blood vessel or portion thereof of claim 67.

71. A method of preserving a blood vessel comprising contacting an isolated blood vessel or portion thereof *ex vivo* with a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase in a solution comprising heparinized blood.

72. The method of claim 71, wherein said contacting is for a time period not longer than four hours.

73. The method of claim 71, wherein the temperature of the solution ranges from about 0.5°C to about 10°C.

74. The method of claim 71, which further comprises a step of removing the solution from contact with the blood vessel or portion thereof.

75. The method of claim 74, wherein said removing step comprises flushing the blood vessel or portion thereof with a second solution lacking said specific inhibitor.

76. The method of claim 71, wherein the blood vessel is a saphenous vein, a mammary artery, or a radial artery; and the vascular graft is a coronary artery bypass graft.

77. The method of claim 76, wherein the blood vessel is a saphenous vein.

78. The method of claim 71, wherein said specific inhibitor is a specific inhibitor of Type III phosphodiesterase.
The method of claim 78, wherein said specific inhibitor is selected from the group consisting of Indolidan (LY 195115), Cilostamide (OPC 3689), Lixazinone (RS 82856), Y-590, Imazobolan (CI 914), SKF 94120, Quazinone, ICI 153,110, Cilostazol (OPC 13013), Bemorand (RJW 22867), Sigauzodan (SK&F 94836), Adibendan (BM 14,478), Milrinone (WIN 47203), Enoximone (MDL 17043), Pimobendan (UD-CG 115, MCI-154), Saterinone (BDF 8634), Amirinone, Olprinone, NSP-153, Zardaverine, quinazolines, benzafentrine, Sulmazole (ARL 115), ORG 9935, and a mixture of any two or more of the foregoing.

The method of claim 71, wherein said specific inhibitor is a specific inhibitor of Type IV phosphodiesterase.

The method of claim 80, wherein the specific inhibitor is selected from the group consisting of Rolipram (ZK 62711; Pyrrolidone), RO 20-1724 (Imidazoaldoxinone), SQ 64442 (Etazolate), Denbufylline (BRL 30892), V11294, EMD 95832/3, SCH351591, KF19514, Thieno [3, 2-d] pyrimidines, RP73401, Cipamfylline, Cilomilast, YM-10335, CDP840, SBZ07499 (ariflo), T-440, CP-80,633, 7-methoxybenzofuran-4-carboxamides, Piclamilast, Rolflumilast, Arofylline, BAY-19-8004, Mesopram, Atizoram, Zardaverine, quinazolines, benzafentrine, Filamist, Cl-1018, C1-1044, D-44,18, IC-485, L-791,943, CD-998, YM-976, YM-58997, V-11294A, CDC-801, CC-7075, R-P-73401, and a mixture of any two or more of the foregoing.

The method of claim 78, wherein the concentration of the specific inhibitor of Type III phosphodiesterase ranges from about 0.1μM to 100μM.

The method of claim 80, wherein the concentration of the specific inhibitor of Type IV phosphodiesterase ranges from about 0.1μM to 100μM.

The method of claim 71, wherein said solution further comprises buffered saline.

The method of claim 71, wherein said solution is the Columbia University solution further comprising said specific inhibitor.

The method of claim 71, wherein said solution is the Euro-Collins solution further comprising said specific inhibitor.

The method of claim 71, wherein said solution is the University of Wisconsin solution further comprising said specific inhibitor.

The method of claim 71, wherein said solution is the low-potassium dextran glucose solution further comprising said specific inhibitor.

The method of claim 71, wherein said solution is the Celsior™ solution further comprising said specific inhibitor.
90. The method of claim 71, wherein said solution further comprises an analog of adenosine 3',5'-cyclic monophosphate or an analog of guanosine 3',5'-cyclic monophosphate.

91. The method of claim 90, wherein said solution further comprises dibutyril adenosine 3',5'-cyclic monophosphate (db cAMP).

92. The method of claim 90, wherein said solution further comprises 8-bromo-adenosine 3',5'-cyclic monophosphate (8-bromo-cAMP).

93. The method of claim 71, wherein said solution further comprises:
   (a) a vasodilator in an amount sufficient to maintain vascular homeostasis, wherein the vasodilator is selected from the group consisting of: adenosine 3',5'-cyclic monophosphate analogues, guanosine 3',5'-cyclic monophosphate analogues, nitroglycerin, and pertussis toxin;
   (b) a sugar in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics;
   (c) magnesium ions in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics;
   (d) a macromolecule of molecular weight greater than 20,000 daltons in an amount sufficient to maintain endothelial integrity and cellular viability;
   (e) potassium ions in a concentration greater than about 110 mM; and
   (f) a buffer in an amount sufficient to maintain the average pH of the blood vessel or portion thereof during said contacting at about the physiologic pH value.

94. The method of claim 71 wherein the blood vessel or portion thereof is a human blood vessel or portion thereof.

95. A method of preserving a blood vessel comprising contacting an isolated blood vessel or portion thereof \textit{ex vivo} with a solution consisting of a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase in heparinized blood.

96. The method of claim 95, wherein the temperature of the solution ranges from about 0.5°C to about 10°C.

97. A method of preserving a blood vessel comprising contacting an isolated blood vessel or portion thereof \textit{ex vivo} with a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase, at a temperature in the range of about 0.5°C to about 10°C.

98. The method of claim 97, wherein said contacting is for a time period not longer than four hours.
99. The method of claim 97, which further comprises a step of removing the solution from contact with the blood vessel or portion thereof.

100. The method of claim 99, wherein said removing step comprises flushing the blood vessel or portion thereof with a second solution lacking said specific inhibitor.

101. The method of claim 97, wherein the blood vessel is a saphenous vein, a mammary artery, or a radial artery; and the vascular graft is a coronary artery bypass graft.

102. The method of claim 101, wherein the blood vessel is a saphenous vein.

103. The method of claim 97, wherein said specific inhibitor is a specific inhibitor of Type III phosphodiesterase.

104. The method of claim 103, wherein said specific inhibitor is selected from the group consisting of Indolidan (LY 195115), Cilostamide (OPC 3689), Lixazinone (RS 82856), Y-590, Imazodan (CI 914), SKF 94120, Quazinone, ICI 153,110, Cilostazol (OPC 13013), Bemorandam (RWJ 22867), Siguazodan (SK&F 94836), Adibendan (BM 14,478), Milrinone (WIN 47203), Enoximone (MDL 17043), Pimobendan (UD-CG 115, MCI-154), Saterinone (BDF 8634), Amirinone, Olprinone, NSP-153, Zardaverine, quinazolines, benzfentrine, Sulmazole (ARL 115), ORG 9935, and a mixture of any two or more of the foregoing.

105. The method of claim 97, wherein said specific inhibitor is a specific inhibitor of Type IV phosphodiesterase.

106. The method of claim 105, wherein the specific inhibitor is selected from the group consisting of Rolipram (ZK 62711; Pyrrolidone), RO 20-1724 (Imidazolidinone), SQ 64442 (Etazololate), Denbufylline (BRL 30892), V11294, EMD 95832/3, SCH351591, KF19514, Thierno [3, 2-d] pyrimidines, RP73401, Cipamfylline, Cilomilast, YM-10335, CDP840, SBZ07499 (ariflo), T-440, CP-80,633, 7-methoxybenzofuran-4-carboxamides, Piclamilast, Rofumlilast, Aroffylone, BAY-19-8004, Mesopram, Atizoram, Zardaverine, quinazolines, benzfentrine, Filamilast, Cl-1018, C1-1044, D-4418, IC-485, L-791,943, CD-998, YM-976, YM-58997, V-11294A, CDC-801, CC-7075, R-P-73401, and a mixture of any two or more of the foregoing.

107. The method of claim 103, wherein the concentration of the specific inhibitor of Type III phosphodiesterase ranges from about 0.1μM to 100μM.

108. The method of claim 105, wherein the concentration of the specific inhibitor of Type IV phosphodiesterase ranges from about 0.1μM to 100μM.

109. The method of claim 97, wherein said solution comprises heparinized blood.

110. The method of claim 97, wherein said solution comprises buffered saline.
111. The method of claim 97, wherein said solution is the Columbia University solution further comprising said specific inhibitor.

112. The method of claim 97, wherein said solution is the Euro-Collins solution further comprising said specific inhibitor.

113. The method of claim 97, wherein said solution is the University of Wisconsin solution further comprising said specific inhibitor.

114. The method of claim 97, wherein said solution is the low-potassium dextran glucose solution further comprising said specific inhibitor.

115. The method of claim 97, wherein said solution is the Celsior™ solution further comprising said specific inhibitor.

116. The method of claim 97, wherein said solution further comprises an analog of adenosine 3',5'-cyclic monophosphate or an analog of guanosine 3',5'-cyclic monophosphate.

117. The method of claim 116, wherein said solution further comprises dibutyryl adenosine 3',5'-cyclic monophosphate (db cAMP).

118. The method of claim 116, wherein said solution further comprises 8-bromo-adenosine 3',5'-cyclic monophosphate (8-bromo-cAMP).

119. The method of claim 97, wherein said solution further comprises:
   (a) a vasodilator in an amount sufficient to maintain vascular homeostasis, wherein the vasodilator is selected from the group consisting of: adenosine 3',5'-cyclic monophosphate analogues, guanosine 3',5'-cyclic monophosphate analogues, nitroglycerin, and pertussis toxin;
   (b) a sugar in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics;
   (c) magnesium ions in an amount sufficient to support intracellular function and maintenance of cellular bioenergetics;
   (d) a macromolecule of molecular weight greater than 20,000 daltons in an amount sufficient to maintain endothelial integrity and cellular viability;
   (e) potassium ions in a concentration greater than about 110 mM; and
   (f) a buffer in an amount sufficient to maintain the average pH of the blood vessel or portion thereof during said contacting at about the physiologic pH value.

120. The method of claim 97, wherein the blood vessel or portion thereof is a human blood vessel or portion thereof.

121. The method of claim 1, wherein the patient is a human.
122. The method of claim 1, wherein the contacting comprises immersing, infusing, flushing, or perfusing.

123. The method of claim 1, wherein the blood vessel is one or a combination of: the internal mammary artery, the radial artery, right gastroepiploic artery, inferior epigastric artery, or the saphenous vein.

124. The method of claim 123, wherein the blood vessel is the saphenous vein.

125. The method of claim 67, wherein the temperature of the solution ranges from about 0.5°C to about 10°C.

126. A method for performing a coronary artery bypass graft in a patient comprising,

   (a) removing from contact with a blood vessel or functional portion thereof a solution comprising a specific inhibitor of Type III phosphodiesterase and/or Type IV phosphodiesterase; and

   (b) grafting the blood vessel or functional portion thereof into the patient so as to serve as a coronary bypass graft.

127. The method of claim 126, wherein the patient is a human patient.